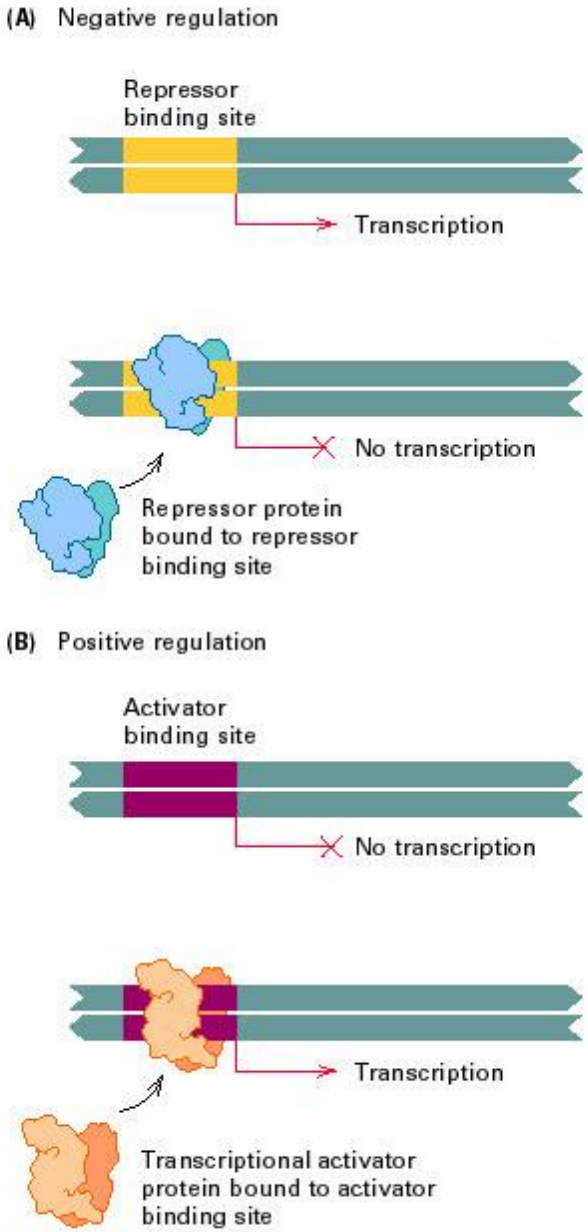
Molecular Mechanisms of Gene Regulation

Prof. Tapan Kr. Dutta
Principal,
Sabang Sajanikanta Mahavidyalaya

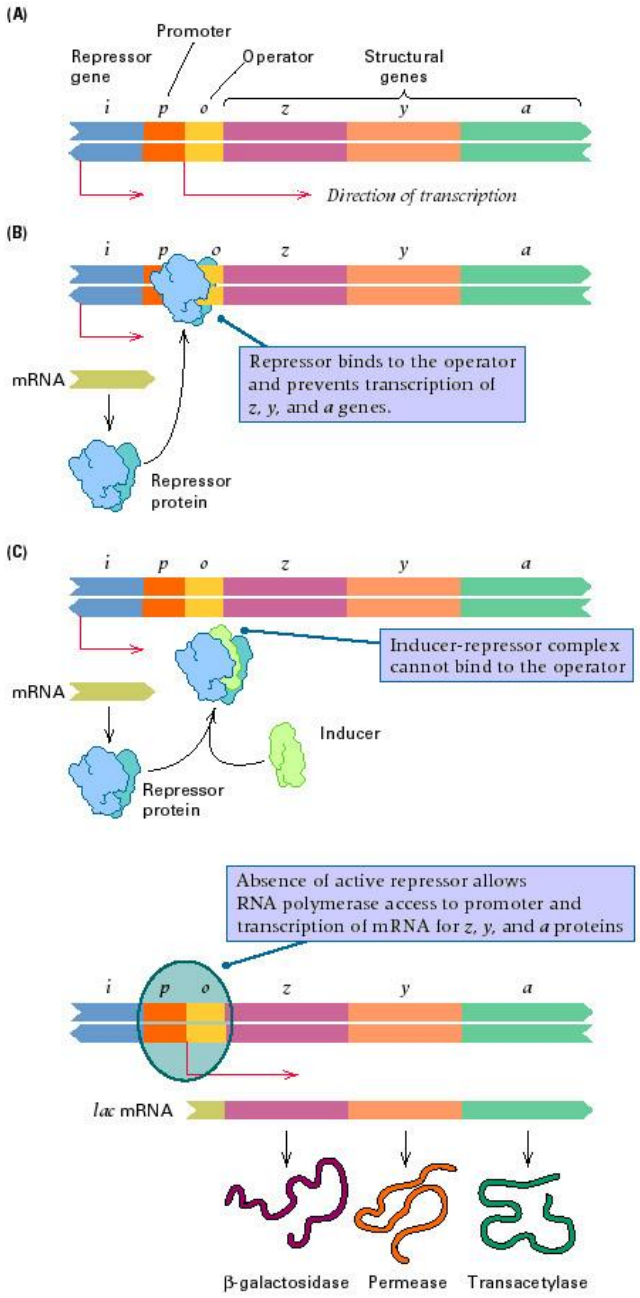
Transcription Regulation: Prokaryotes

- Negative Regulation = repressor binds to regulatory site to block transcription of genes
- Positive Regulation = Activator binds to regulatory site to stimulate transcription of genes


(A) Negative regulation
Repressor binding site
Transcription
No transcription
Repressor protein bound to repressor binding site
(B) Positive regulation
Activator binding site
No transcription
Transcription
Transcriptional activator protein bound to activator binding site

Operon Model of Regulation

- The operon - several structural genes under the control of a single regulatory domain, ie. coordinate regulation
- Repressor binding site = operator
- RNAP binding site = promoter


(A)
Promoter
Repressor gene
Operator
Structural genes
i
p
o
z
y
a
Direction of transcription
(B)
i
p
o
z
y
a
mRNA
Repressor binds to the operator and prevents transcription of z, y, and a genes.
Repressor protein
(C)
i
p
o
z
y
a
Inducer-repressor complex cannot bind to the operator
mRNA
Inducer
Repressor protein
Absence of active repressor allows RNA polymerase access to promoter and transcription of mRNA for z, y, and a proteins
i
p
o
z
y
a
lac mRNA
β-galactosidase
Permease
Transacetylase

Operon Model of Regulation

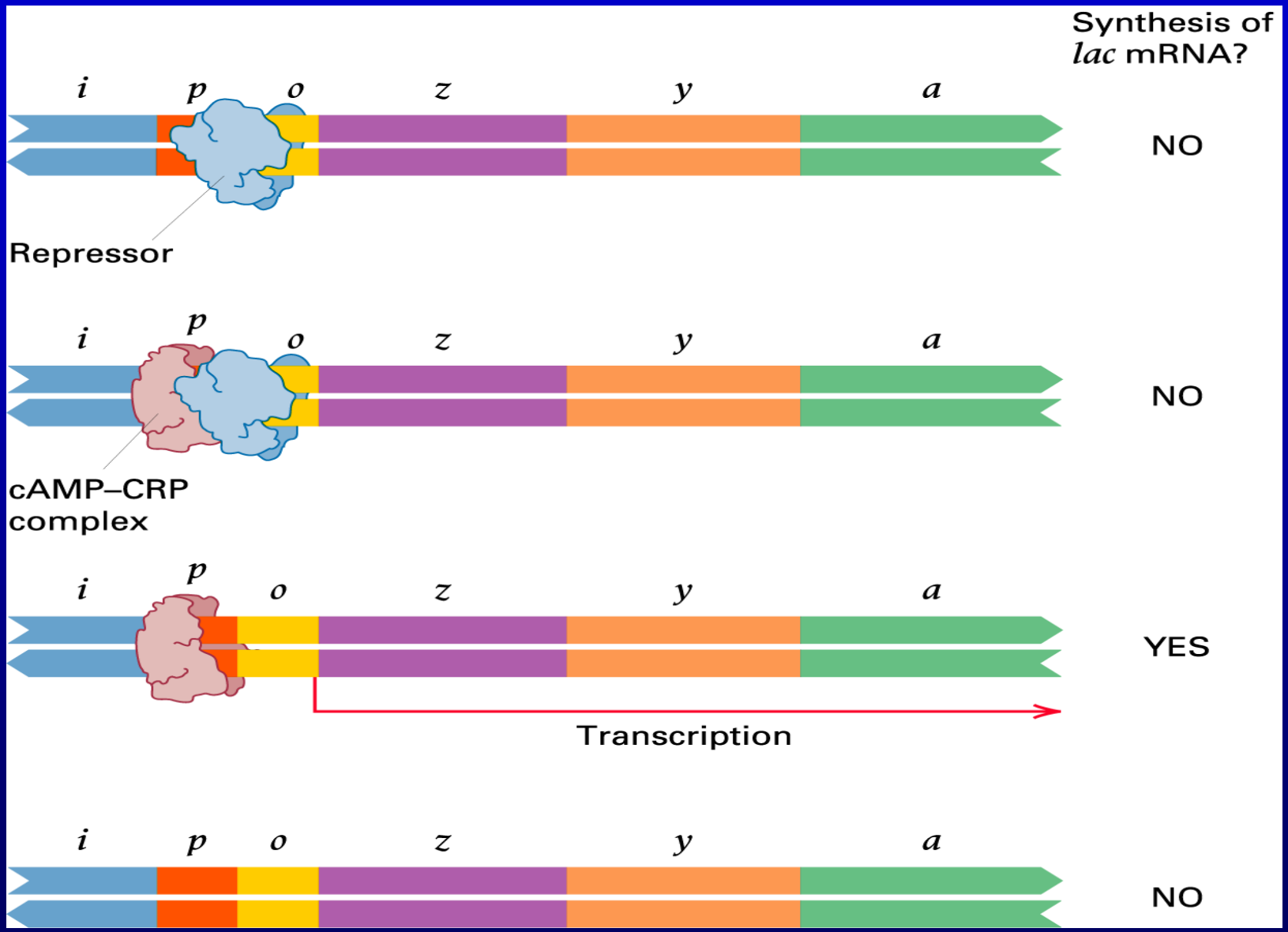
- Inducible operon - activated by small molecule inducers, eg. *Lac* operon
- Repressible operon - shut off by small molecule co-repressors, eg. *Trp* operon

Gene Regulation Mechanisms

- Positive Regulation: transcription occurs only if promoter is activated by transcriptional activator
- Negative regulation is more common in prokaryotes
- Positive regulation is more common in eukaryotes
- Autoregulation: protein regulates its own transcription

Lac Operon

- Lactose degradation is regulated by the lactose (lac) operon
- The first regulatory mutations discovered affect lactose metabolism
- Structural proteins of the lac operon:
  - *B*- galactosidase
  - lactose permease
  - Lactose acetylase


Synthesis of *lac* mRNA?
i
p
o
z
y
a
NO
Repressor
i
p
o
z
y
a
NO
cAMP–CRP complex
i
p
o
z
y
a
YES
Transcription
i
p
o
z
y
a
NO

Lac Operon

- Gene expression can be inducible or constitutive
- Repressor is expressed constitutively from the i gene
- Repressor binds to the operator to block transcription of structural genes
- Inducer lactose binds and inactivates the repressor to permit transcription initiation

Lac Operon

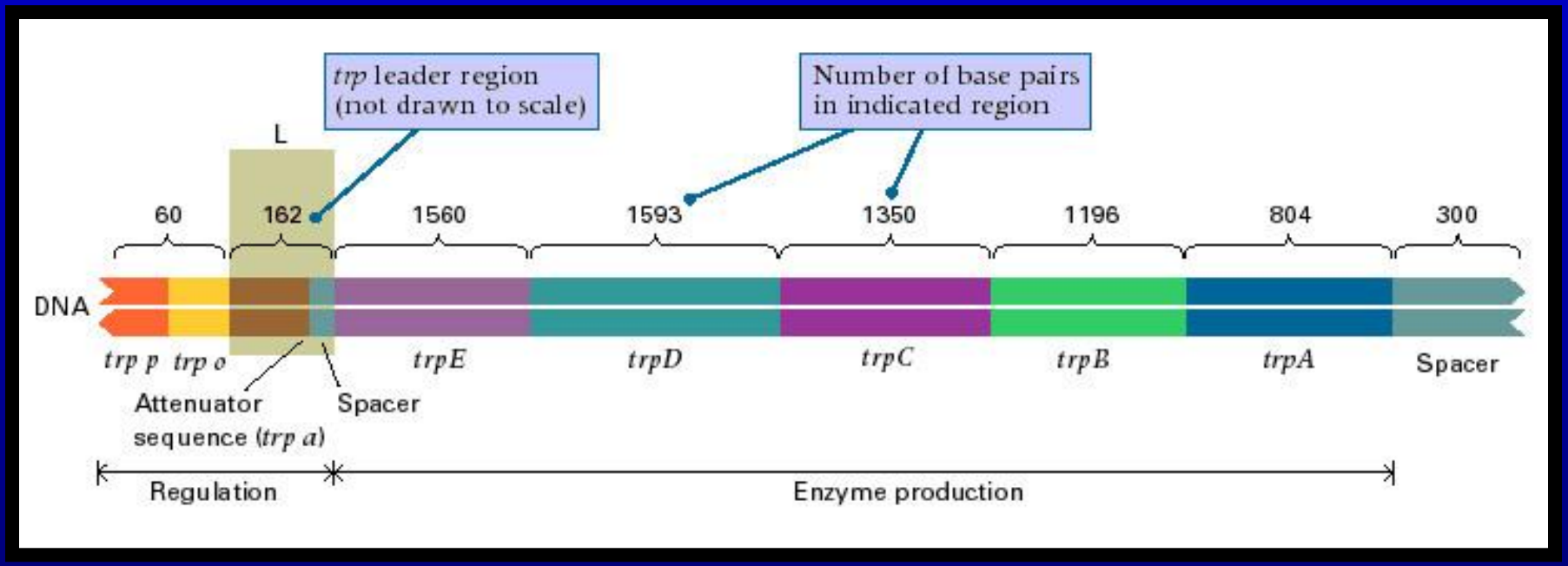
- Lactose operator is essential site for repression
- Operator mutations are cis-dominant because only genes on the same genetic unit are affected
- Lactose promoter is essential site for transcription
- Lac operon contains linked structural genes and regulatory sites

Lac Operon

- Lactose operon is also subject to positive regulation
- Positive regulation of the lac operon involves cAMP-CRP (cyclic AMP receptor protein) which binds to the promoter to activate transcription by RNA polymerase
- cAMP-CRP complex regulates the activity of the lac operon

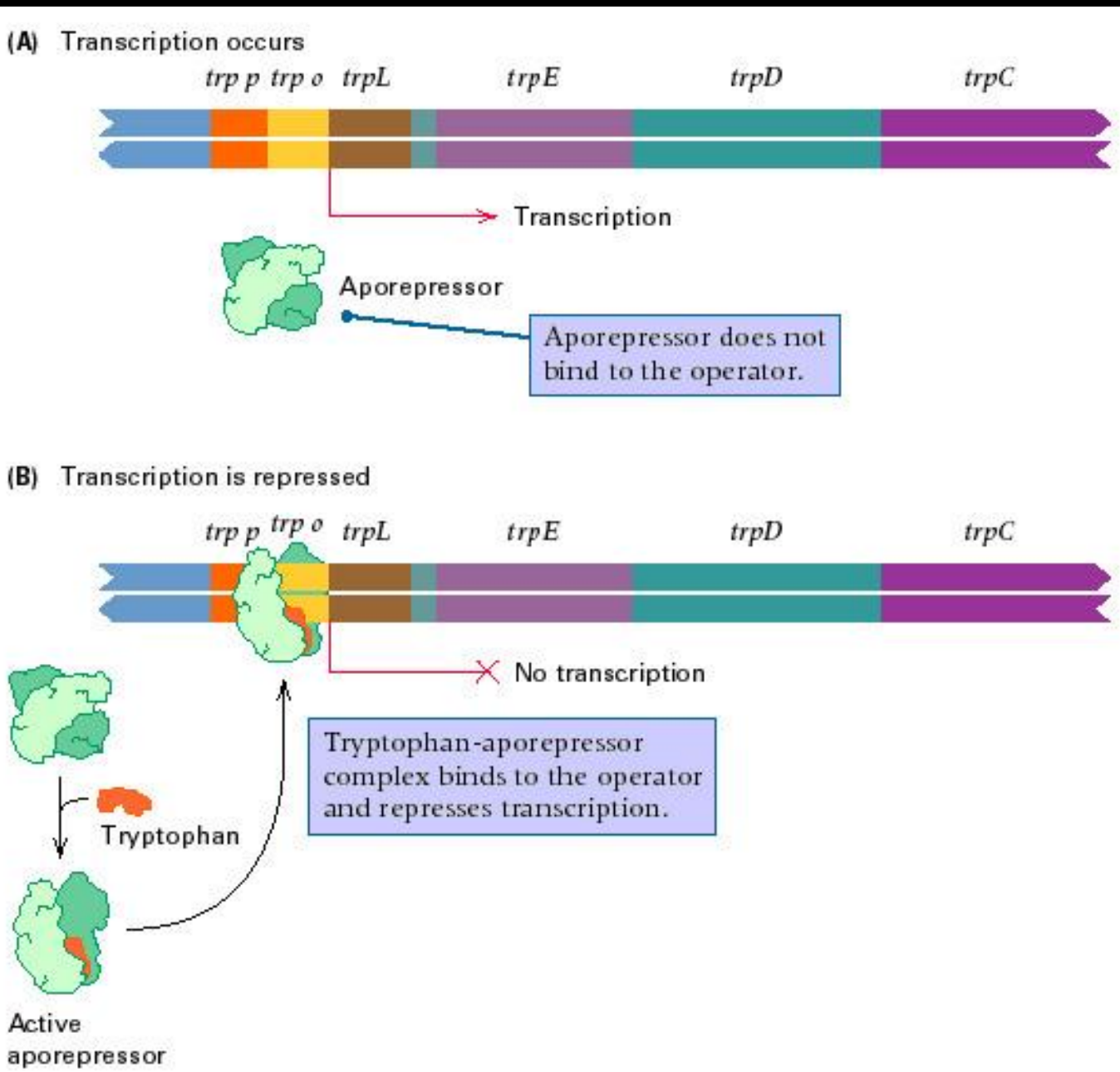
Trp Operon

- Trp operon contains structural genes required for the synthesis of the tryptophan
- Trp operon is transcriptionally active unless tryptophan is present
- Repressible system regulated by a negative feedback loop


trp leader region
(not drawn to scale)
Number of base pairs
in indicated region
L
60
162
1560
1593
1350
1196
804
300
DNA
trp p
trp o
trpE
trpD
trpC
trpB
trpA
Spacer
Attenuator
sequence (trp a)
Spacer
Regulation
Enzyme production

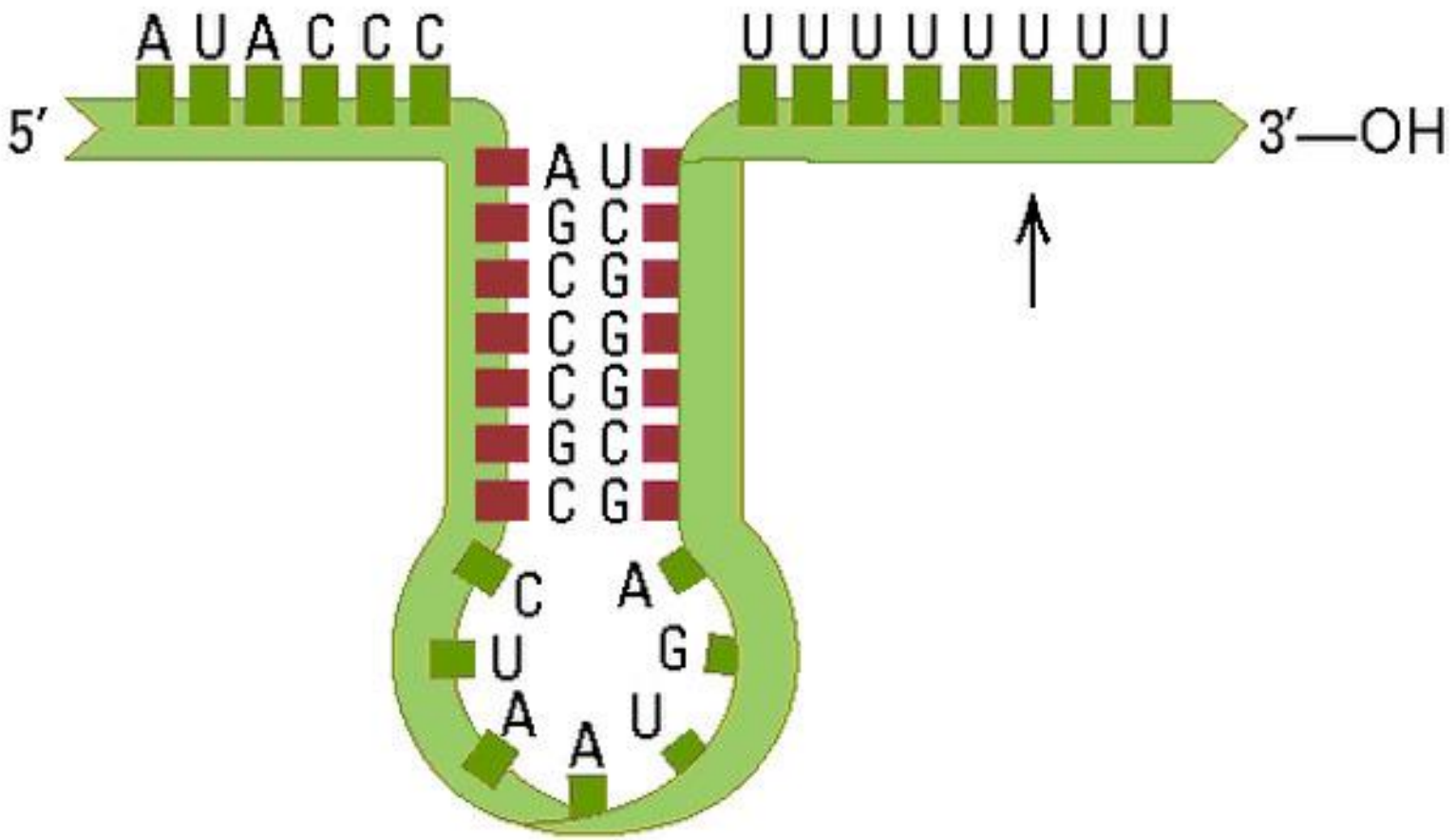
Trp Operon

- Trp operon is shut off when tryptophan binds to inactive aporepressor
- Tryptophan-repressor complex binds to operator to block transcription when tryptophan levels are high
- If tryptophan levels fall, trp-repressor complex dissociates from operator


(A) Transcription occurs
trp p
trp o
trpL
trpE
trpD
trpC
Transcription
Aporepressor
Aporepressor does not bind to the operator.
(B) Transcription is repressed
trp p
trp o
trpL
trpE
trpD
trpC
No transcription
Tryptophan-aporepressor complex binds to the operator and represses transcription.
Tryptophan
Active aporepressor

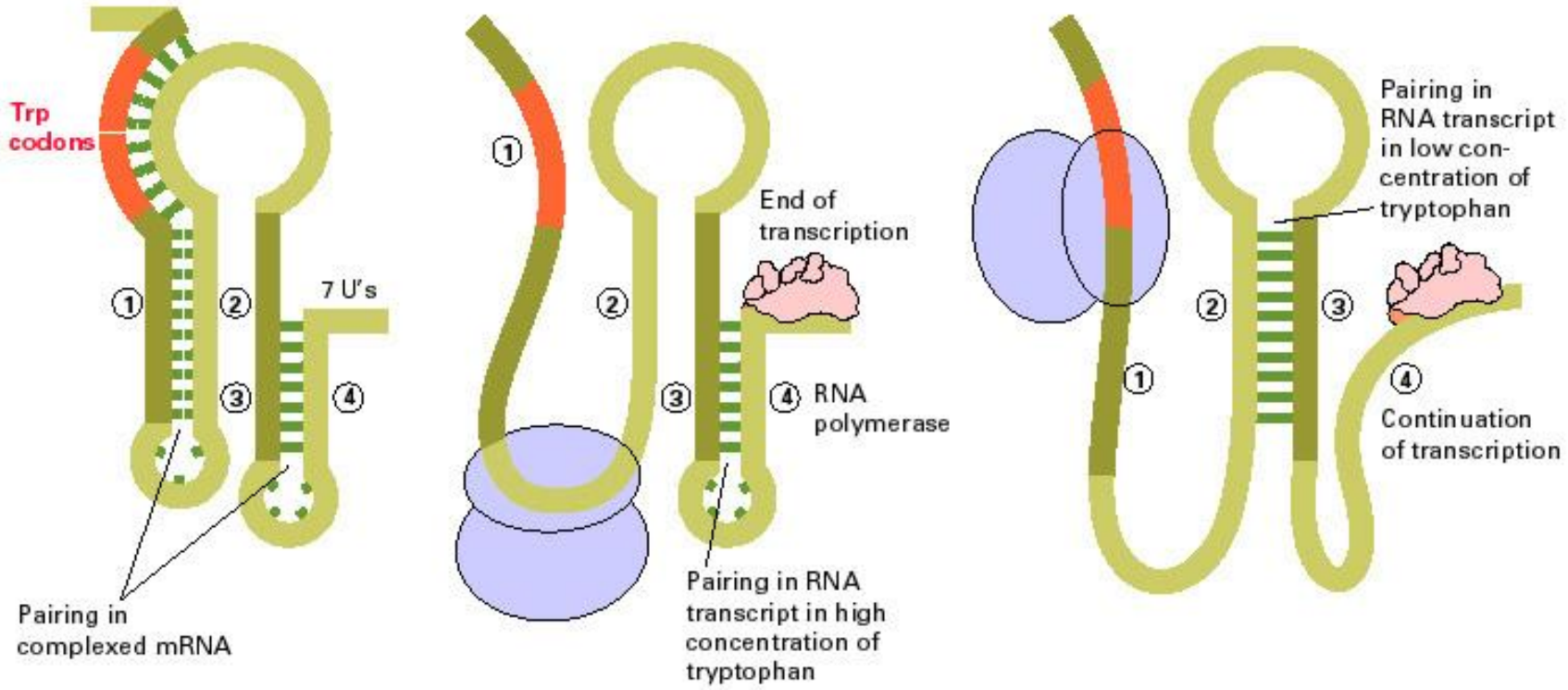
Attenuation

- Attenuation - a very sensitive form of translational regulation of the trp operon
- The trp attenuator sequence consists of 5’ base sequence in mRNA which is complementary and can base pair to form a stem and loop structure


A U A C C C
U U U U U U U U
5′
3′—OH
A U
G C
C G
C G
C G
C G
G C
C G
C A
U G
A U
A

Attenuation

- Attenuation results in the premature termination of mRNA synthesis due to stem and loop formation in the 5’ region of mRNA
- If tRNA-trp is present , synthesis of the leader peptide results in pairing of mRNA which blocks the action of RNAP



(A) Free mRNA. Base pairs between 1 and 2 and between 3 and 4.

(B) High concentration of tryptophan. Ribosome reaches region 2 and pairing of 3–4 causes termination of transcription.

(C) Low concentration of tryptophan. Ribosome stalled in region 1 at Trp codons permits pairing of 2–3 and transcription is not terminated after region 4.

Attenuation

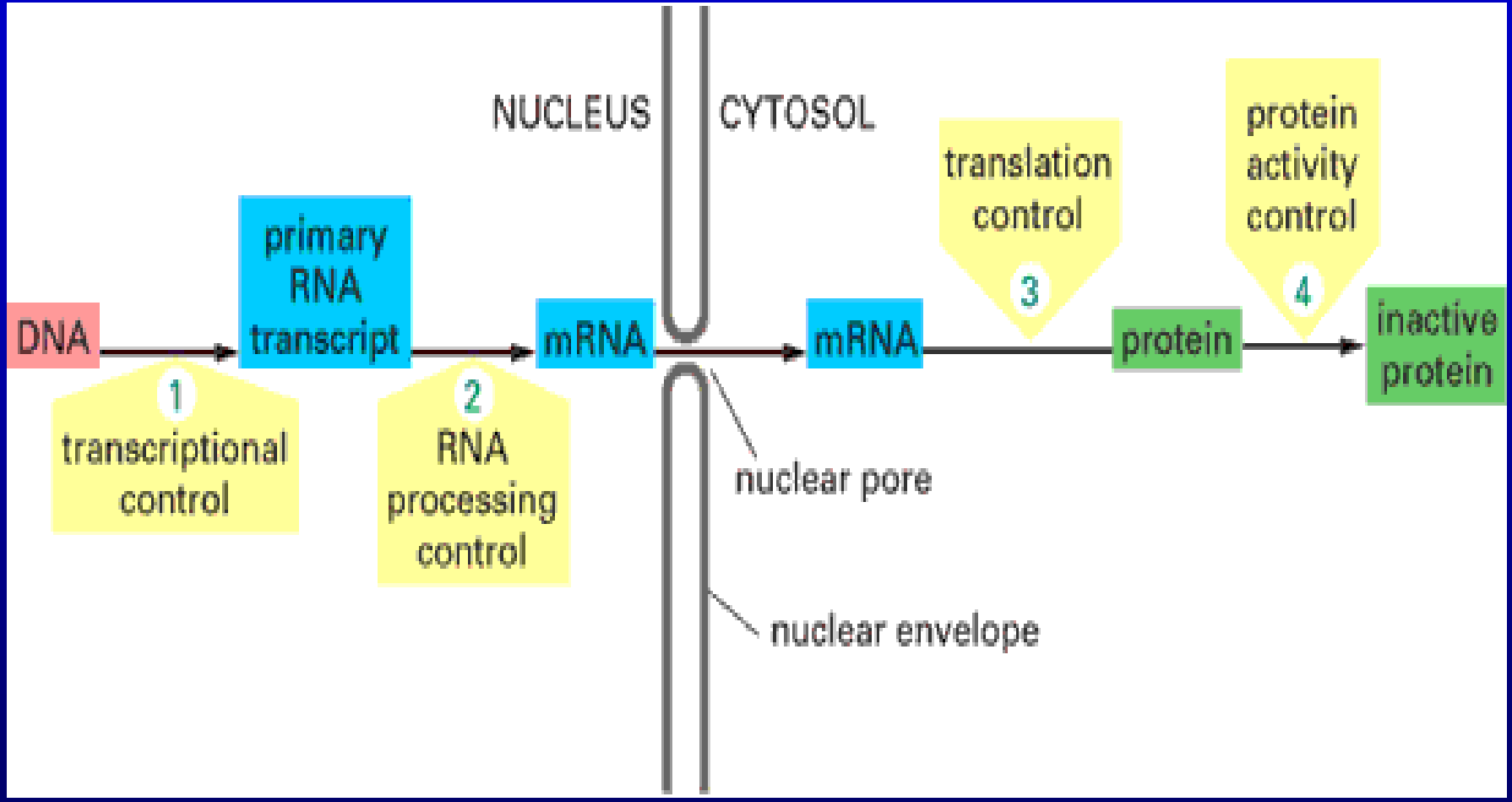
- At low concentrations of tRNA-trp, the ribosome stalls, and the mRNA opens so that transcription continues
- Attenuation permits the cell to respond to tryptophan levels by expressing the genes needed for its synthesis when needed

Eukaryotic Gene Regulation

- cellular differentiation
- homeostasis
- response to environment

Levels of Regulation

- transcription
- RNA processing
- transport
- mRNA stability
- selective translation
- post-translational modification


NUCLEUS
CYTOSOL
DNA
primary RNA transcript
mRNA
mRNA
protein
inactive protein
translation control
3
protein activity control
4
1
transcriptional control
2
RNA processing control
nuclear pore
nuclear envelope

Examples of Regulation

- transcription factors
- DNA methylation
- steroid hormones
- alternative splicing

Gene Regulation: Eukaryotes

Transcription regulation in eukaryotes differs from prokaryotes:

- The processes of transcription and translation are separate
- Many genes are "split" genes; alternative splicing may occur
- Each gene is regulated by a separate promoter

Gene Regulation: Eukaryotes

Gene organization in eukaryotes:

- DNA is bound to histones and non-histone regulatory proteins
- Significant fraction of DNA is moderately or highly repetitive
- Large fraction of DNA does not code for proteins
- Gene regulation can occur by DNA rearrangements

Transcriptional Regulation

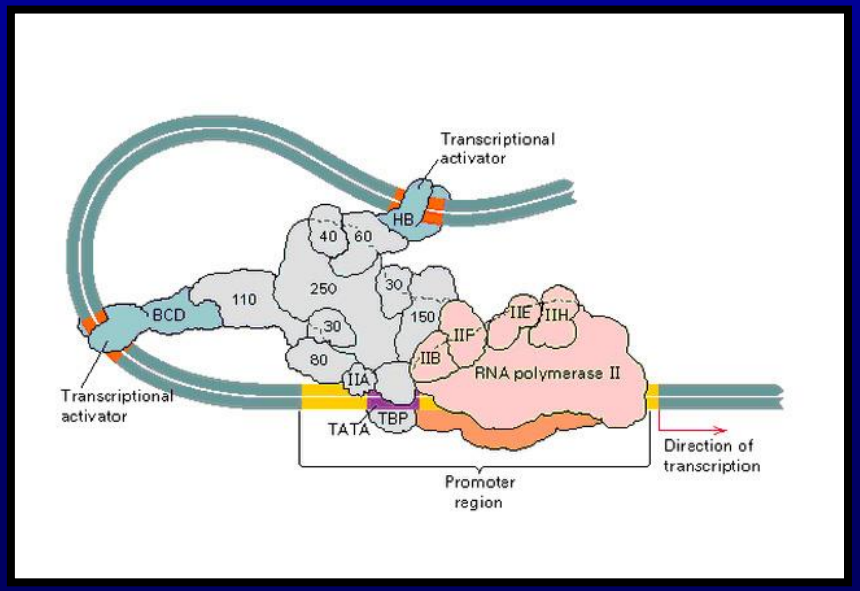
- Transcriptional control is a frequent mode of regulation in eukaryotes
- Housekeeping genes are essential for metabolic activity and are expressed constitutively
- Many specialized genes are under transcriptional control
- Genes controlling yeast mating type regulate transcription

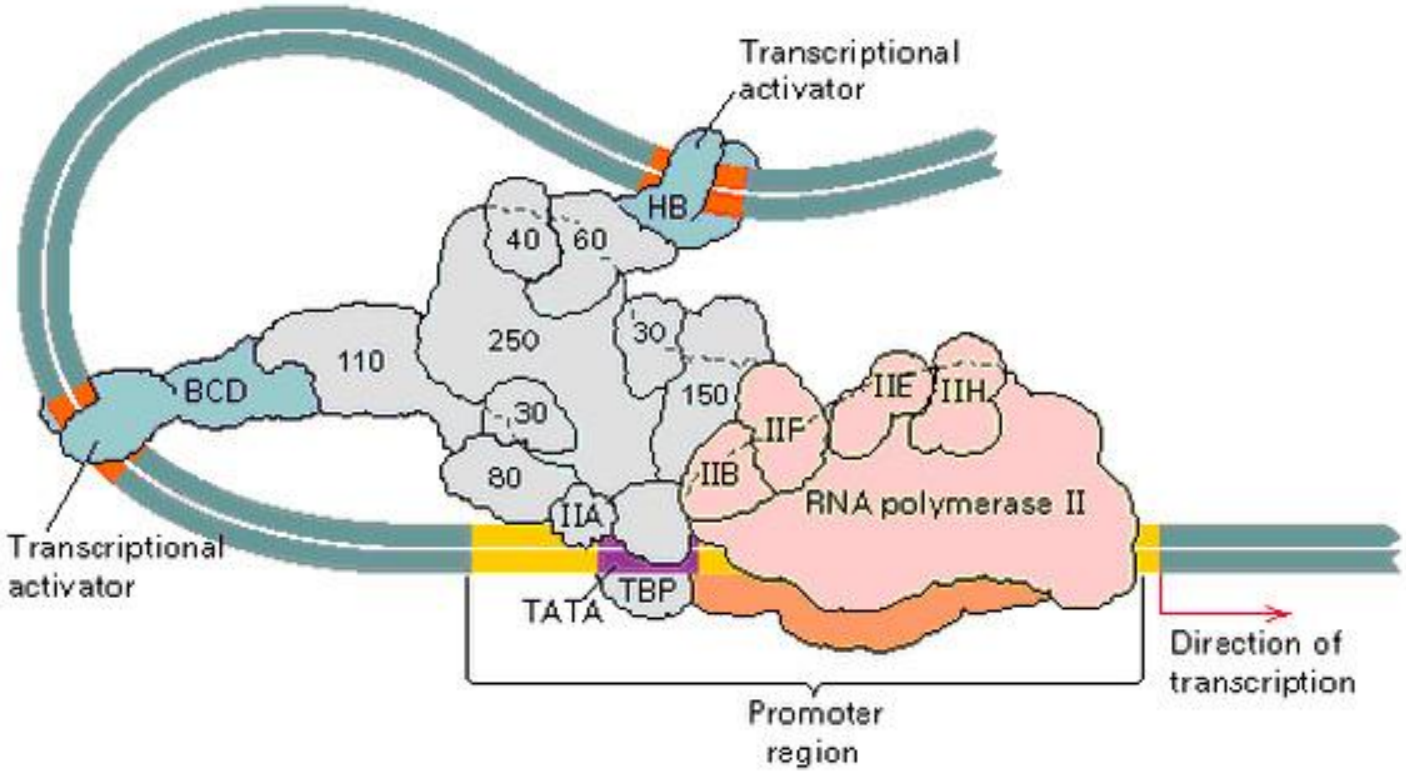
Eukaryotic Transcription

- Eukaryotic transcription complex includes many proteins called

general transcription factors common to the promoters of many genes

- Transcriptional activation occurs by a mechanism called recruitment involving the interaction of transcription factors with promoter and enhancer elements




Transcriptional activator
HB
40
60
250
30
110
BCD
30
150
IIE
IIH
IIF
80
IIB
RNA polymerase II
IIA
Transcriptional activator
TBP
TATA
Direction of transcription
Promoter region

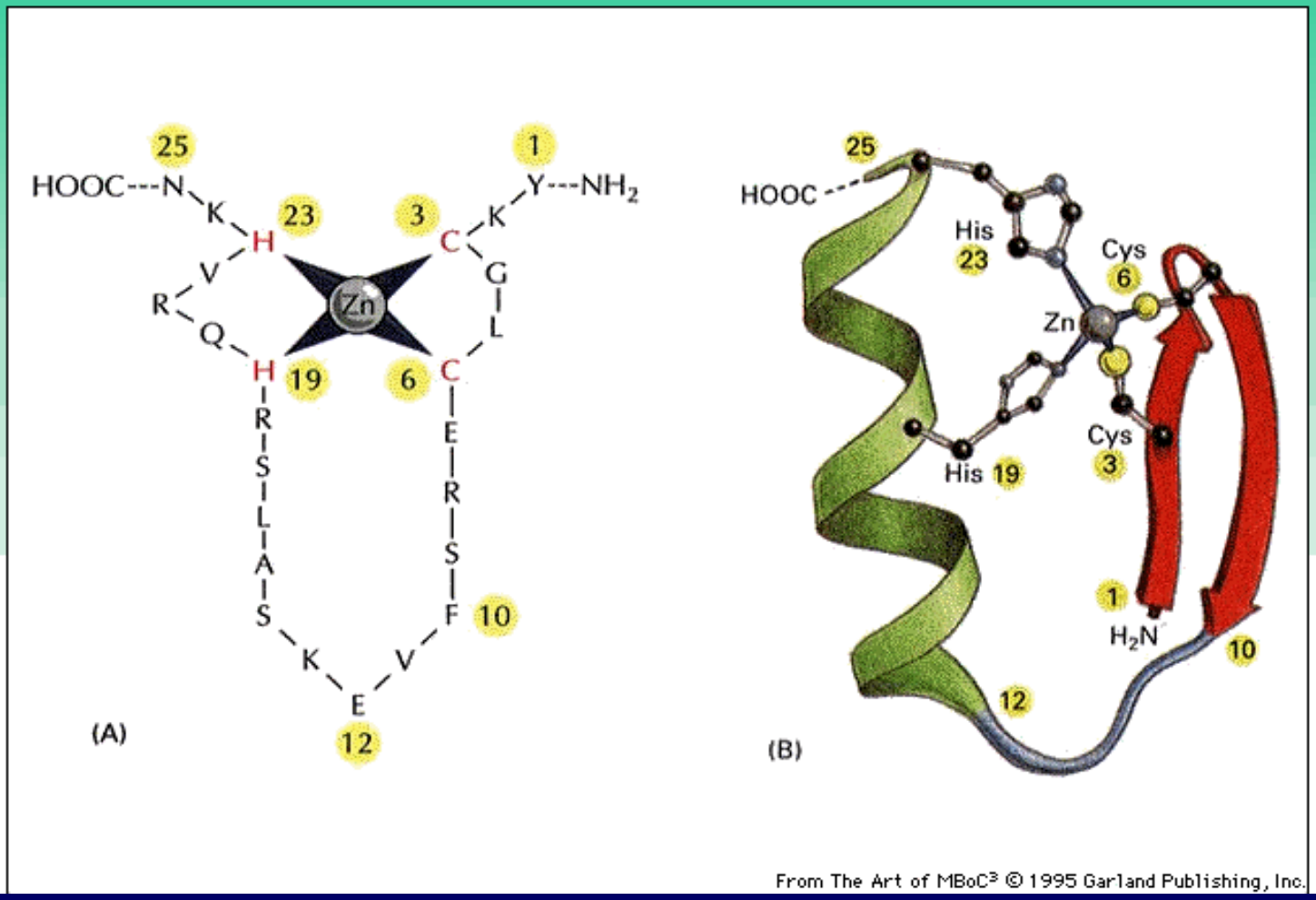
Transcription Regulation

- Combinatorial control means that a few genes can control many others
- Strategically placed enhancers can act as genetic switches
- One gene can have two or more promoters that are regulated differently

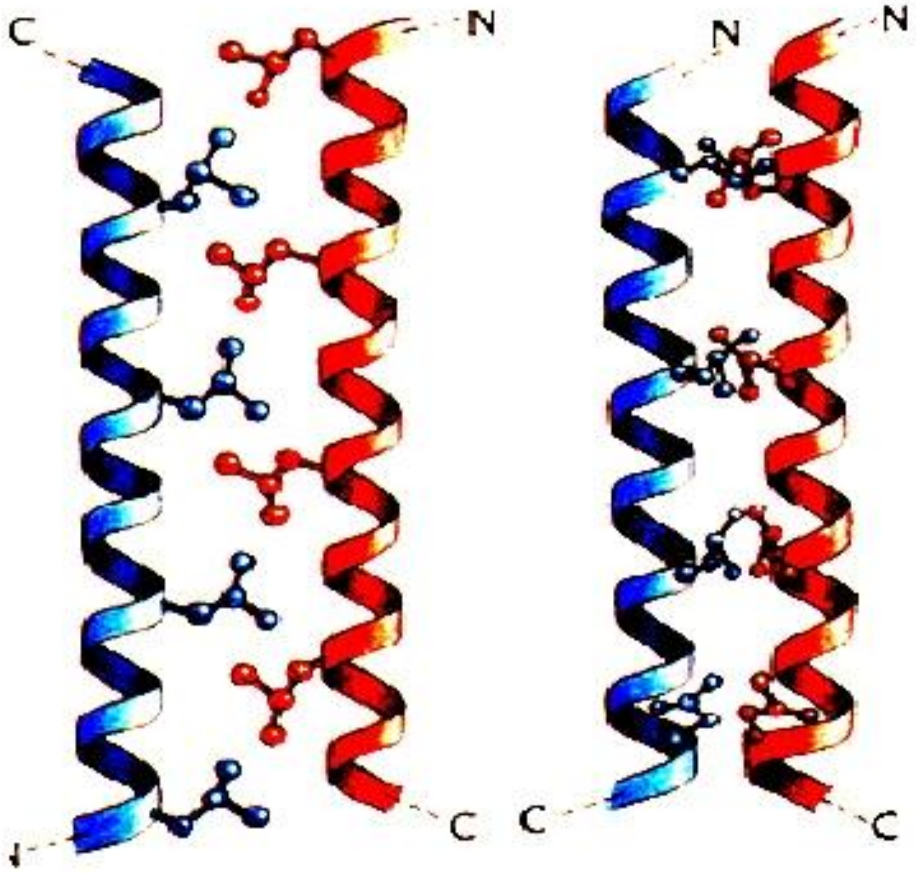
Transcription Factors

- GAL4 example
  - DNA binding domain / UAS
  - *trans*-activating domain
- TATA box binding protein example
- structural motifs
  - helix-turn-helix
  - zinc finger
  - leucine zipper

Zinc Finger


25
HOOC---N
K
H
23
3
C
K
1
Y---NH2
V
R
Q
Zn
G
L
H
19
6
C
R
I
S
I
L
A
S
E
R
S
F
10
K
V
E
12
(A)
25
HOOC
His
23
Cys
6
Zn
Cys
3
His 19
1
H2N
10
12
(B)
From The Art of MBoC3 © 1995 Garland Publishing, Inc.

The Leucine Zipper



Original concept

More correct

DNA Methylation

- DNA methylation occurs often at 5’-CG-3’ dinucleotides and is associated with a low level of transcriptional activity
- DNA methylases catalyze the the formation of 5-methylcytosine
- Methylation represents a form of transcriptional regulation

DNA methylation

- 5-methyl cytosine
- methyl group in major groove
- 5% of many genomes methylated
- correlated with gene inactivity
- heterochromatic X

- methylation usually at 5’ CpG 3’
- CpG at 1/5 expected frequency of 4%
- spontaneous deamination of CpG gives TpG
- CpG islands

  unmethylated

  at 5’ ends of genes

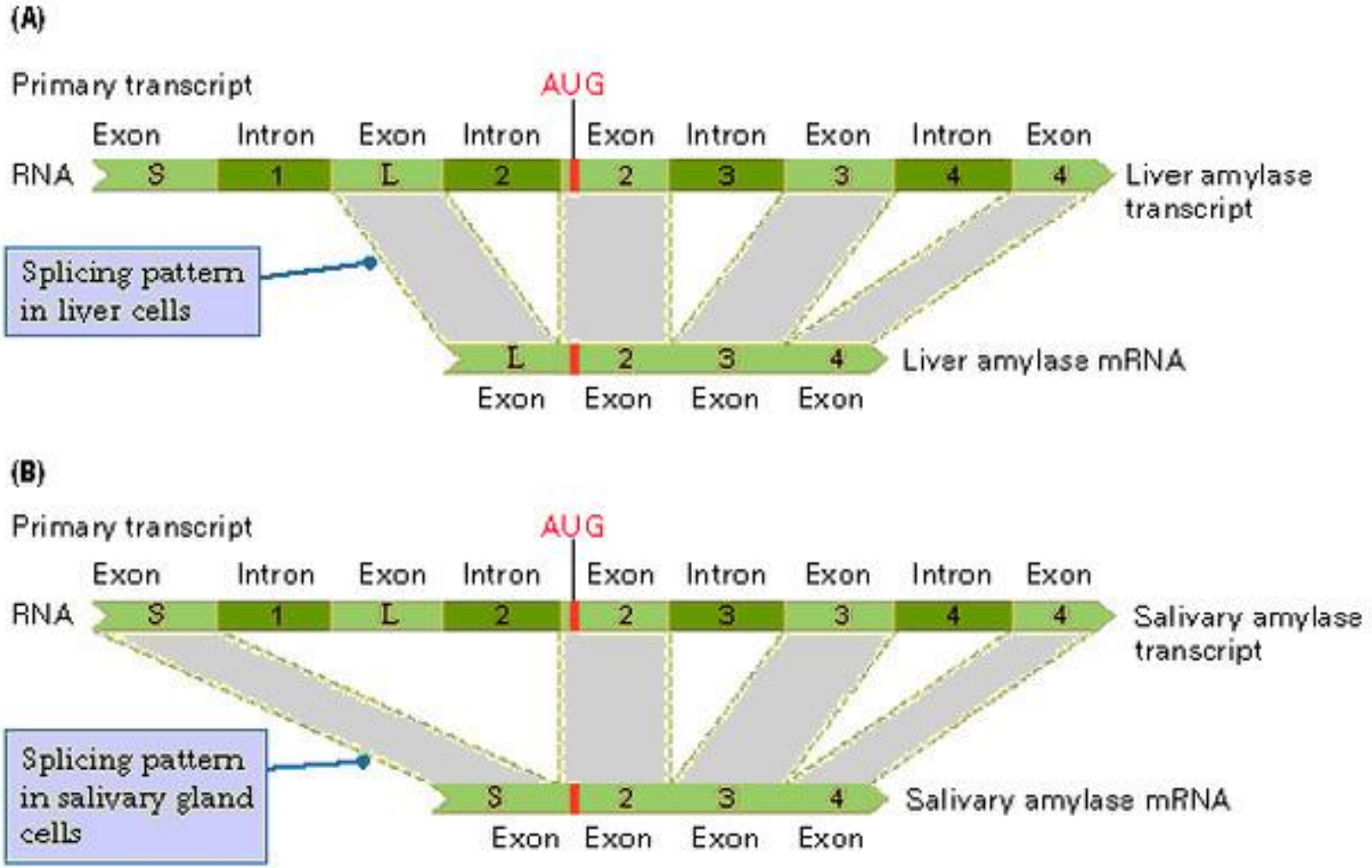
  distribution in genome vaires

Genomic Imprinting

- Unusual epigenetic silencing in mammals
- Occurs in germ line
- Imprints methylated differently in the sexes
- Evident in Prader-Willi and Angelman syndromes

Alternative Splicing

- Alternative splicing is a form of gene regulation which results in the generation of alternative mRNAs from a single gene
- Different splice patterns may occur in different tissues resulting in tissue-specific gene expression


(A)
Primary transcript
AUG
Exon
Intron
Exon
Intron
Exon
Intron
Exon
Intron
Exon
RNA
S
1
L
2
2
3
3
4
4
Liver amylase transcript
Splicing pattern in liver cells
L
2
3
4
Liver amylase mRNA
Exon
Exon
Exon
Exon
(B)
Primary transcript
AUG
Exon
Intron
Exon
Intron
Exon
Intron
Exon
Intron
Exon
RNA
S
1
L
2
2
3
3
4
4
Salivary amylase transcript
Splicing pattern in salivary gland cells
S
2
3
4
Salivary amylase mRNA
Exon
Exon
Exon
Exon

Alternative Splicing

- example preprotachykinin gene
- tachykinins
  - sensory neurotransmitters
  - P neuropeptide in nervous system
  - K neuropeptide in instestine and thyroid

DNA Rearrangements

- Yeast cells carry out programmed DNA rearrangements as part of a mating system called homothallism in which some cells undergo a conversion to opposite mating type
- In vertebrate immune system DNA splicing between variable region and constant region domains takes place in the formation of antibodies

Translational Regulation

- Gene regulation can occur at the level of translation

Types of translational control include:

- Absence of mRNA translation without molecular signal
- Regulation of mRNA half-life
- Regulation of the rate of protein synthesis
- Aborted translation