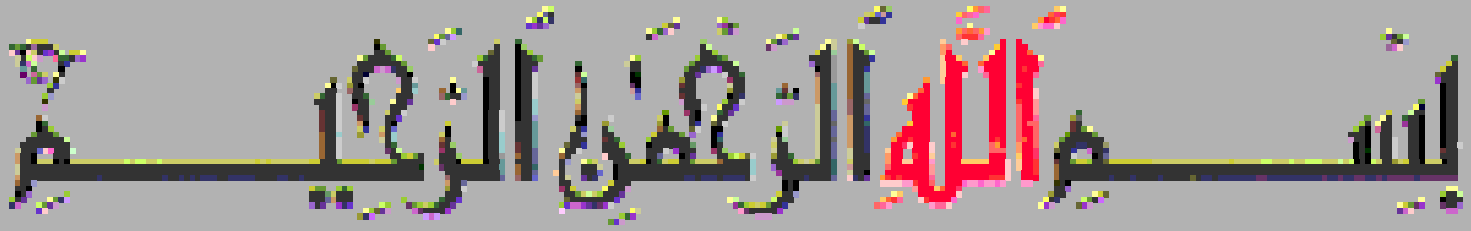
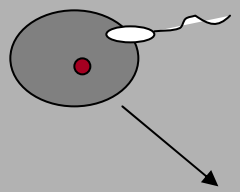


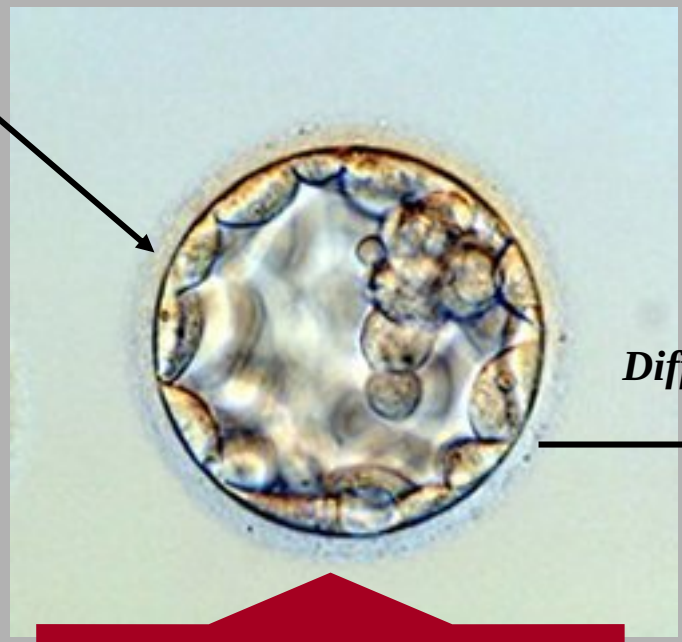
In the Name of Allah, Most Compassionate, Most Merciful

Differentiation and Development



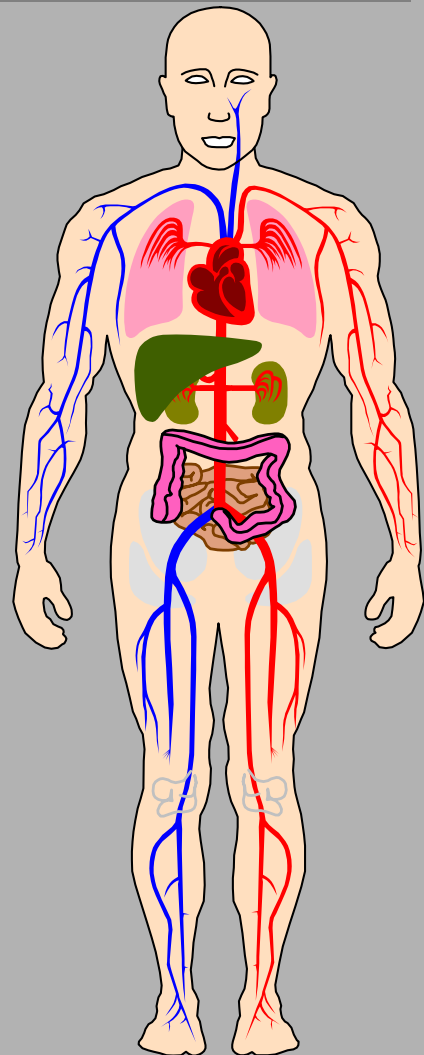
Fertilized cell

Blastocyte



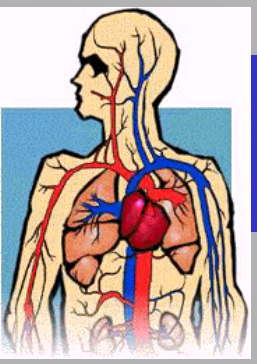
Differentiation

Human body



Note: ***Every cell in the body has the same genetic material***

How do different tissues differ from each other?



Tissues have different shape, function, metabolic features

As tissues make different proteins and enzymes and the enzymes Synthesise different substances, thus producing tissue variability.

Tissues differ in gene expression

GENES AND THEIR EXPRESSION

There are estimated 25,000 – 30,000 genes in the human genome in each cell

- Only a fraction are expressed.
- Different genes are expressed in different tissues
- Route of expression of genes differs significantly where some gene products are produced in large amounts, others in very small amounts& some are not produced at all in some cells

- This gives each tissue its distinctive character and function.
- This is the basic mechanism underlying cell differentiation

CONTROL OF GENE EXPRESSION

Types of Genes in Cells

House-keeping genes

Genes for products that are required at all times and hence the gene is expressed at almost a constant rate.

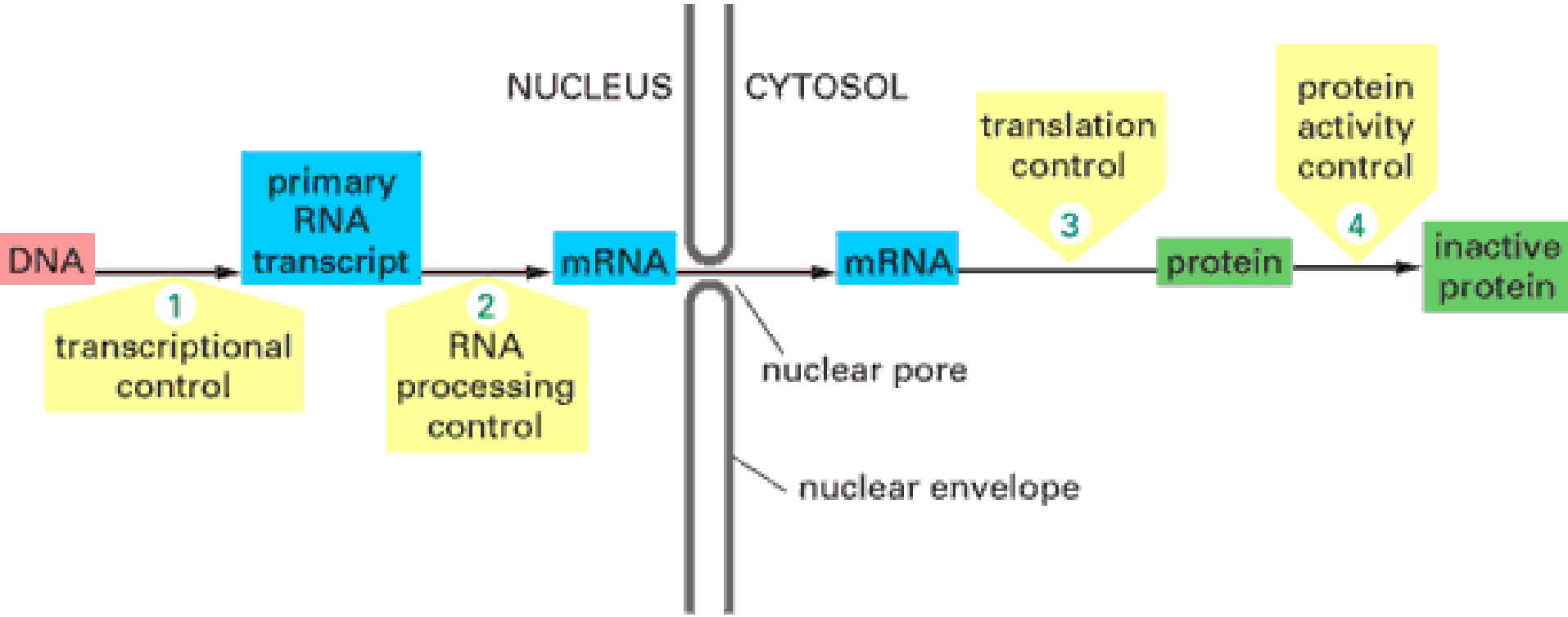
Inducible Genes

Genes for some gene products that rise and fall in response to molecular signals.

These genes can be induced and repressed.

HOW IS GENE EXPRESSION REGULATED?

Levels of regulation of protein Synthesis

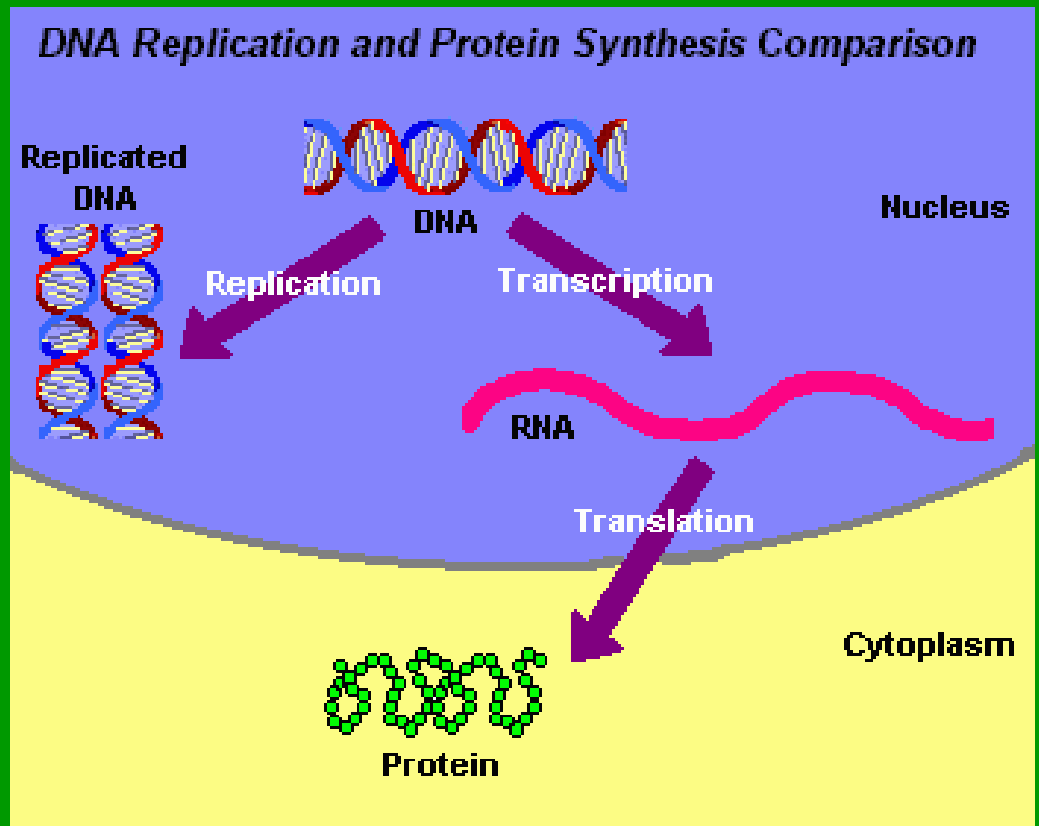


REGULATION OF THE LEVEL OF GENE PRODUCT (PROTEINS) in CELLS

- Synthesis of the primary RNA transcript (transcription).
- Post-transcriptional processing of mRNA.
- mRNA degradation.
- Protein synthesis (translation).
- Post-translational modifications.
- Protein degradation.

Regulation of Gene Expression

Regulation of Transcription



GENE EXPRESSION

Gene expression depends on:

- Chromatin structure.
- Activity of RNA polymerase.
- Sequence in the upstream activator and enhancer.
- Regulatory proteins.

CHROMATIN STRUCTURE

- Transcriptionally active chromatin is structurally distinct.
- Before transcription, several changes occur in chromatin.
  - Increased sensitivity of DNA to nuclease mediated degradation (hypersensitivity sites).
  - Low number of nucleosomes.
  - Undermethylation of CPG sequences in the DNA.
  - Low level of histone HI.

RNA POLYMERASE AND GENE

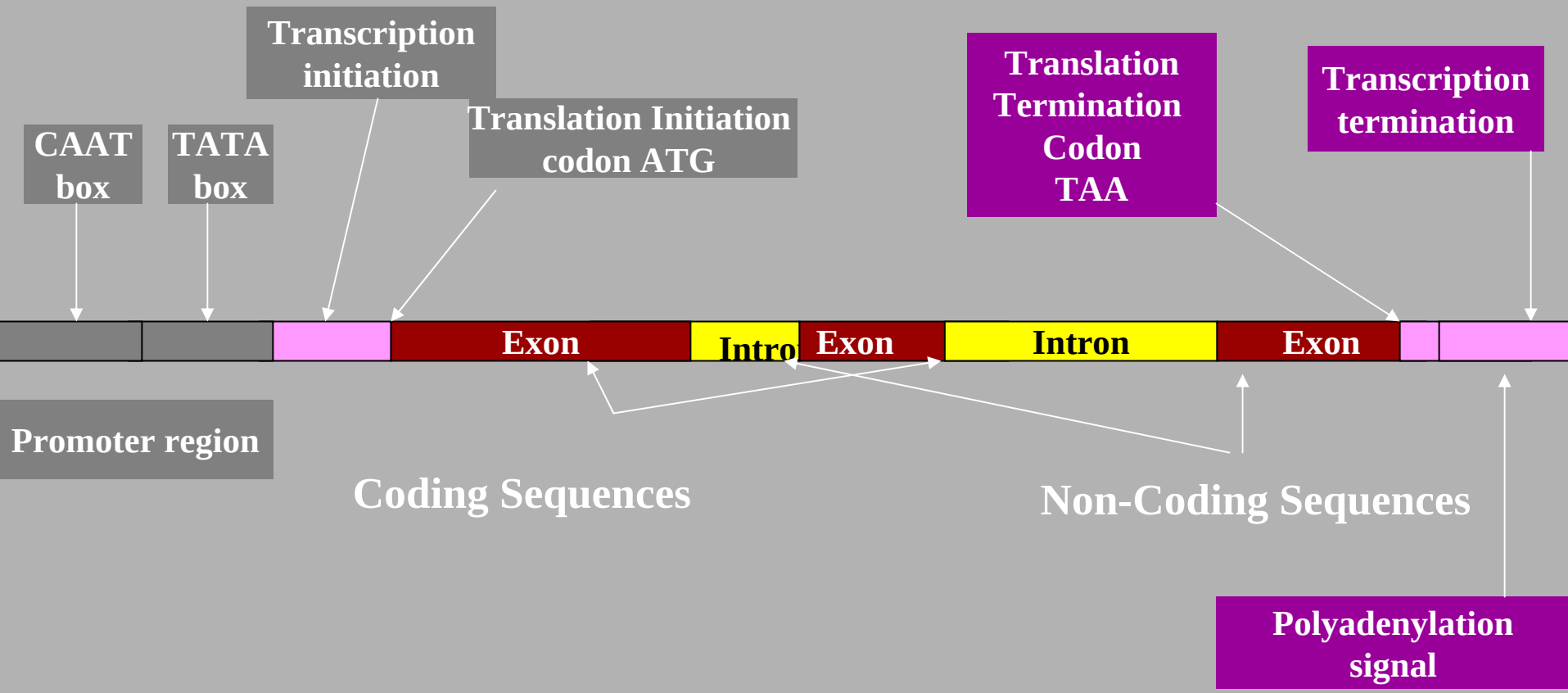
- RNA polymerase is the main enzyme controlling expression of transcription.
  - It binds to specific sites on DNA called "Promoters" near the initiation site.
- Transcription regulation is achieved by regulation of interaction of RNA polymerase with the promoters.

Contd……

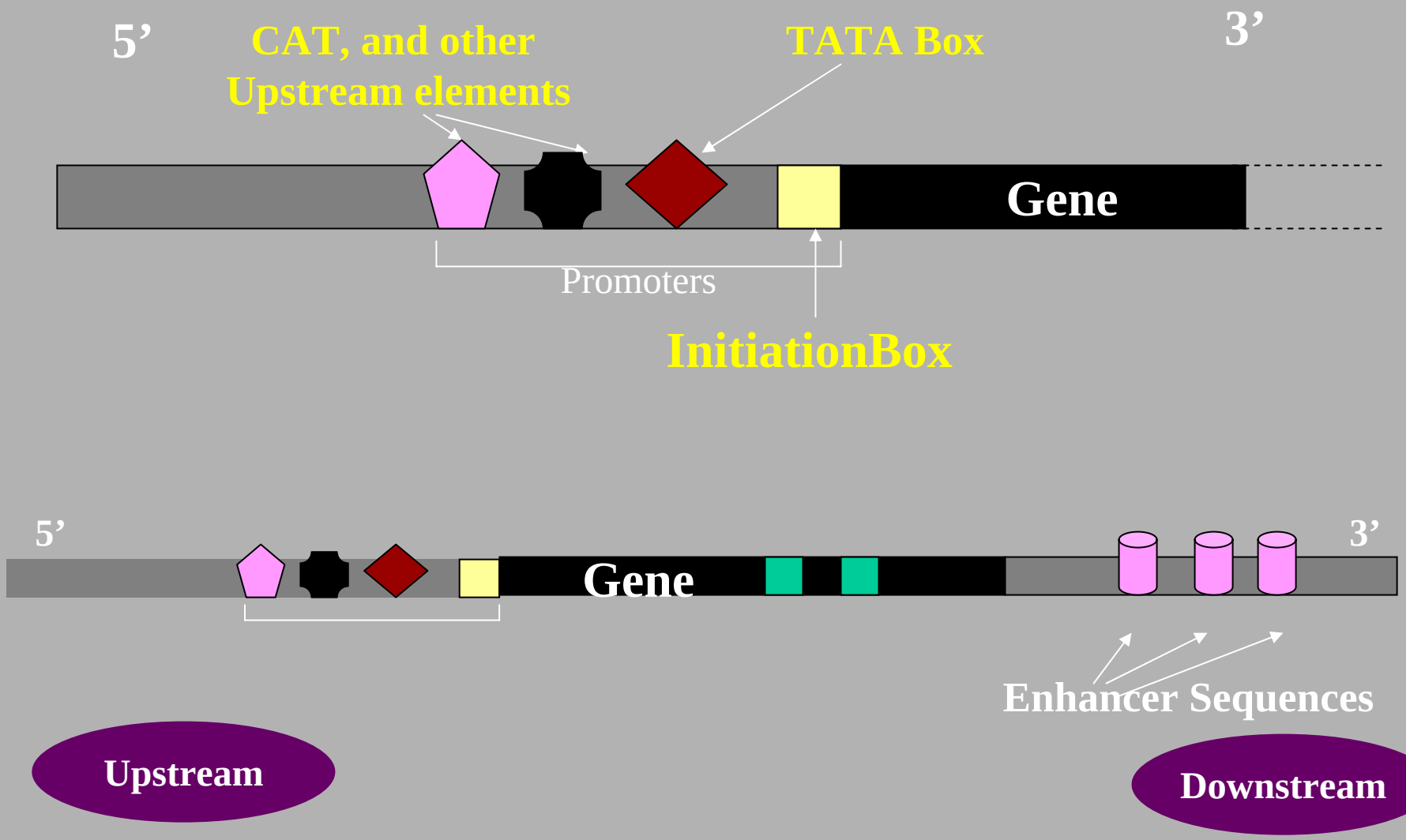
RNA POLYMERASE AND GENE ….Contd

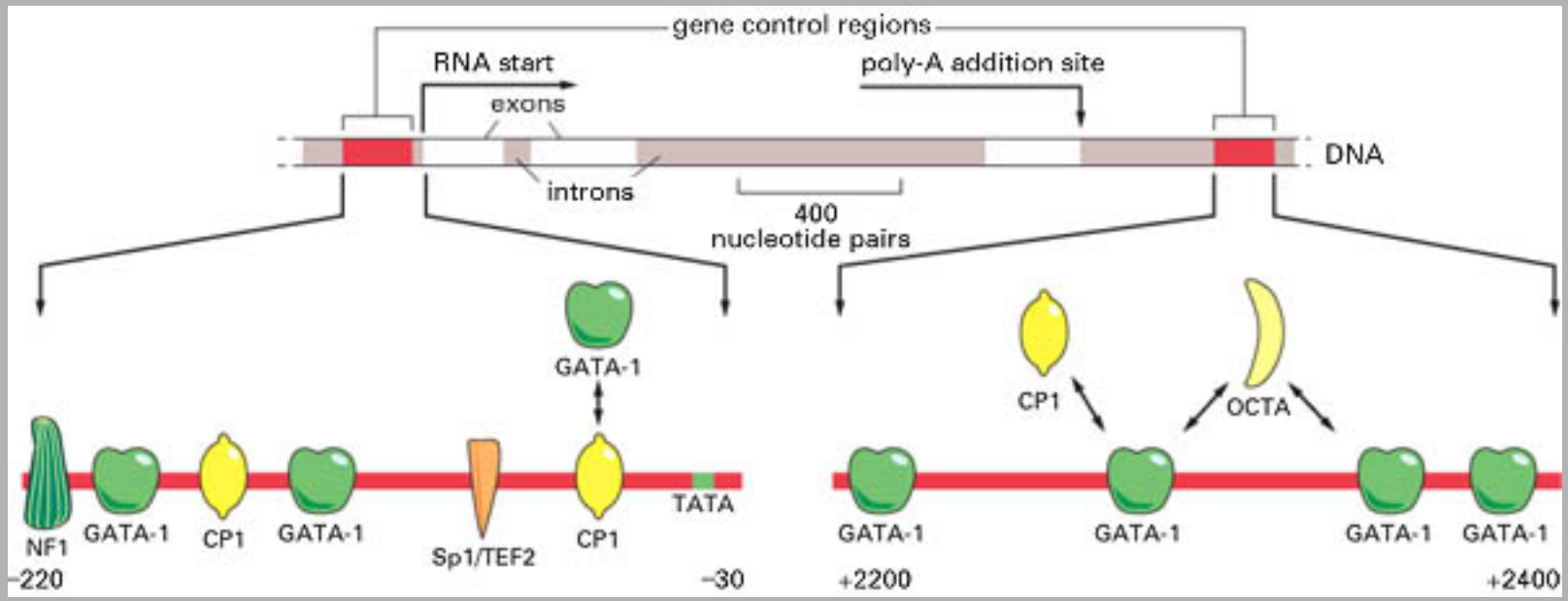
- Promoters vary in their sequence and this influences binding affinity of RNA polymerase and hence frequency of transcription.
- Interaction between RNA polymerase and the promoters is affected by several regulatory proteins.

Genes-exons and introns and promoter regions



Promoter and Enhancer layouts in Eucaryotes




gene control regions
RNA start
poly-A addition site
exons
introns
DNA
400
nucleotide pairs
GATA-1
NF1
GATA-1
CP1
GATA-1
Sp1/TEF2
CP1
TATA
−220
−30
CP1
OCTA
GATA-1
GATA-1
GATA-1
GATA-1
+2200
+2400

RNA POLYMERASE AND PROMOTERS

RNA Pol I: Synthesis of pre-ribosomal RNA transcript a precursor for 18S, 5.8S and 28S rRNA.

RNA Pol II: Synthesis of mRNA:

- Recognizes thousands of different promoter sequences.
- Binding is influenced by transcription factors, which bind to consensus sequences.

RNA POLYMERASE AND PROMOTERS …….Contd

RNA Pol III: Synthesis of tRNA and 5S rRNA. Recognizes a few special promoter sequences.

REGULATION OF PROMOTERS

- Most eucaryotic promoters are *positively regulated*,i.e.
  - RNA polymerase has no or very little affinity for their promoter.
  - One or more activator proteins bind to promoter.
  - This influences binding of RNA polymerase.

Contd…….

REGULATION OF PROMOTERS ….Contd

- Some promoters are *negatively regulated*, i.e.
  - RNA polymerase binds promoter and initiates transcription.
  - Repressor proteins bind to promoter and inhibits transcription.

Transcription Factors

- Specialized proteins that **regulate gene expression**.
- Have four **Domains**:
  - for **binding** to a specific sequence on the **DNA**
  - for **binding** to the **RNA polymerase II** complex
  - for **entering the nucleus**
  - for **responding to stimuli** which signal switching on or off a gene.
- Transcription factors for a specific gene **appear** mainly **in cells** where that **gene has to be expressed**.

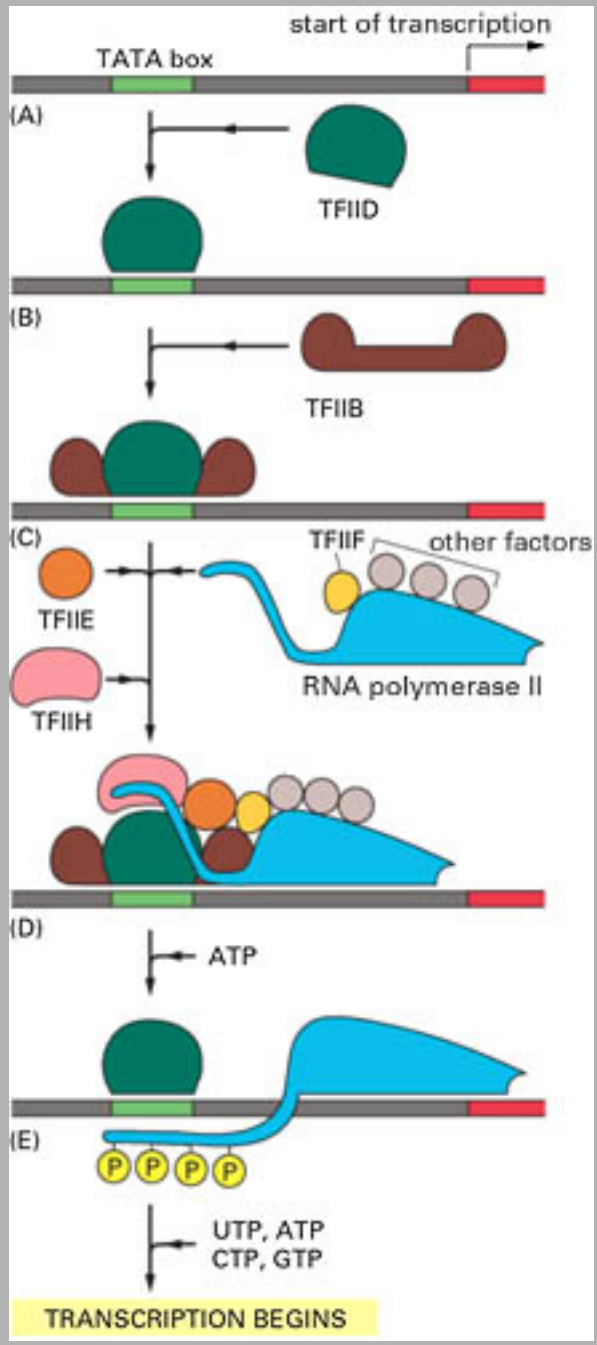
  e.g. MyoD appears only in cells destined to become muscle cells, and switches on a wide selection of genes needed in the muscles, but not on other cells.

TRANSCRIPTION FACTORS

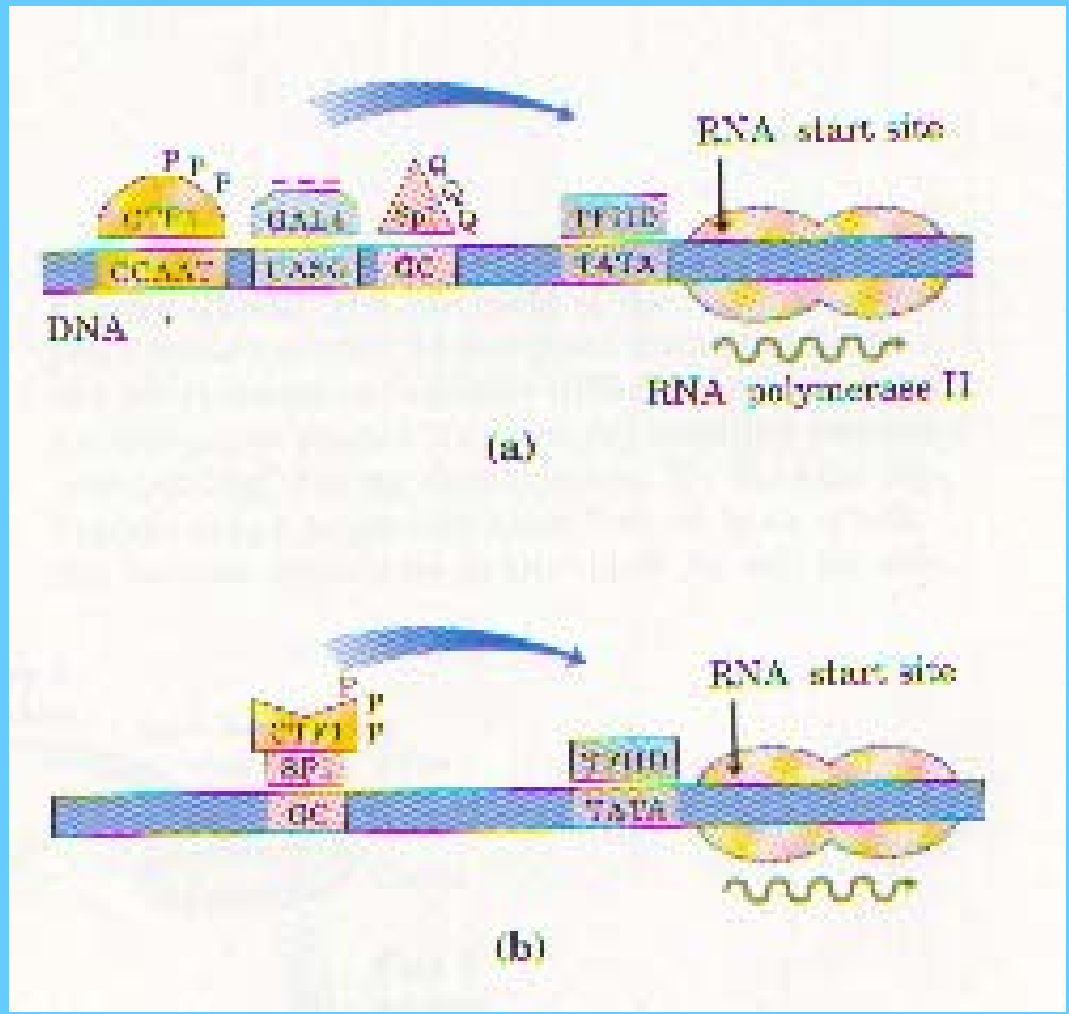
- Proteins
- May be:
  - inducers - increase transcription, or
  - repressors – decrease transcription.
- Recognizes and bind to:
  - Consensus sequences i.e.
    - TATA box (TATAAA)
    - GC Box (GGGCGG)
    - CCAAT box (GCCAAT)
  - Enhancers

REGULATION OF TRANSCRIPTION BY TRANSCRIPTION FACTORS

- Interaction with promoters.
- Protein – protein interactions between different regulatory proteins.
- Interaction with RNA polymerase.
- Often require DNA looping for protein-protein contact.


start of transcription
TATA box
(A)
TFIID
(B)
TFIIB
(C)
TFIIF
other factors
TFIIE
TFIIH
RNA polymerase II
(D)
ATP
(E)
P
P
P
P
UTP, ATP
CTP, GTP
TRANSCRIPTION BEGINS

Positive Control of Gene Expression



EXAMPLES OF TRANSCRIPTION FACTORS

- TF 11 A
- TF 11 B
- TF 11 D
- TF 11 E
- TF 11 E
- TF 11F
- Sp1 (Has Gln-rich domain; Zn finger protein).
- CTF1 (Has pro-rich domain)
- C/EBP (Has pro-rich domain, Lencine Zipper)
- GALA (Zn finger, Acidic activation domain)

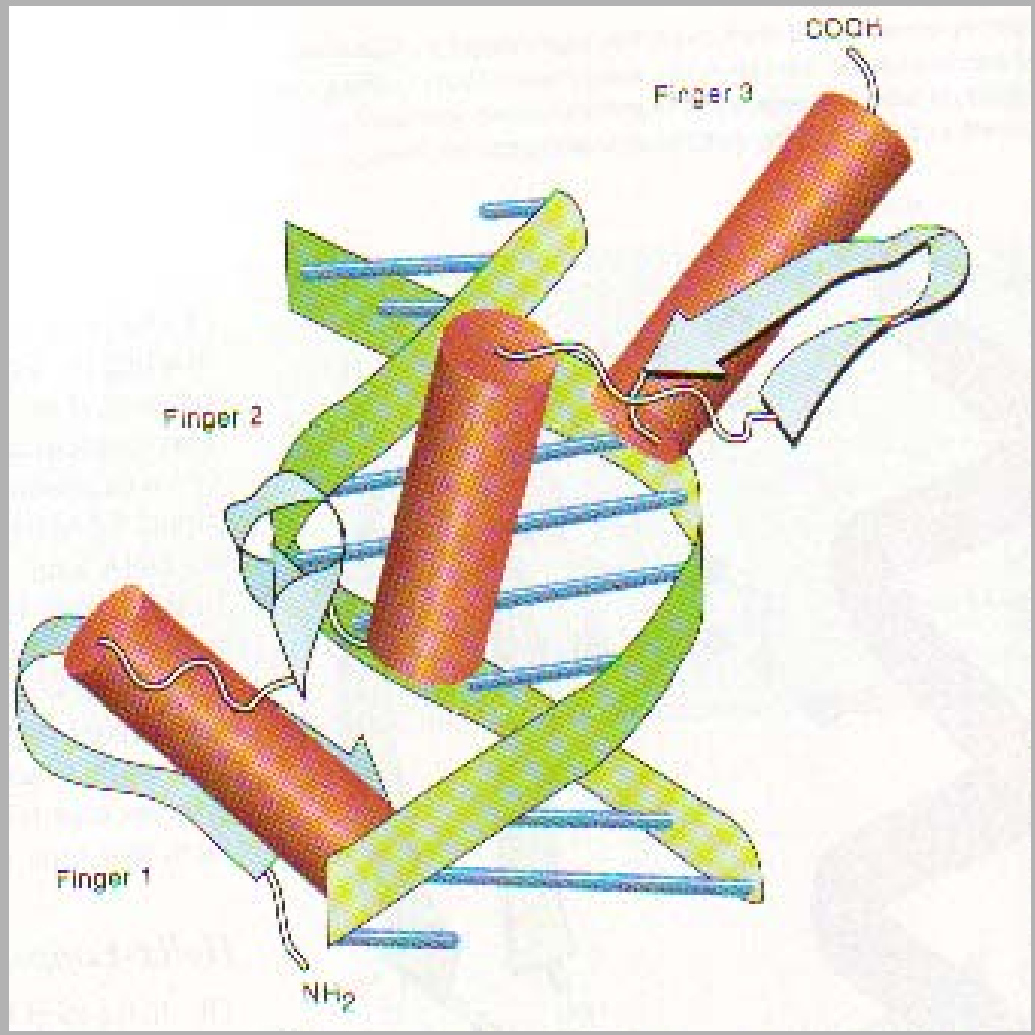
DNA BINDING DOMAIN OF REGULATORY PROTEINS

- Zinc finger: About 30 a.a residues,

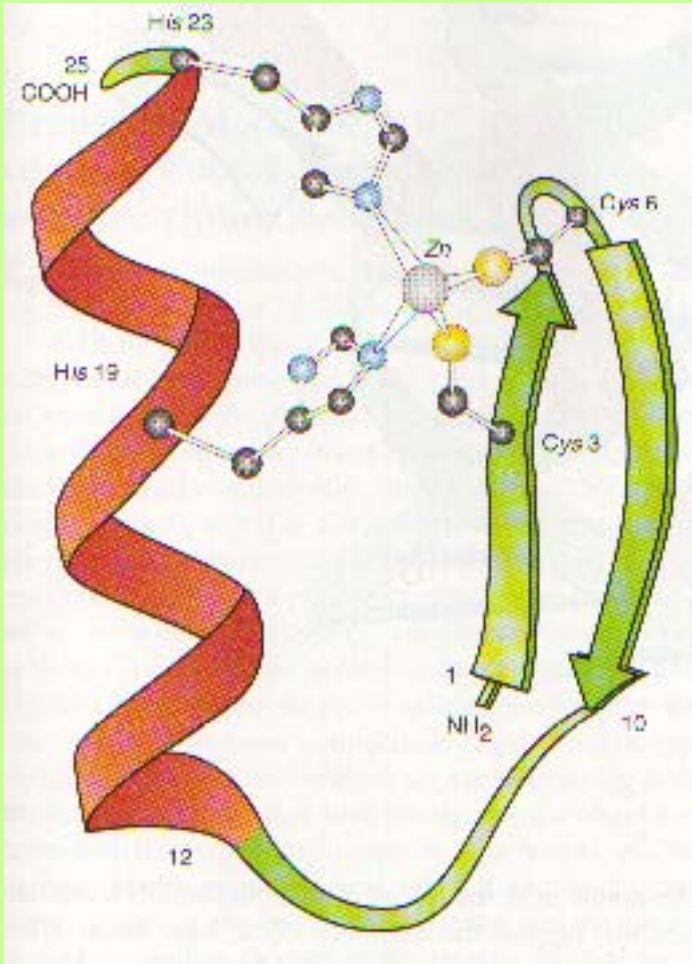
  Four are either Cys or 2Cys and 2 His, coordinated to single Zn^{++}

Contd….

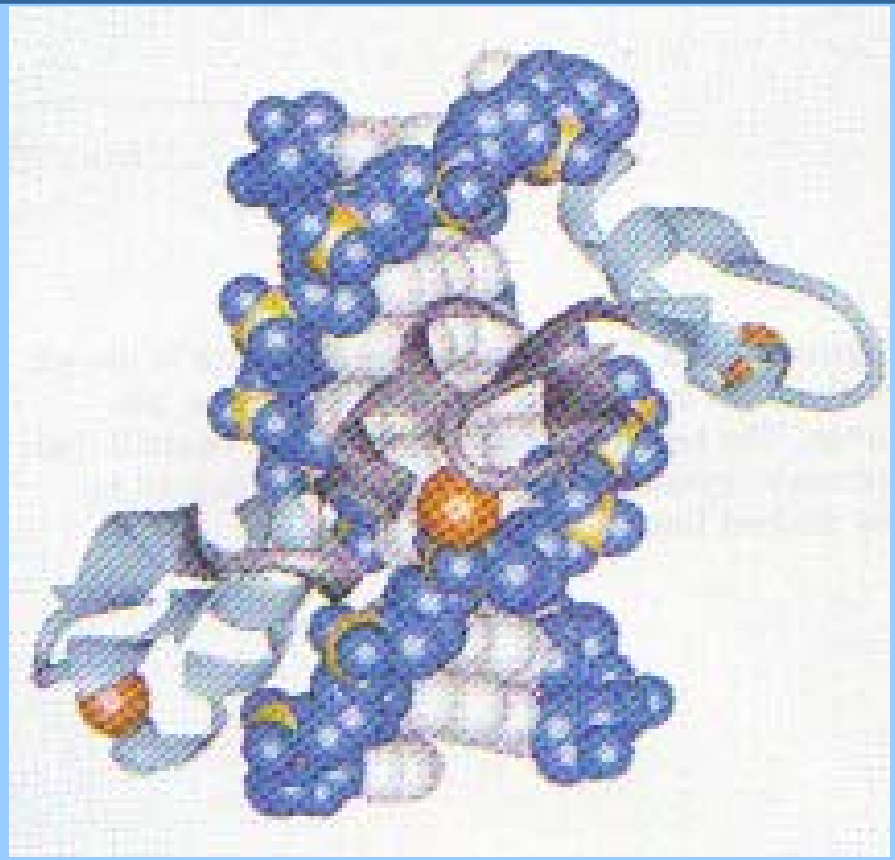
Zinc Finger Motif & DNA Interaction



A Zinc Finger Motif



Interaction Between Zinc Finger Motif and DNA



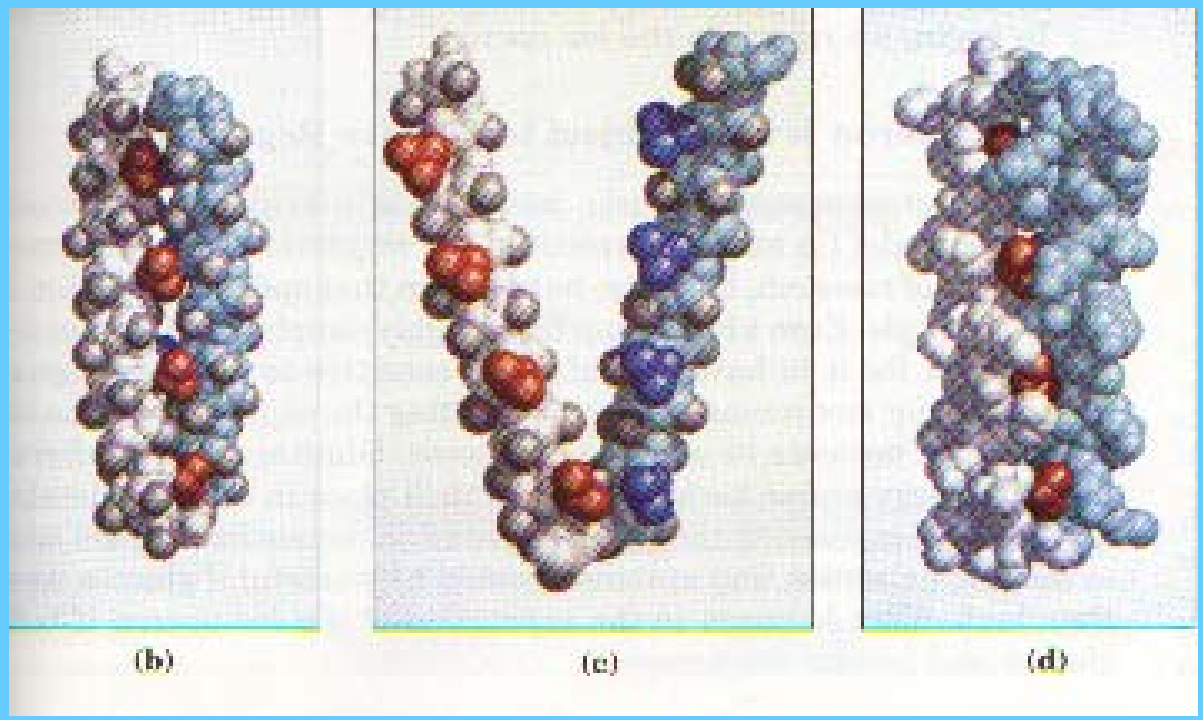
DNA BINDING DOMAIN OF REGULATORY PROTEINS …. Contd

- The Helix – turn Helix: Two short α-helical sequences (7-9 a.a. long) separated by a β turn (~ 20 a.a). One of the α-helices is the recognition helix and binds DNA major groove.

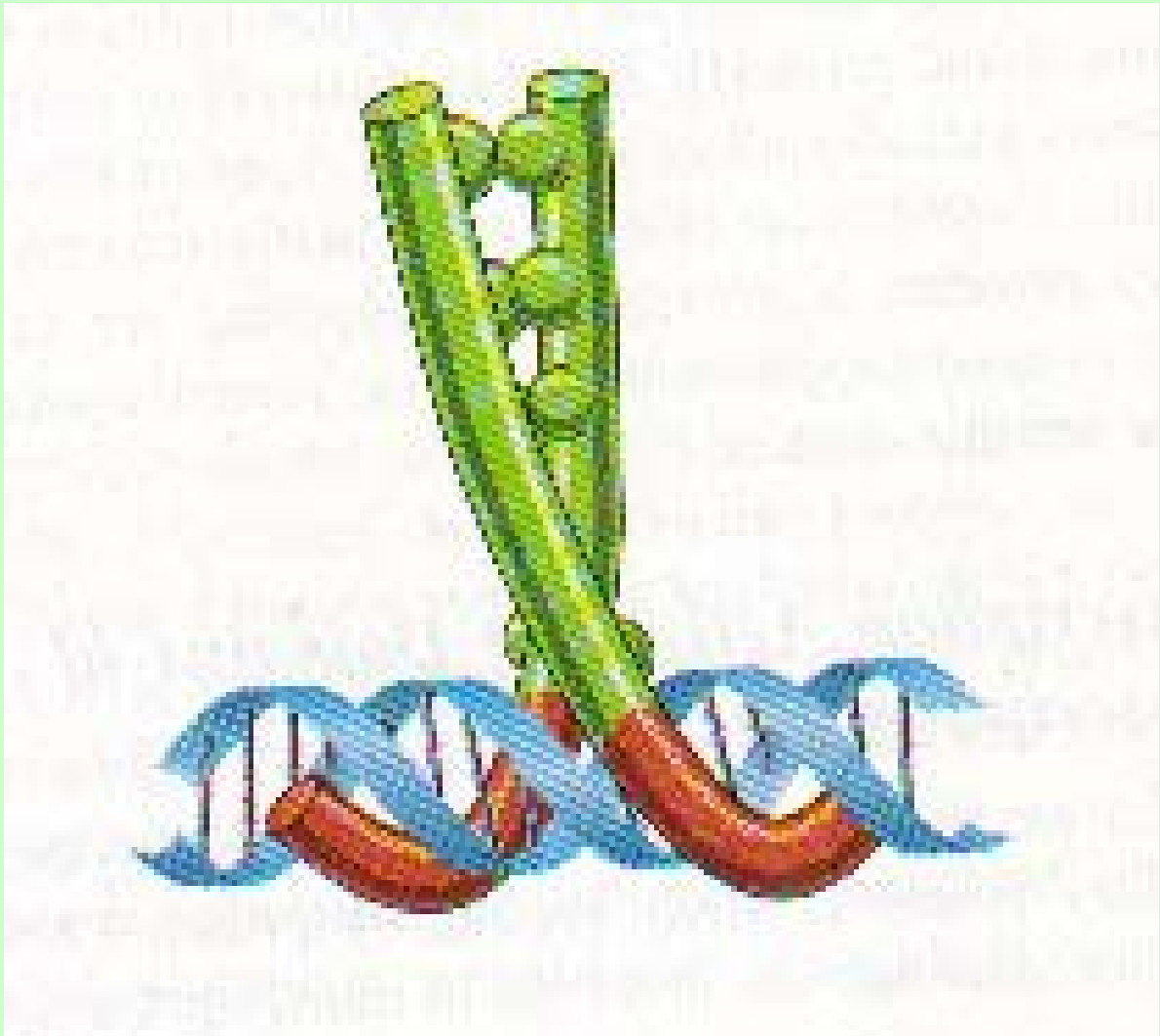
DNA BINDING DOMAIN OF REGULATORY PROTEINS …. Contd

- The leucine zipper: Has amphipathic α-helix, with hydrophobic a.a. on one size. Every 7^{th} a.a. is a leucine.

Leucine Zipper Motif



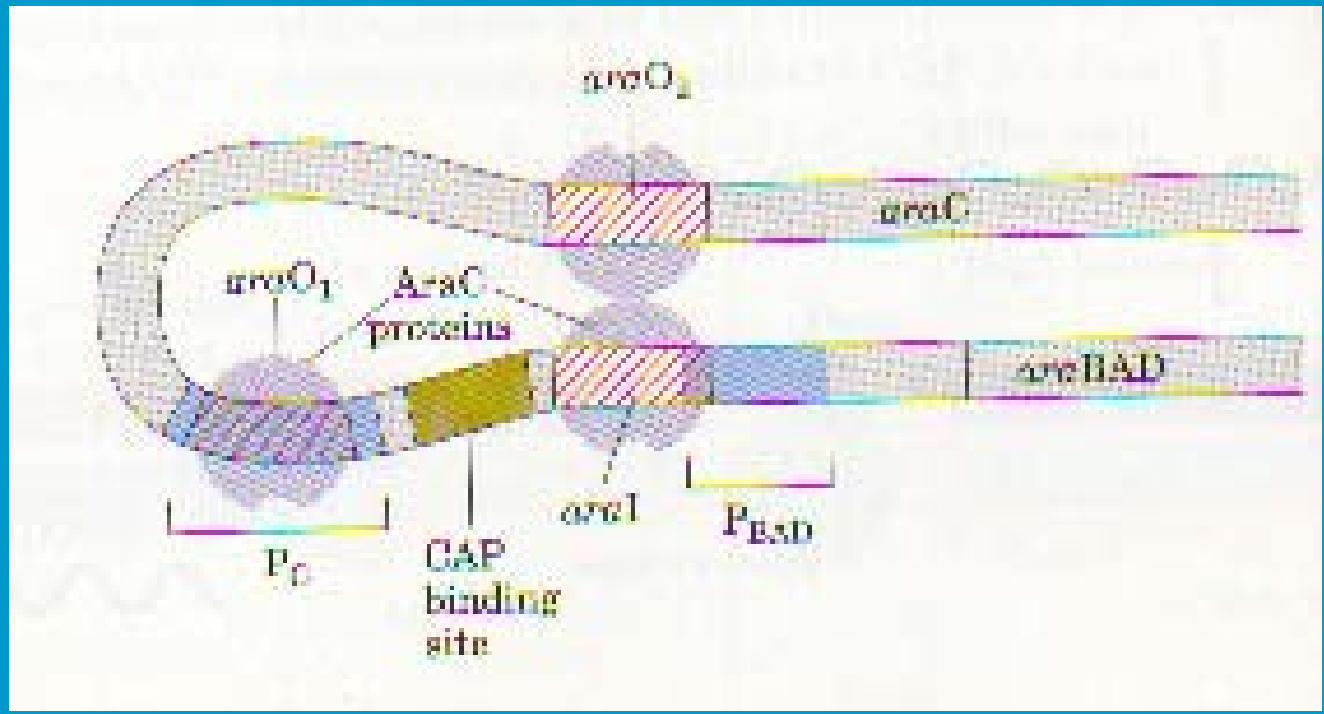
A Leucine Zipper Motif

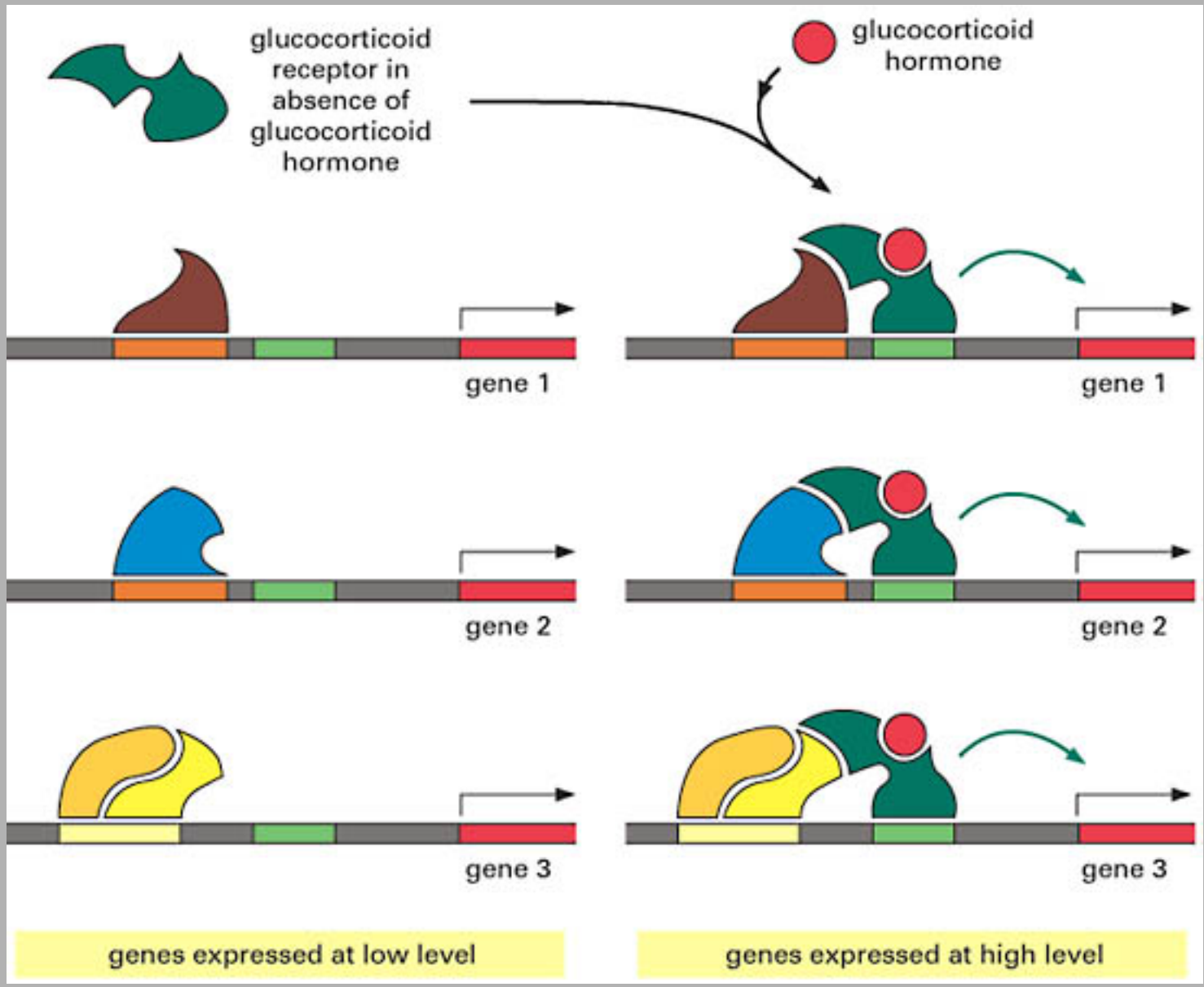


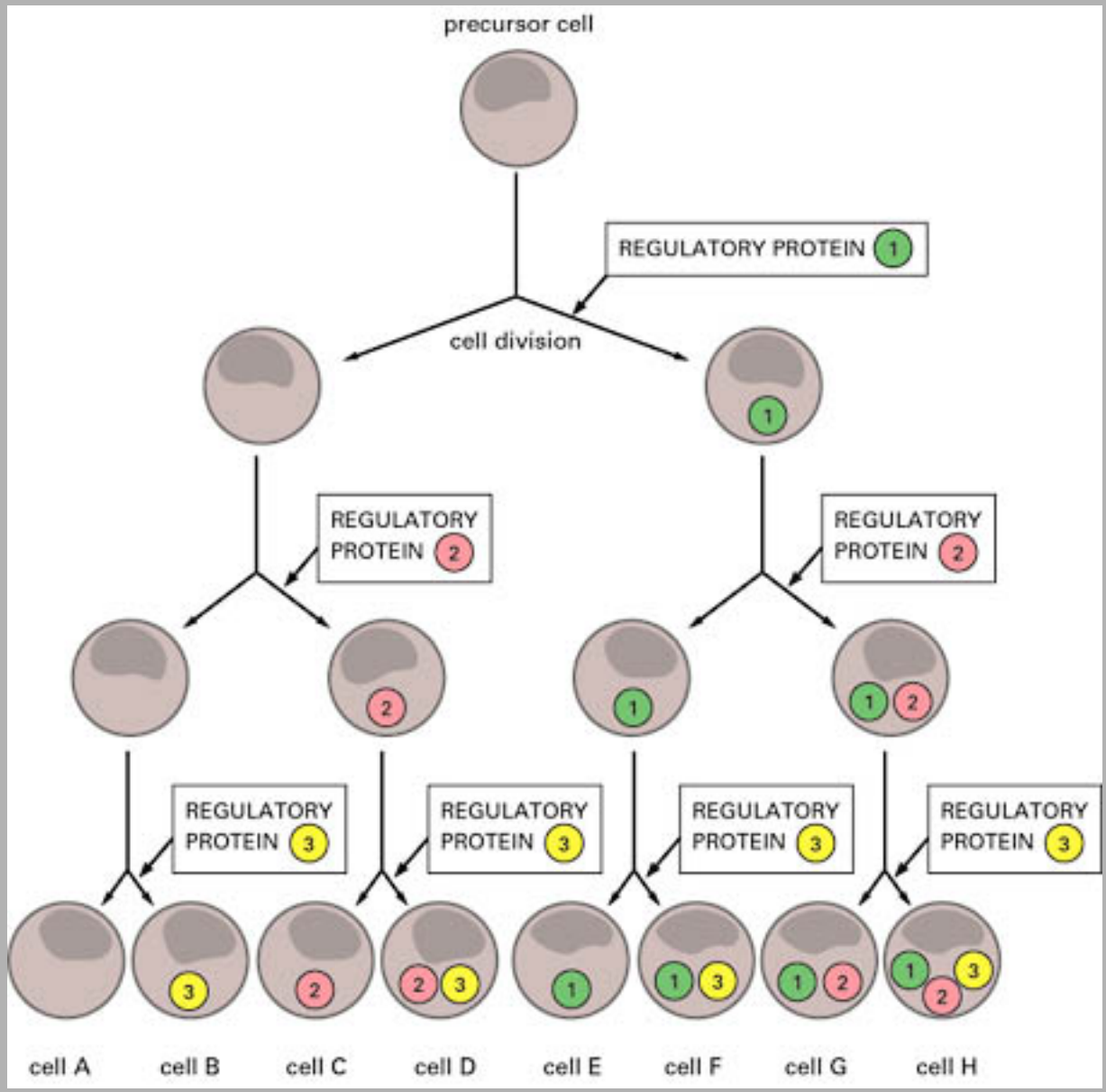
DNA BINDING DOMAIN OF REGULATORY PROTEINS …. Contd

- Regulatory proteins react with RNA polymerase and with other unrelated regulatory proteins. Domains for protein-protein interaction:
  - Glutamic-rich domain.
  - Proline-rich domain
  - Acidic domain

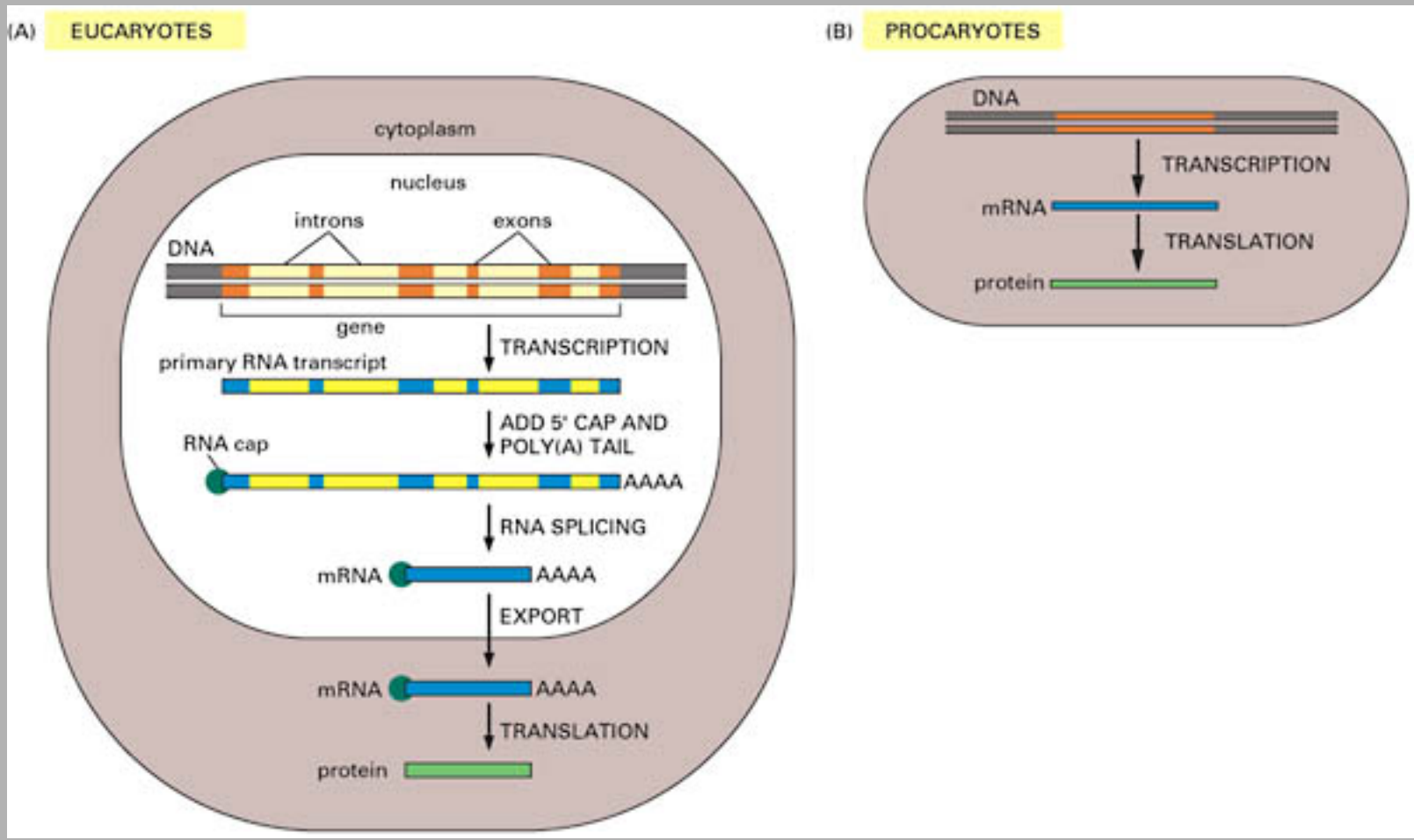
Protein-Protein Interaction




glucocorticoid receptor in absence of glucocorticoid hormone
glucocorticoid hormone
gene 1
gene 1
gene 2
gene 2
gene 3
gene 3
genes expressed at low level
genes expressed at high level


precursor cell
REGULATORY PROTEIN 1
cell division
REGULATORY PROTEIN 2
REGULATORY PROTEIN 2
REGULATORY PROTEIN 3
REGULATORY PROTEIN 3
REGULATORY PROTEIN 3
REGULATORY PROTEIN 3
cell A
cell B
cell C
cell D
cell E
cell F
cell G
cell H

Control of gene expression by RNA processing


(A) EUCARYOTES
cytoplasm
nucleus
introns
exons
DNA
gene
TRANSCRIPTION
primary RNA transcript
ADD 5' CAP AND
POLY(A) TAIL
RNA cap
AAAA
RNA SPLICING
mRNA
AAAA
EXPORT
mRNA
AAAA
TRANSLATION
protein
(B) PROCARYOTES
DNA
TRANSCRIPTION
mRNA
TRANSLATION
protein