Gene Expression

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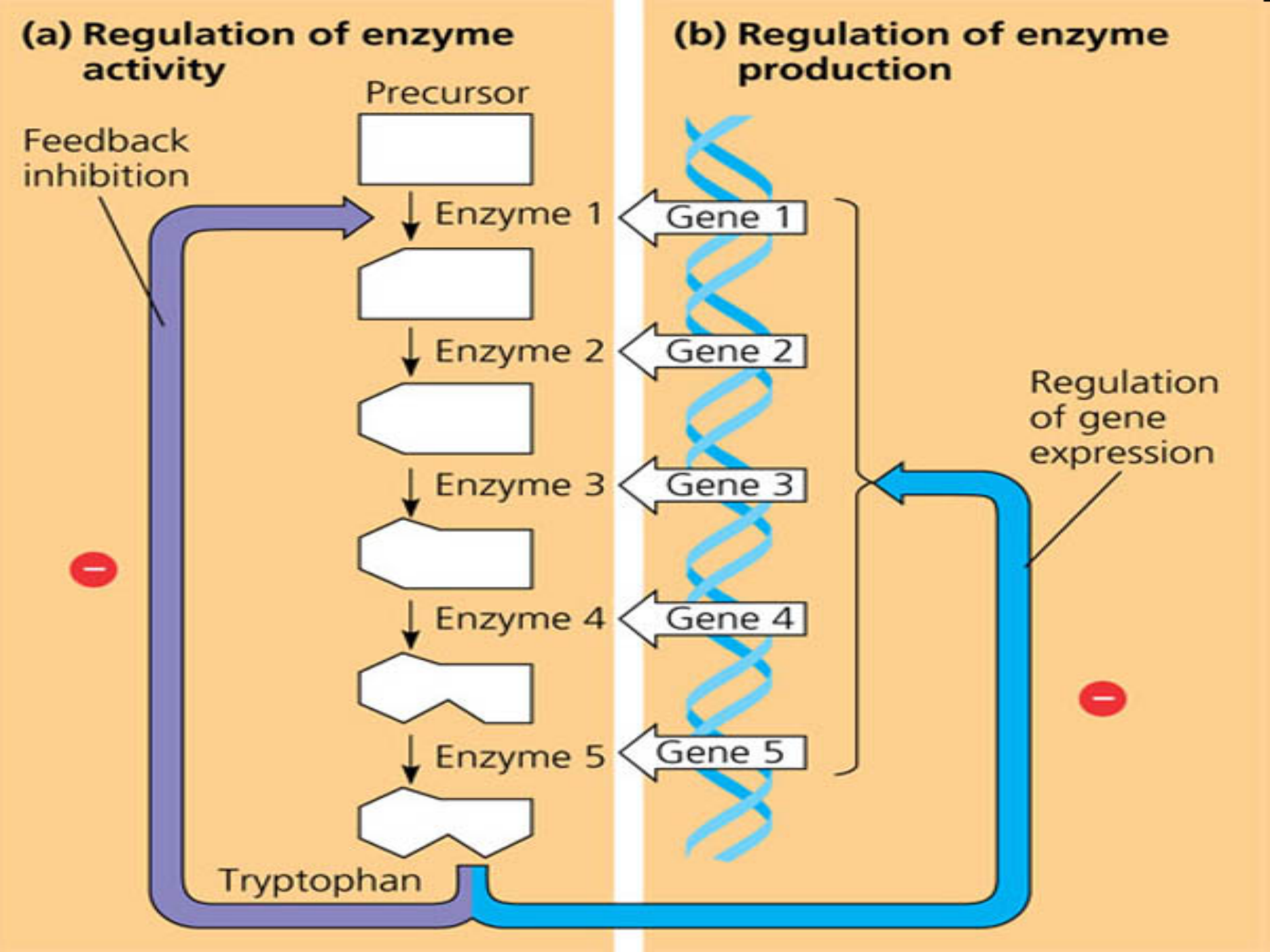
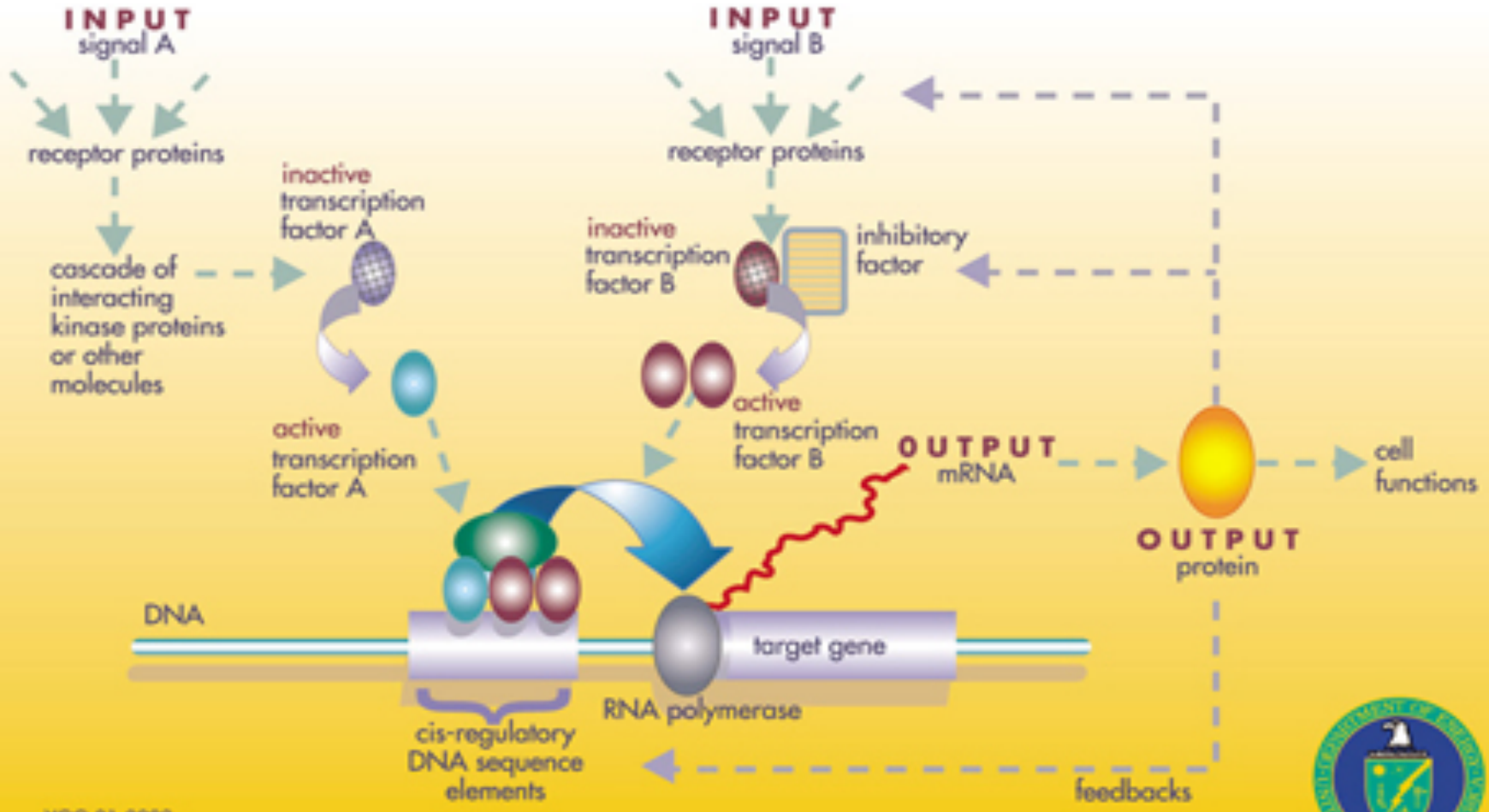
Surat

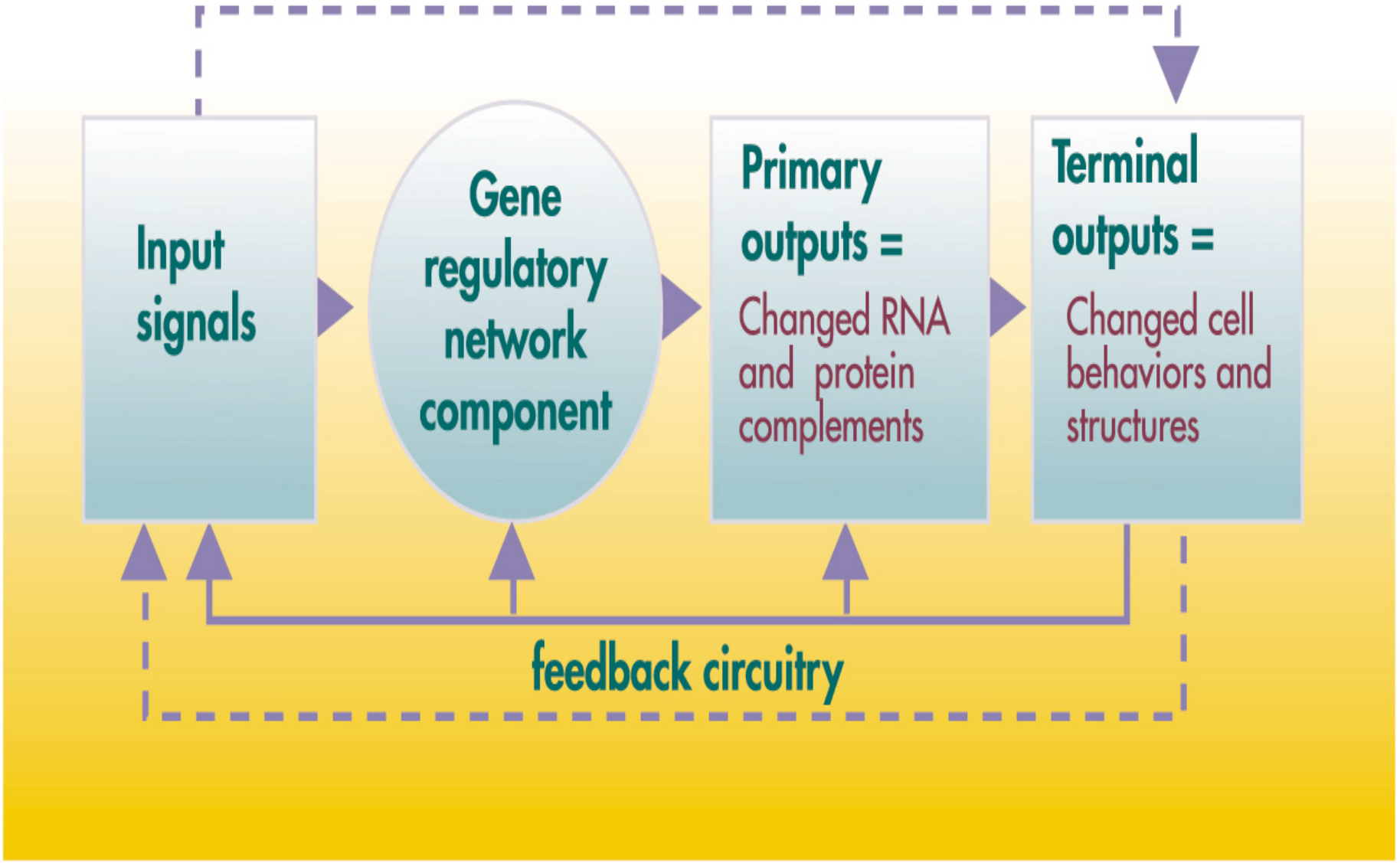

(a) Regulation of enzyme activity
(b) Regulation of enzyme production
Precursor
Feedback inhibition
Enzyme 1
Enzyme 2
Enzyme 3
Enzyme 4
Enzyme 5
Gene 1
Gene 2
Gene 3
Gene 4
Gene 5
Regulation of gene expression
Tryptophan

GENOMES to LIFE

A GENE REGULATORY NETWORK



YGG 01-0083


Input signals
Gene regulatory network component
Primary outputs =
Changed RNA and protein complements
Terminal outputs =
Changed cell behaviors and structures
feedback circuitry

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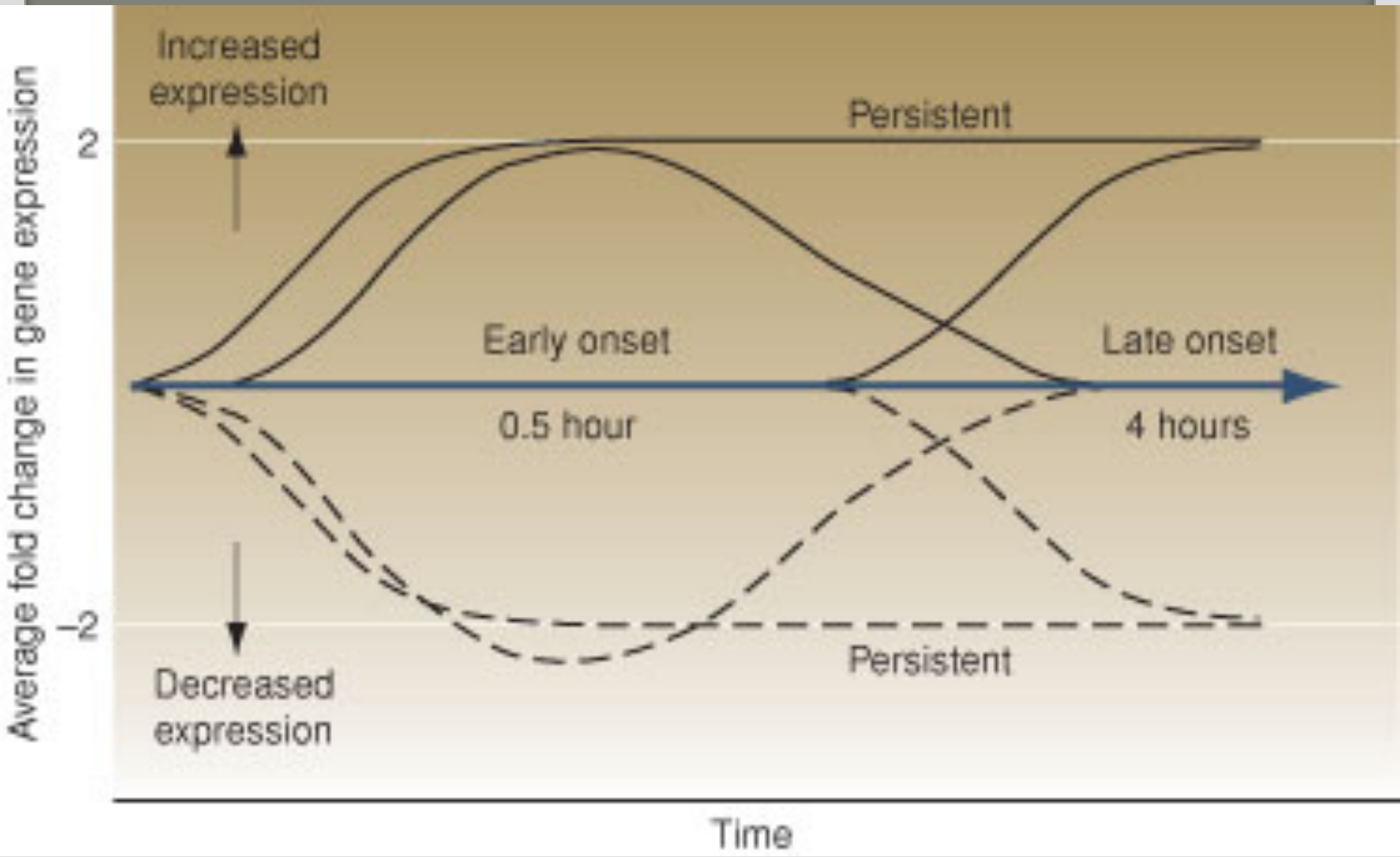
Biological Responce through Regulatory Signal



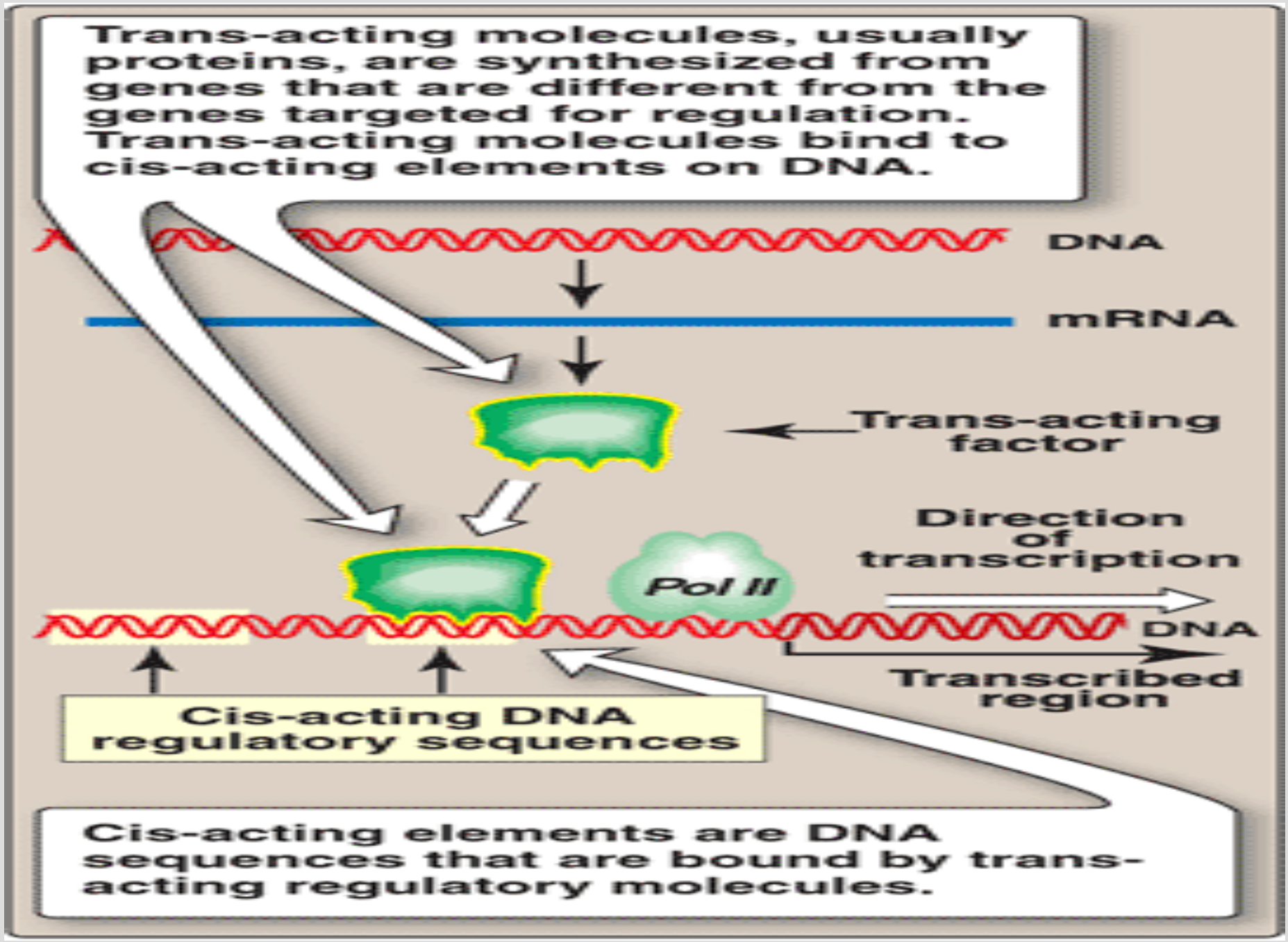
Regulatory Sequences & Molecules

Cis-acting factor - Sequence

- Sequence of genes
- present on
  - transcription gene
  - same chromosome.

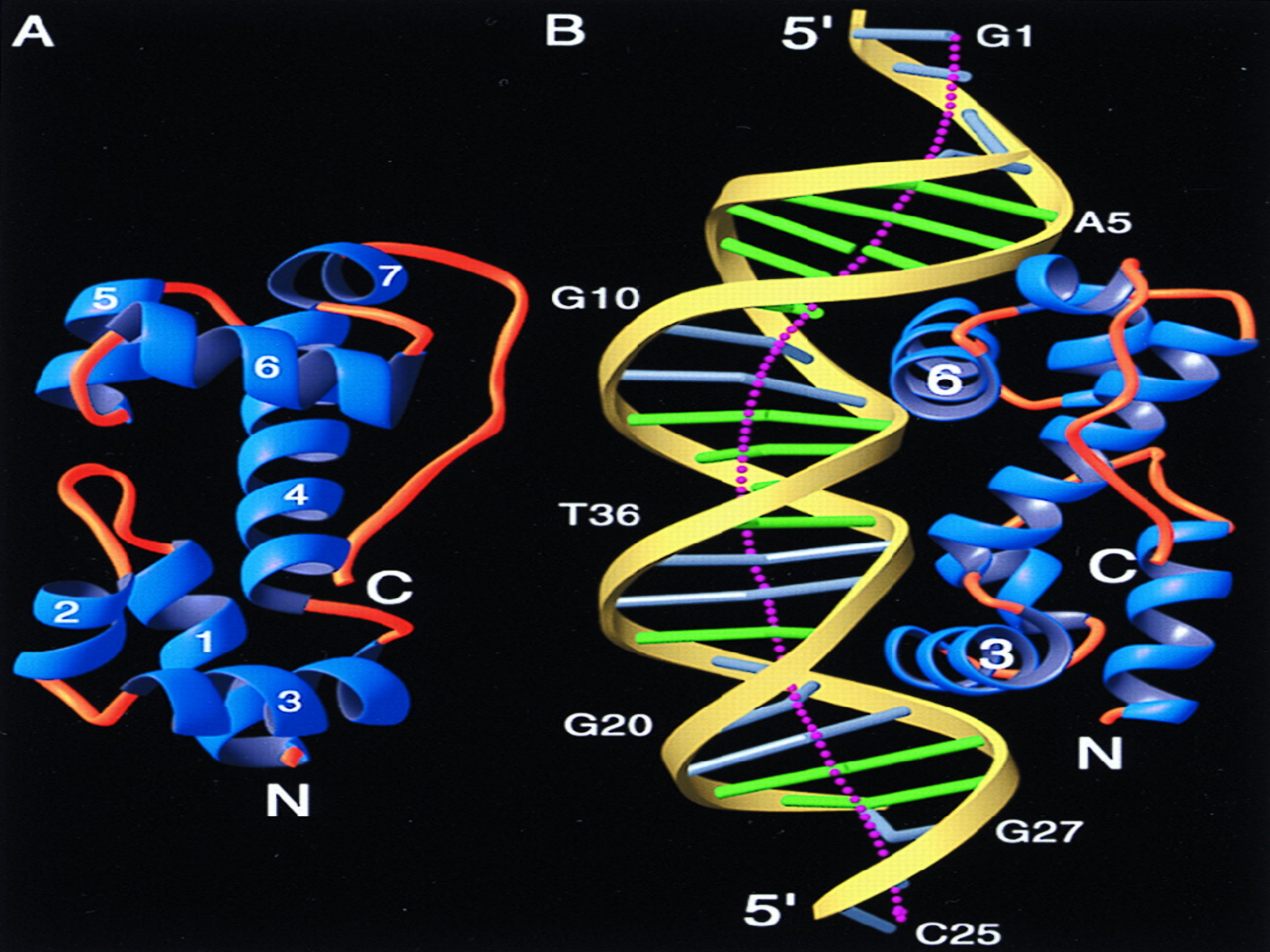
Trans-acting factor – Molecule - Protein

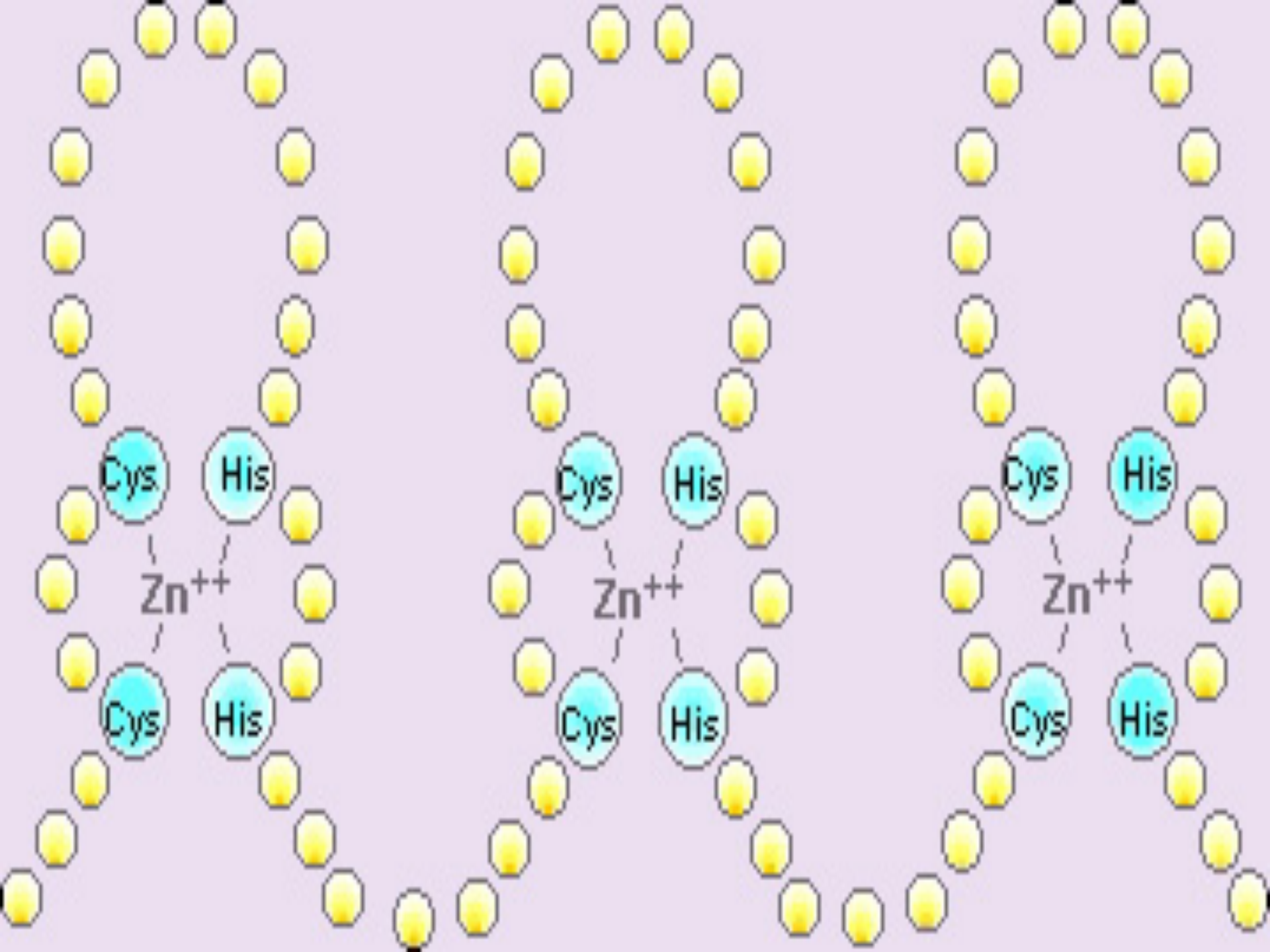
- This gene makes protein molecule
- That molecule make influence on Cis-acting sequence, which is on other site (chromosome)
- It's gene present at different site or at different chromosome.

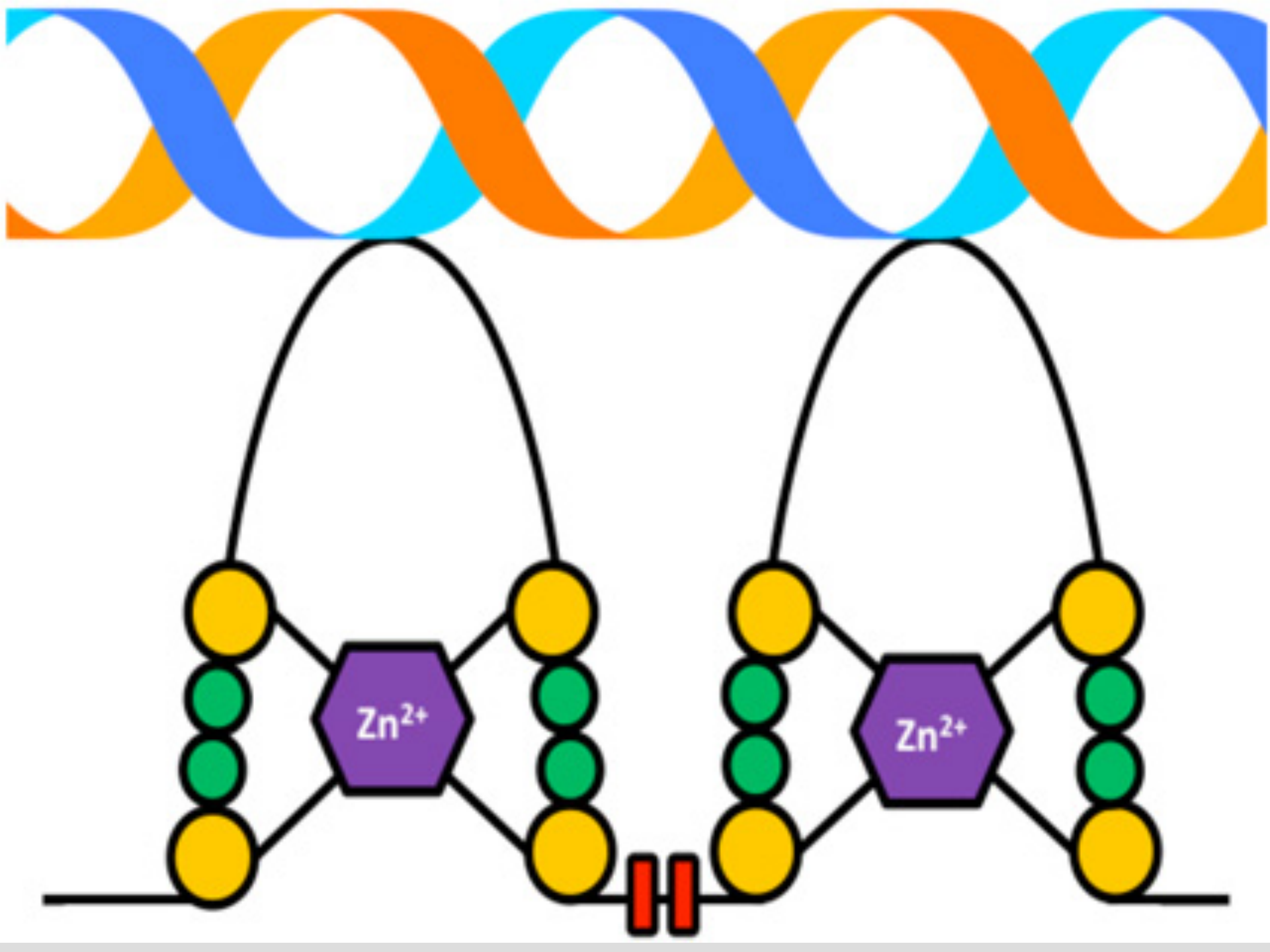

Trans-acting molecules, usually proteins, are synthesized from genes that are different from the genes targeted for regulation. Trans-acting molecules bind to cis-acting elements on DNA.
DNA
mRNA
Trans-acting factor
Direction of transcription
Pol II
DNA
Transcribed region
Cis-acting DNA regulatory sequences
Cis-acting elements are DNA sequences that are bound by trans-acting regulatory molecules.

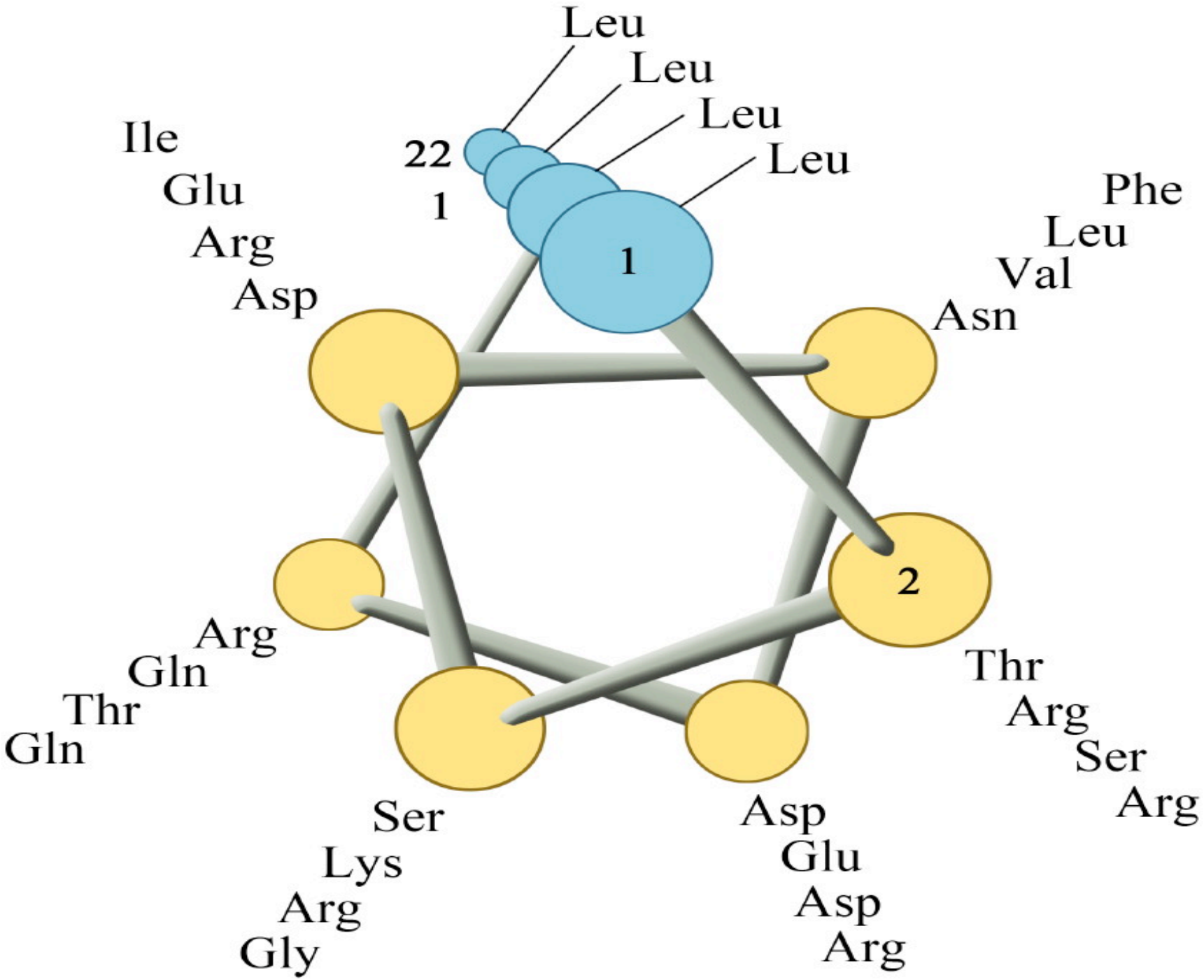
Gene Expression Theories

Zinc finger
Leucine zipper
Helix-turn-helix in the protein.


A
B
5'
G1
A5
G10
T36
G20
G27
5'
C25
N
C
N
C
1
2
3
4
5
6
7
1
3
6


Cys
His
Zn++
Cys
His
Cys
His
Zn++
Cys
His
Cys
His
Zn++
Cys
His


Zn^{2+}
Zn^{2+}


Leu
Leu
Leu
Leu
22
1
1
Ile
Glu
Arg
Asp
Phe
Leu
Val
Asn
2
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Thr
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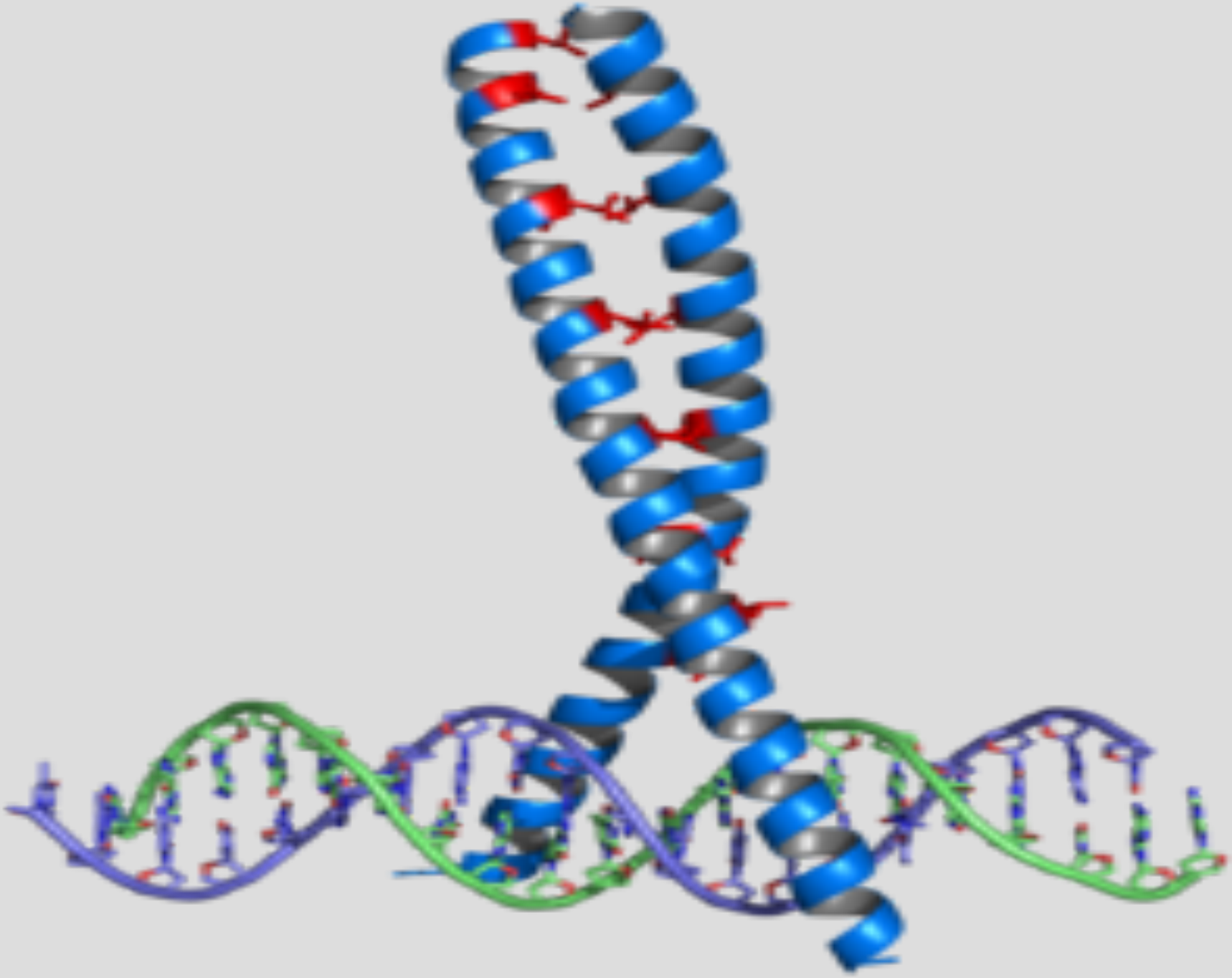
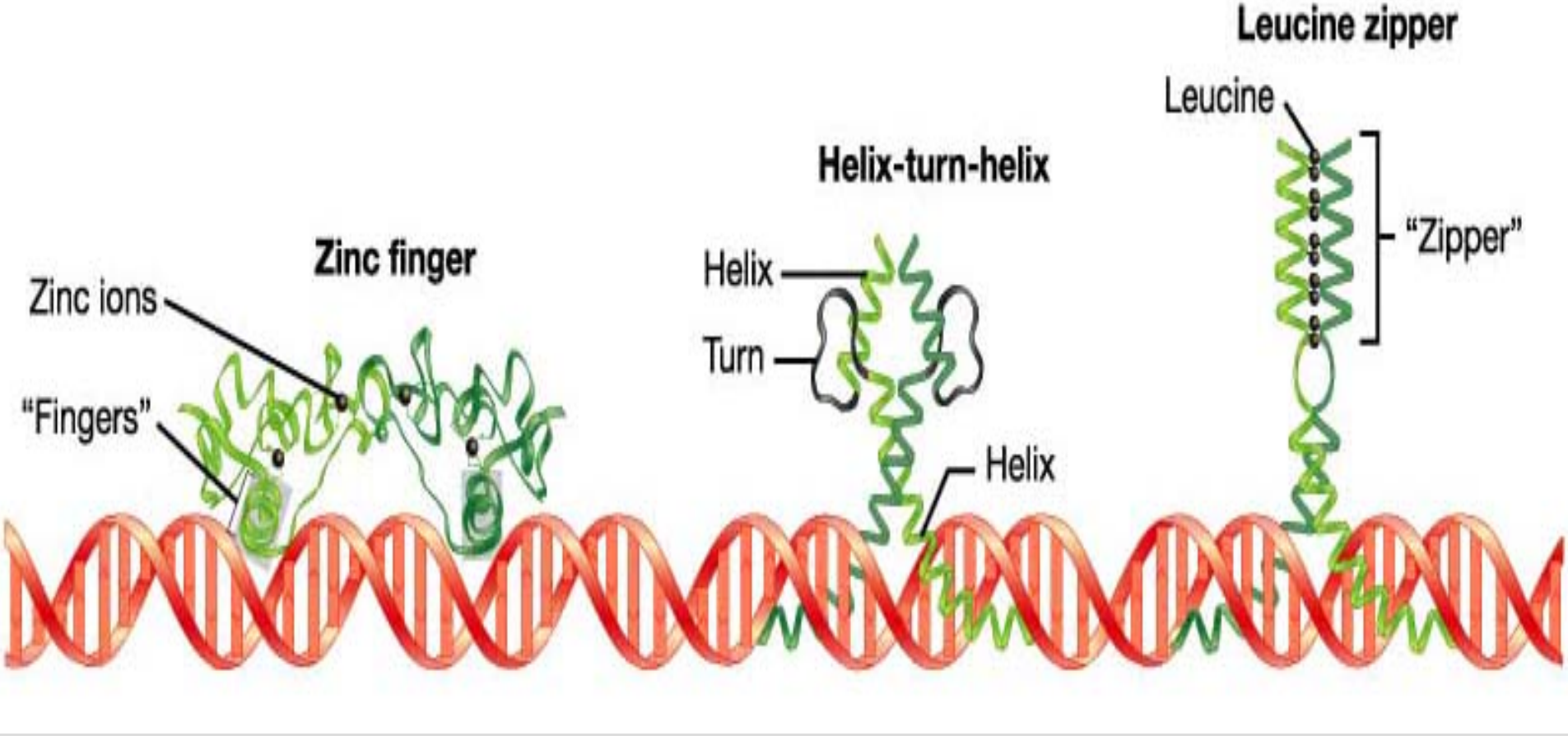
Arg
Ser
Lys
Arg
Gly
Asp
Glu
Asp
Arg




Zinc finger
Zinc ions
"Fingers"
Helix-turn-helix
Helix
Turn
Helix
Leucine zipper
Leucine
"Zipper"

Gene Expression in Prokaryotics

1. Lac operon
2. Tryptophane operon
3. Co-ordination of transcription & translation
   a) Stringent Response
   b) Regulatory Ribosomal protein

Operon

Cis-acting regulatory elements
Structural genes that code for proteins
The transcription product is a single polycistronic messenger RNA (mRNA).
Controlled turned on or off .
Entire package = Operon.

Mechanism of Operon

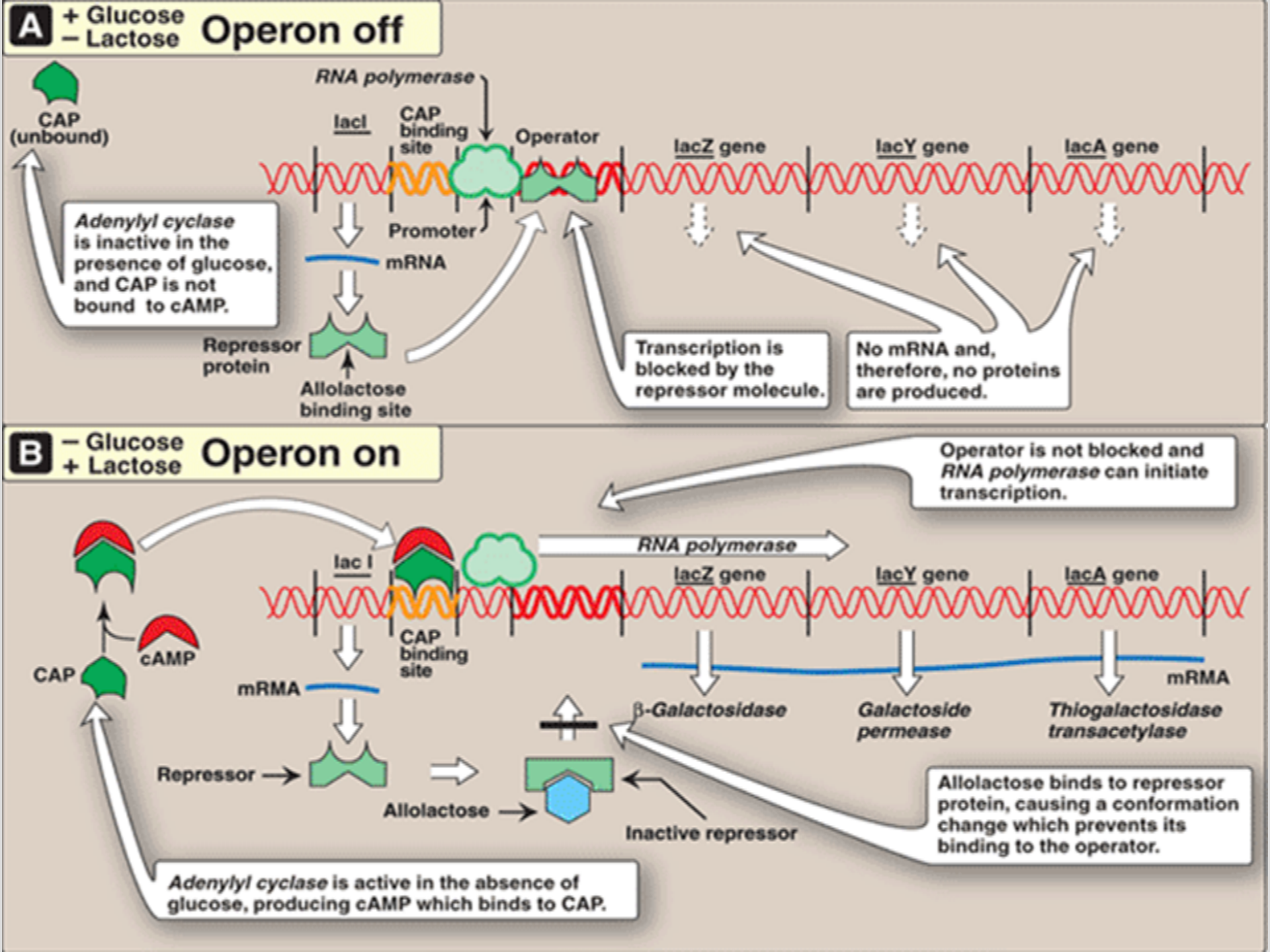
Repressor bind to Operon

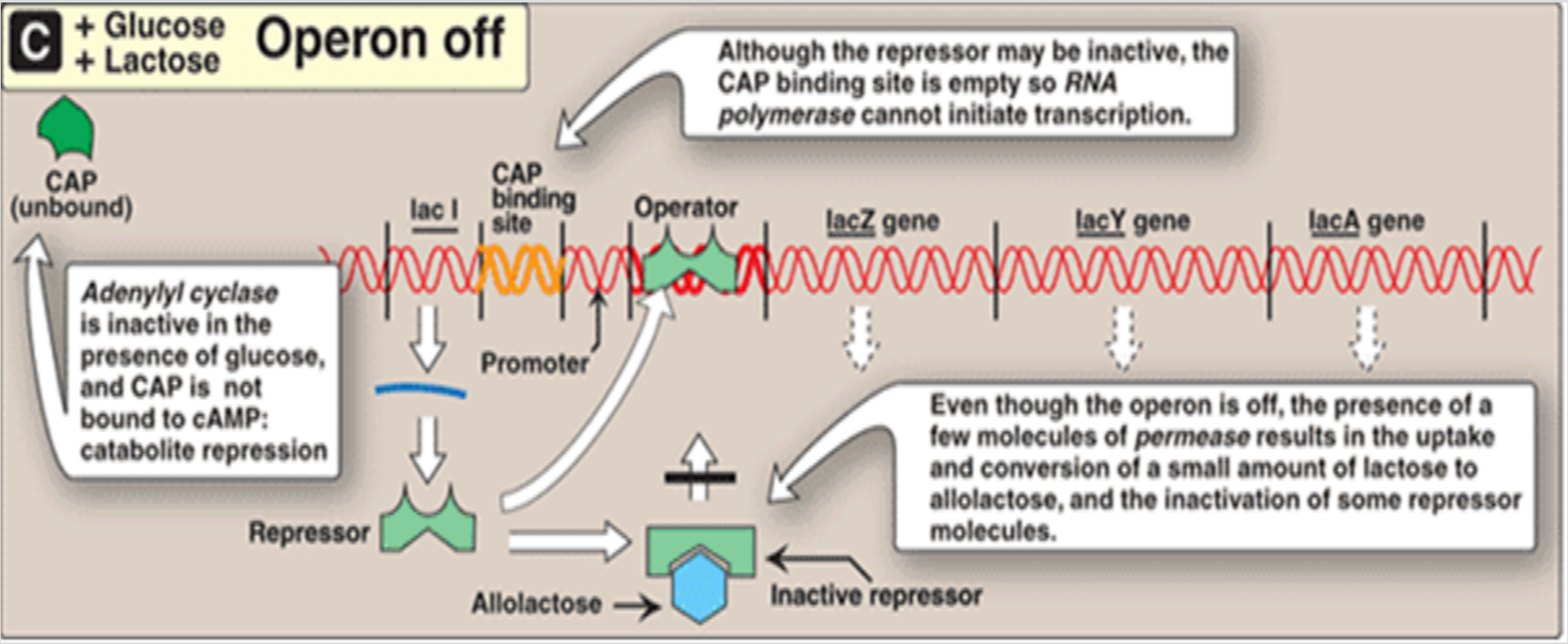
- = No Transcription
- = RNA Polymerase cannot make m-RNA
- = No Tranlation = No Protein

Operator bind to Operon

- = Remove repression
- = Transcription
- = Translation
- = Protein Synthesis occur(Enzyme synthesis)
- = Pathway proceed

Lactose (Lac) Operon


A + Glucose – Lactose Operon off
CAP (unbound)
RNA polymerase
lacI
CAP binding site
Operator
lacZ gene
lacY gene
lacA gene
Promoter
mRNA
Adenylyl cyclase is inactive in the presence of glucose, and CAP is not bound to cAMP.
Repressor protein
Allolactose binding site
Transcription is blocked by the repressor molecule.
No mRNA and, therefore, no proteins are produced.
B – Glucose + Lactose Operon on
Operator is not blocked and RNA polymerase can initiate transcription.
RNA polymerase
lac I
lacZ gene
lacY gene
lacA gene
CAP binding site
cAMP
CAP
mRMA
mRMA
β-Galactosidase
Galactoside permease
Thiogalactosidase transacetylase
Repressor
Allolactose
Inactive repressor
Allolactose binds to repressor protein, causing a conformation change which prevents its binding to the operator.
Adenylyl cyclase is active in the absence of glucose, producing cAMP which binds to CAP.


C
+ Glucose
+ Lactose
Operon off
CAP
(unbound)
Although the repressor may be inactive, the CAP binding site is empty so *RNA polymerase* cannot initiate transcription.
lac I
CAP binding site
Operator
lacZ gene
lacY gene
lacA gene
Promoter
Adenylyl cyclase is inactive in the presence of glucose, and CAP is not bound to cAMP: catabolite repression
Even though the operon is off, the presence of a few molecules of *permease* results in the uptake and conversion of a small amount of lactose to allolactose, and the inactivation of some repressor molecules.
Repressor
Allolactose
Inactive repressor

Lactose Operon

Lactose (lac) operon codes for three proteins

Lac Z gene

- β-galactosidase
- hydrolyzes lactose to galactose and glucose

Lac Y gene

- Permease
- facilitates the movement of lactose into the cell

Lac A gene

- Thiogalactoside transacetylase
- unknown function

All of these proteins are produced when lactose is available to the cell but glucose is not.

Bacteria use glucose as a fuel in preference to any other sugar

Lactose Operon

With the operon, there is three structural genes

- Promoter (P) region
- Operator (O) site
- CAP site

The lacZ, lacY and lacA genes are expressed only when

- O site is empty
- CAP site is bound by a complex of cAMP
- CAP bind to O site

Note : CAP (catabolite gene activator protein) = CRP = (cAMP regulatory protein)

Lac I gene = for the repressor protein

Tryptophan operon

The tryptophan (trp) operon

- Codes for five proteins.
- Required for synthesis of tryptophan.

Negative feedback control

Tryptophan bind to repressor
Facilitate binding of repressor to the operator.
Process name = **Attenuation**.

With attenuation, transcription is initiated but is terminated well before completion.

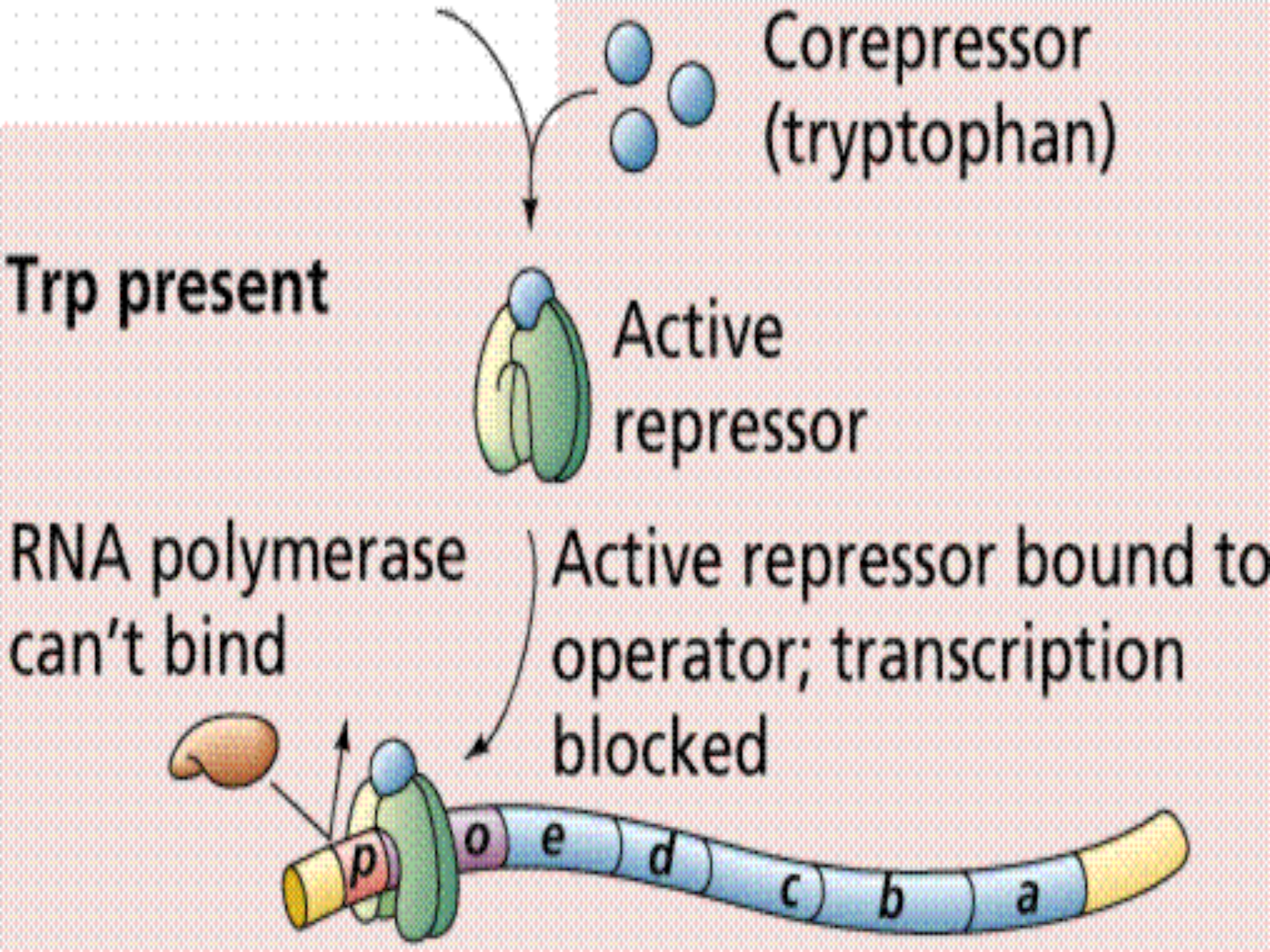
Tryptophan operon

Tryptophane = Excess

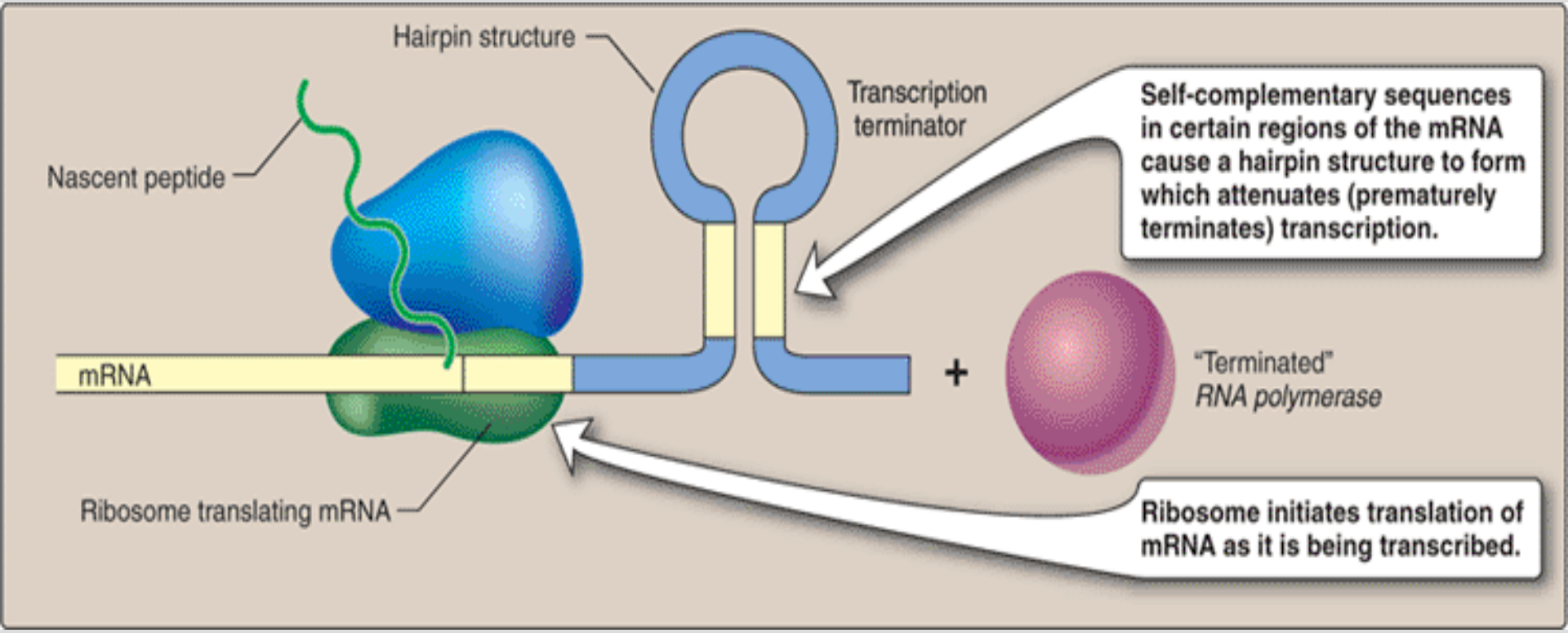
- Initiation of Transcription start
- But Formation of a hairpin (stem-loop) structure at the 5′-end of the mRNA
- Pre-mature termination.
- Results in the formation of a truncated, nonfunctional peptide product
- That is rapidly degraded.

Tryptophane = Less

- Operon is expressed.
- Prevents attenuation
- Thus allows transcription to continue.
- Protein Synthesis occur.


Corepressor
(tryptophan)
Trp present
Active
repressor
RNA polymerase
can't bind
Active repressor bound to
operator; transcription
blocked
p
o
e
d
c
b
a

Tryptophane Operon



Coordination of Transcription & Translation

Stringent response

Amino acid starvation is known as the stringent response.

Binding of uncharged tRNA

Phosphorylation of GDP = Poly-Phosphorylated Guanosine (ppGpp).

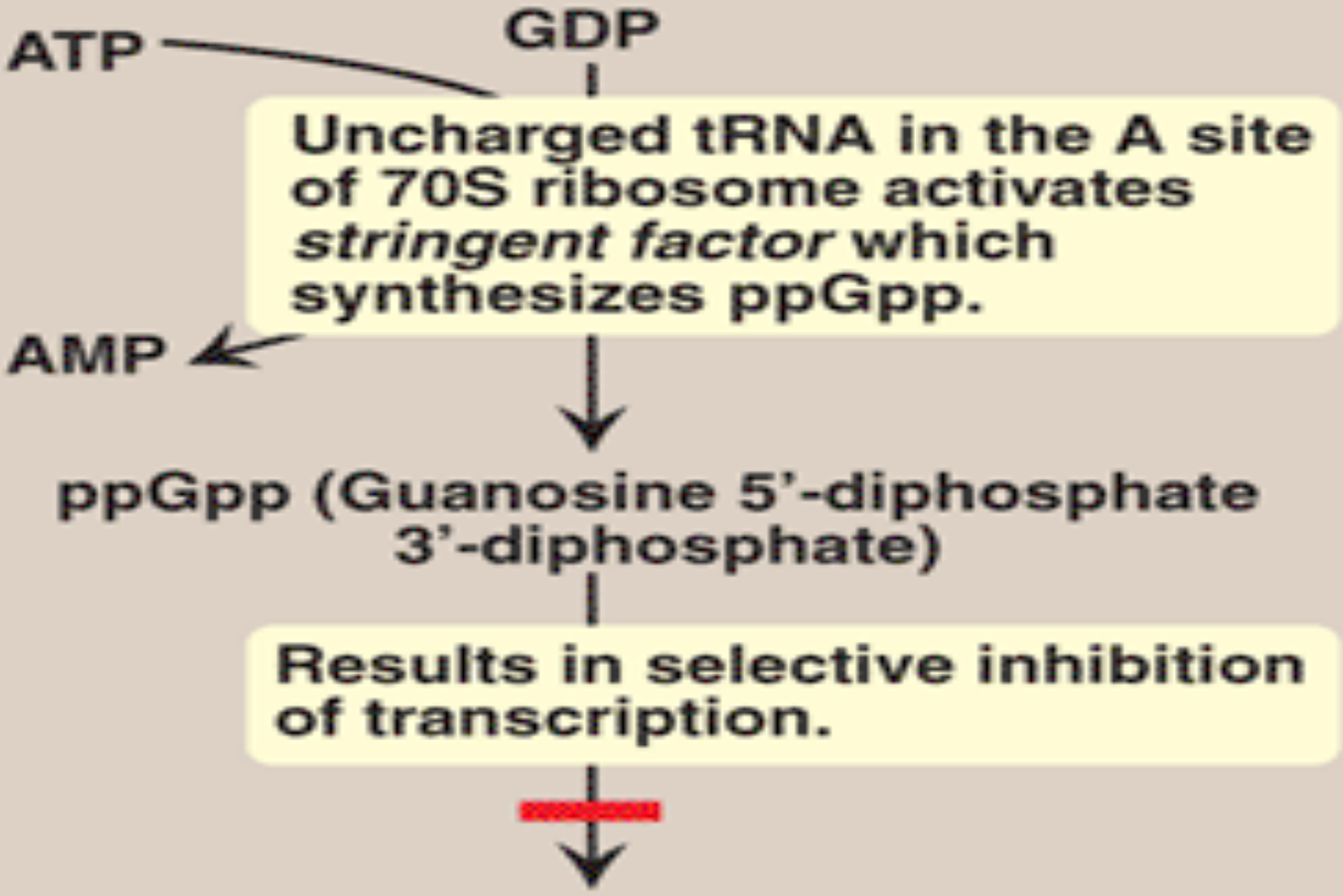
Phosphorylated GDP is usually catalyzed by stringent factor.

Stringent Factor = Enzyme associated with ribosomes.

Elevated ppGpp = inhibition of rRNA synthesis

rRNA, tRNA ,mRNA synthesis = inhibited

Ultimately results in down-regulation of protein synthesis until amino acids are again available


ATP
GDP
Uncharged tRNA in the A site of 70S ribosome activates *stringent factor* which synthesizes ppGpp.
AMP
ppGpp (Guanosine 5'-diphosphate 3'-diphosphate)
Results in selective inhibition of transcription.
rRNA, tRNA, some mRNAs

Regulatory ribosomal proteins

Ribosomal protein = r-Protein

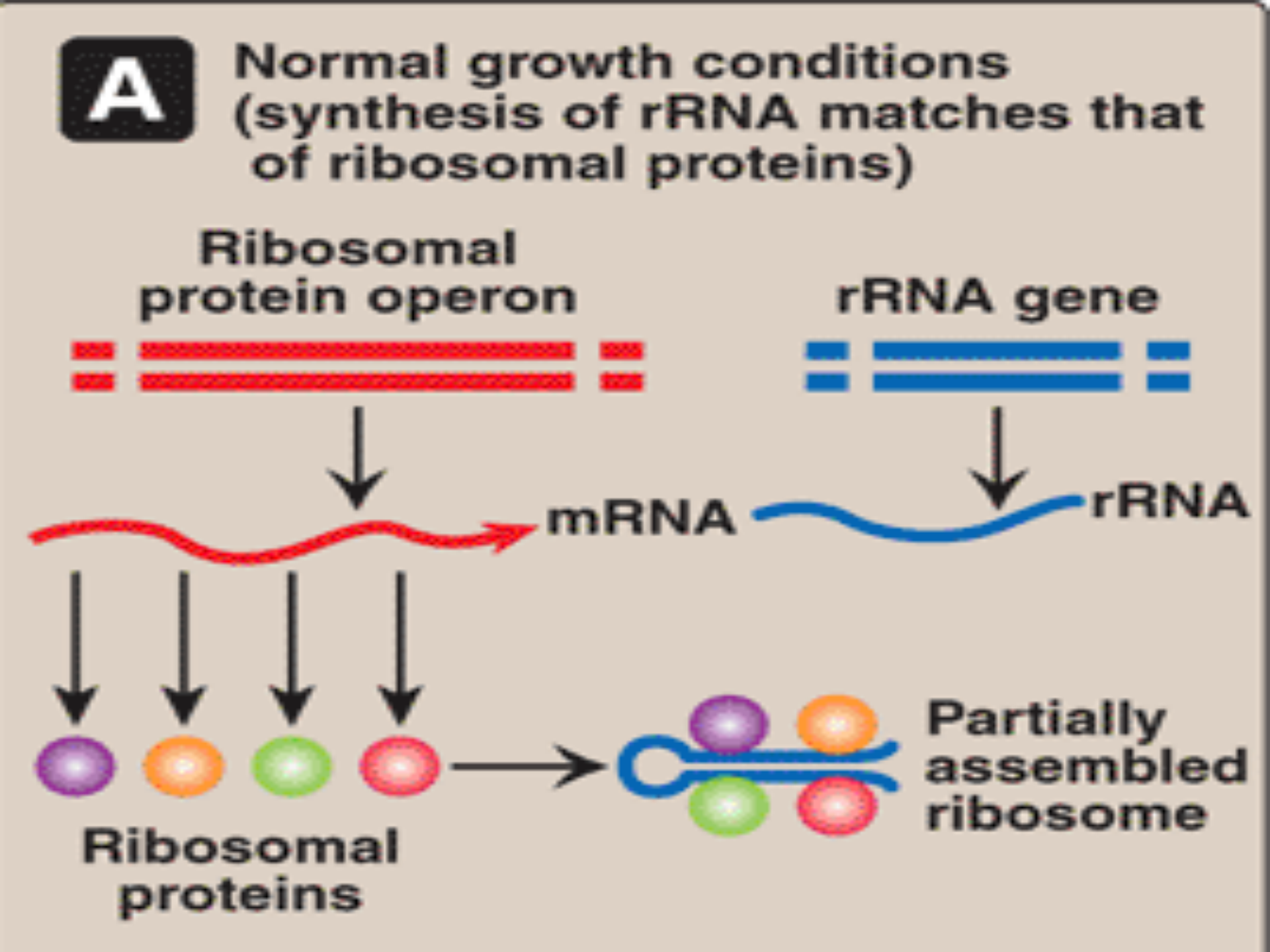
Excess r-protein = inhibited their own production

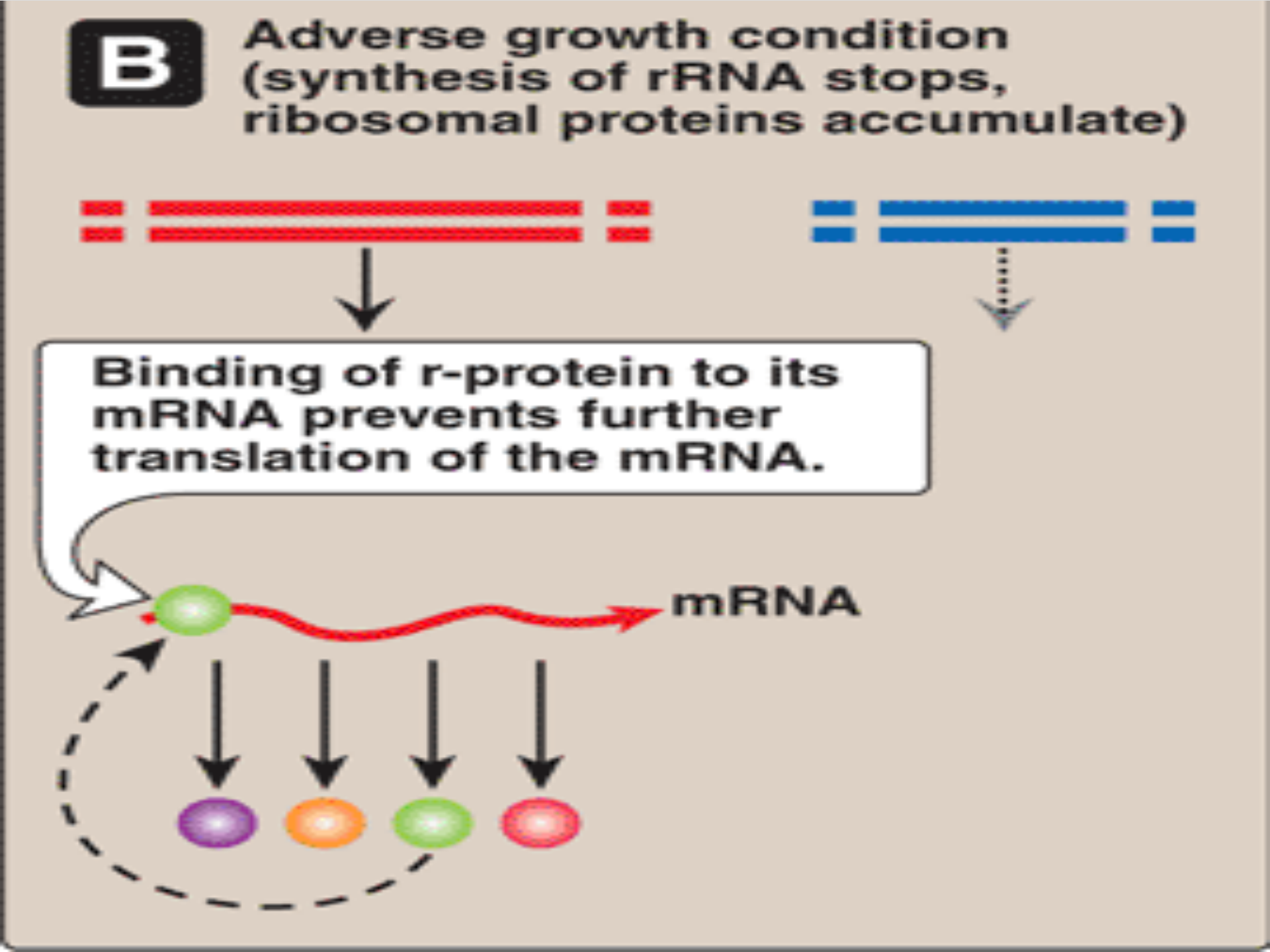
Bind with

- r-RNA (more affinity)
- m-RNA – initiation sequence

Acting as a physical obstacle .

One r-protein inhibits synthesis of all r-proteins.


A
Normal growth conditions (synthesis of rRNA matches that of ribosomal proteins)
Ribosomal protein operon
rRNA gene
mRNA
rRNA
Ribosomal proteins
Partially assembled ribosome


B
Adverse growth condition (synthesis of rRNA stops, ribosomal proteins accumulate)
Binding of r-protein to its mRNA prevents further translation of the mRNA.
mRNA

Regulation of Eukaryotic Gene Expression

Regulation of Eukaryotic Gene Expression

1. Trans-acting molecules
2. Cis-acting regulatory elements
   a) Intracellular receptors
   b) Cell-surface receptors
3. Regulation by post-transcriptional processe
   a) Post -Transcription Modification
   b) Splice-site choice:
   c) mRNA editing:
4. Regulation through modification in DNA
   a) Access of DNA
   b) Amount of DNA
   c) Arrangement of DNA

Regulation of Eukaryotic Gene Expression

Trans-acting molecules + Cis-acting elements

Operons are not found in eukaryotes

Regulated at multiple levels

For example,

Post-transcriptional regulation level

At the protein level

1. Trans-acting molecules

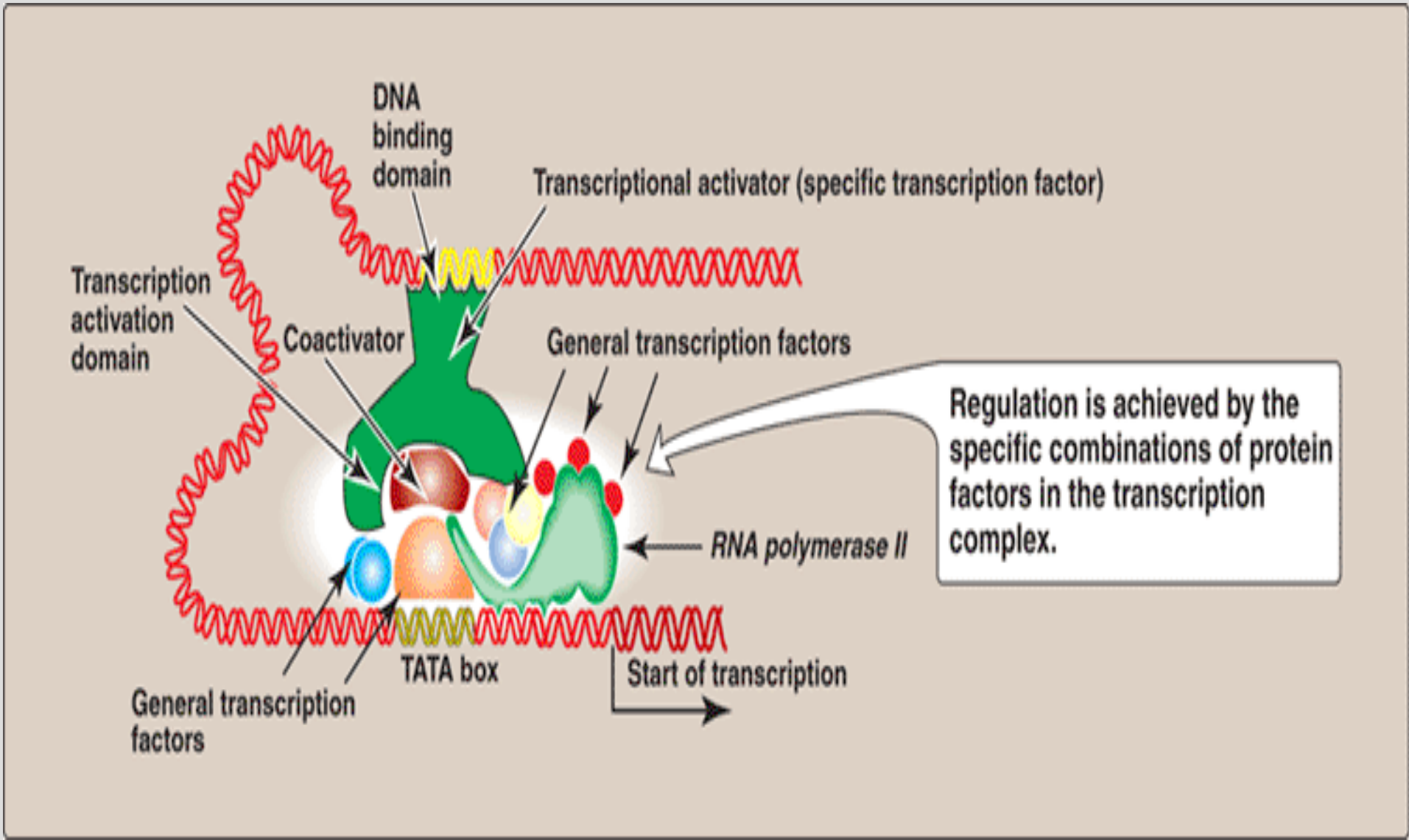
DNA-binding proteins = Trans-acting molecules
Work as transcriptional activators

They have at least two binding domains

- DNA-binding domain
- Transcription activation domain.

Formation of multiprotein complex bound to DNA.

Trans-acting molecules

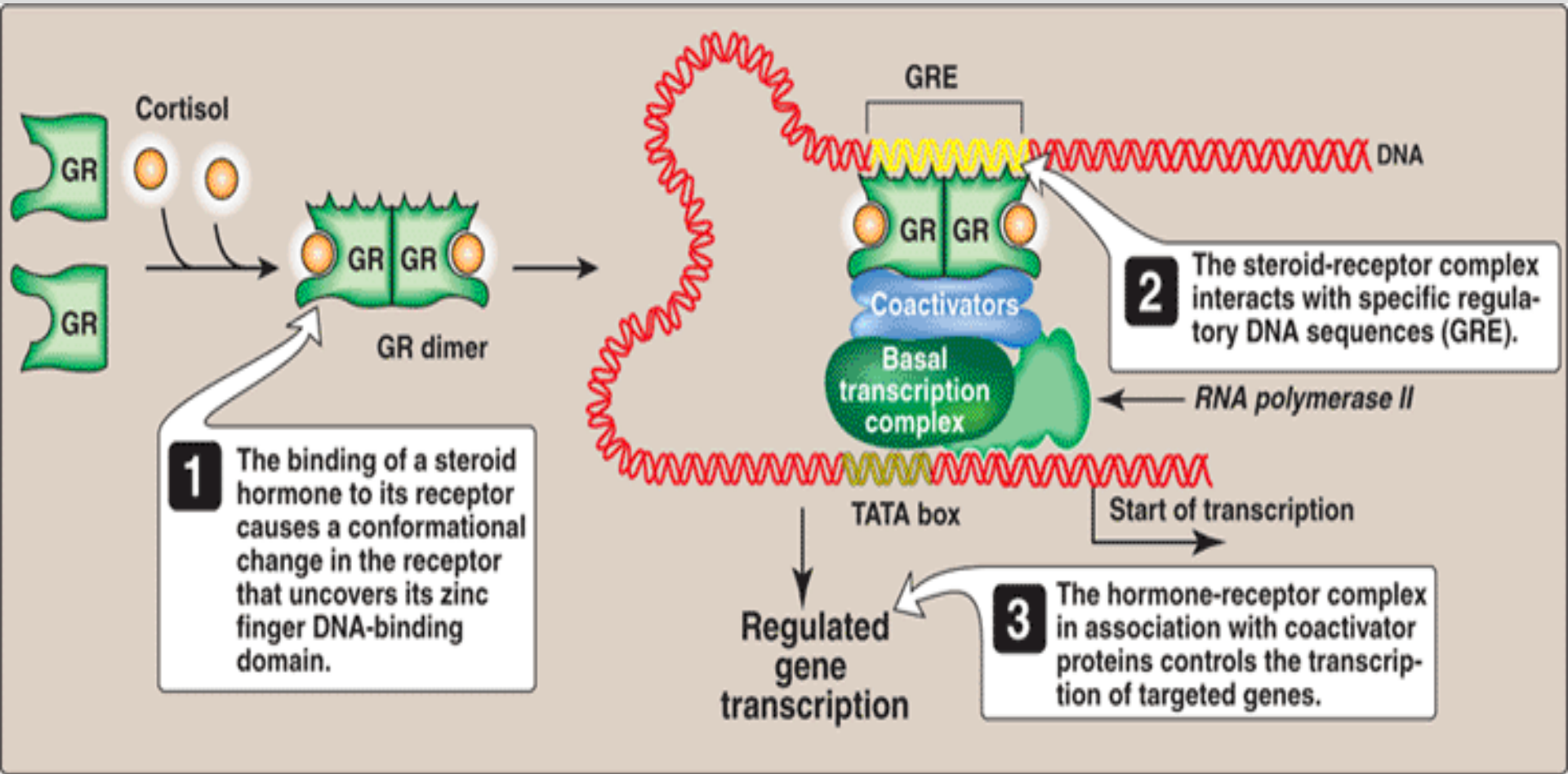


2. Cis-acting regulatory elements

A protein binds to a regulatory element
Affects the expression of those genes.

For example,
Hormone-response elements (HREs) are cis-acting DNA sequences
Trans-acting protein factors bind with HRE
And regulate gene expression in response to hormonal signals.

Cis-acting regulatory elements



Cis-acting Regulatory elements

Regulatory signals mediated by

A.Intra-cellular receptors

B. Cell-surface receptors

A. Regulatory signals mediated by intracellular receptors:

Glucocorticoid, Mineralocorticoid, Sex hormone, Vitamin D, Retinoic acid,and thyroid hormone receptors
Directly influence activity of transcription factors
Alteration of DNA-binding affinity of the factors.
Steroid hormones bind to soluble receptors
Ligand causes a conformational change
Receptor–ligand complex enters nucleus, in association with coactivators,
Complex bind with nuclear DNA at a cis-acting regulatory element, (GRE)
Glucocorticoid-response element (GRE)—an example of an HRE.
GRE can be located upstream or downstream of the genes it regulates, and is able to function at great distances from those genes.
The GRE, then, can function as a true enhancer

Regulatory signals mediated by cell-surface receptors:

Insulin, epinephrine, and glucagon.

Glucagon = peptide hormone

Binds to G-protein at plasma membrane receptor.

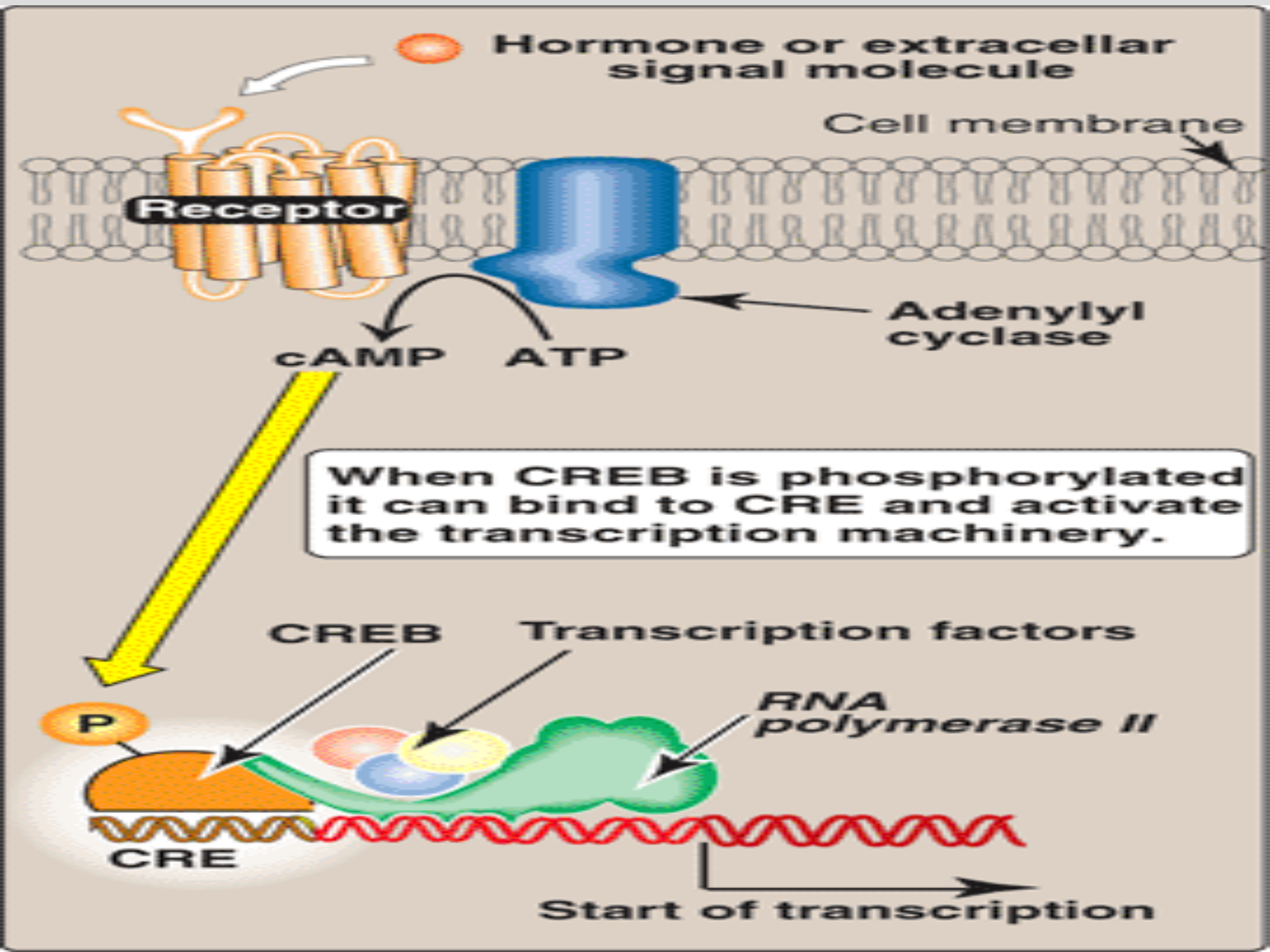
Extracellular signal is then transduced to intracellular cAMP

Affect protein expression through protein kinase A–mediated covalent modification.

CREB = cAMP-response element-binding protein = phosphorylated.

Via a leucine zipper CREB bind to a cis-acting element, the cAMP-response element (CRE)

Resulting transcription of target genes with CREs in their promoters.


Hormone or extracellar signal molecule
Cell membrane
Receptor
Adenylyl cyclase
cAMP
ATP
When CREB is phosphorylated it can bind to CRE and activate the transcription machinery.
CREB
Transcription factors
RNA polymerase II
P
CRE
Start of transcription

3. Regulation by post-transcriptional processe

Post -Transcription Modification

- Capping at the 5′-end,
- Polyadenylation at the 3′-end,
- Splicing

Splice-site choice:

- Protein isoforms – through splicing.

mRNA editing:

Base altered.

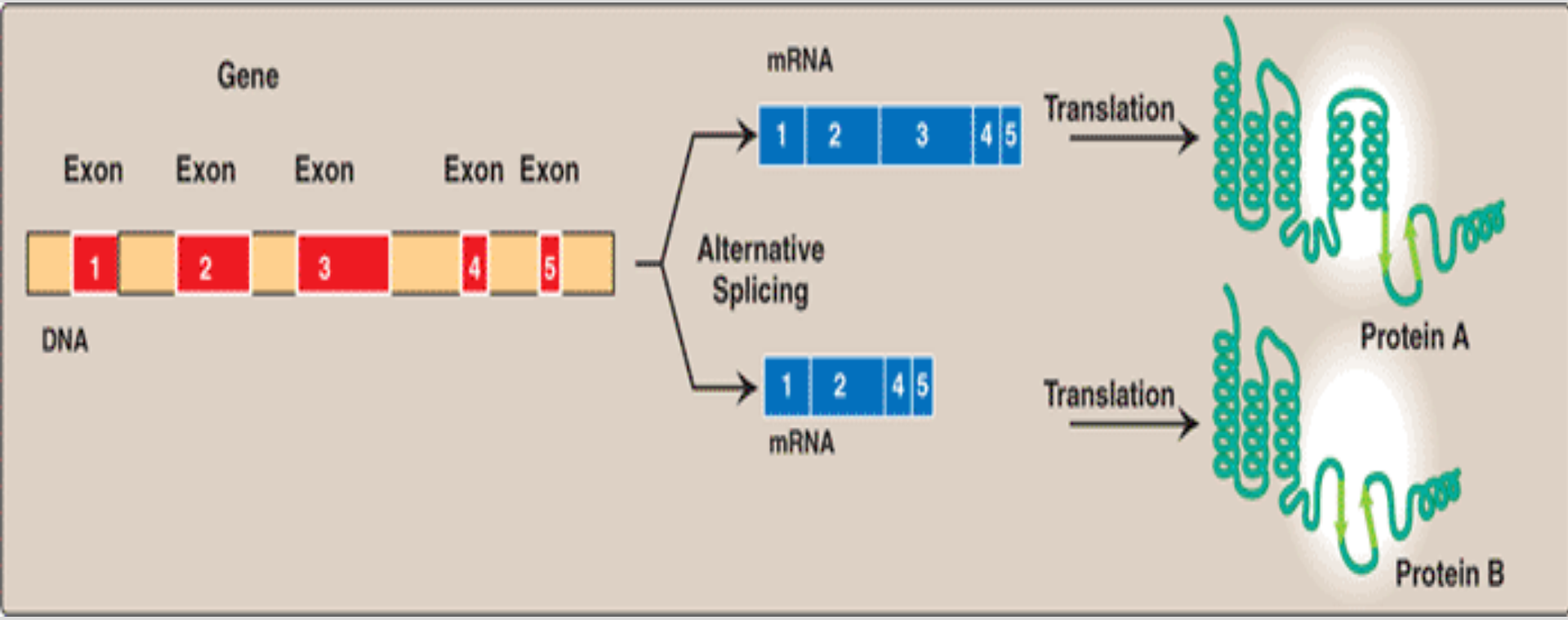
Example in humans = Apoprotein (apo) B

In intestine = Shorter protein = Apo B-48

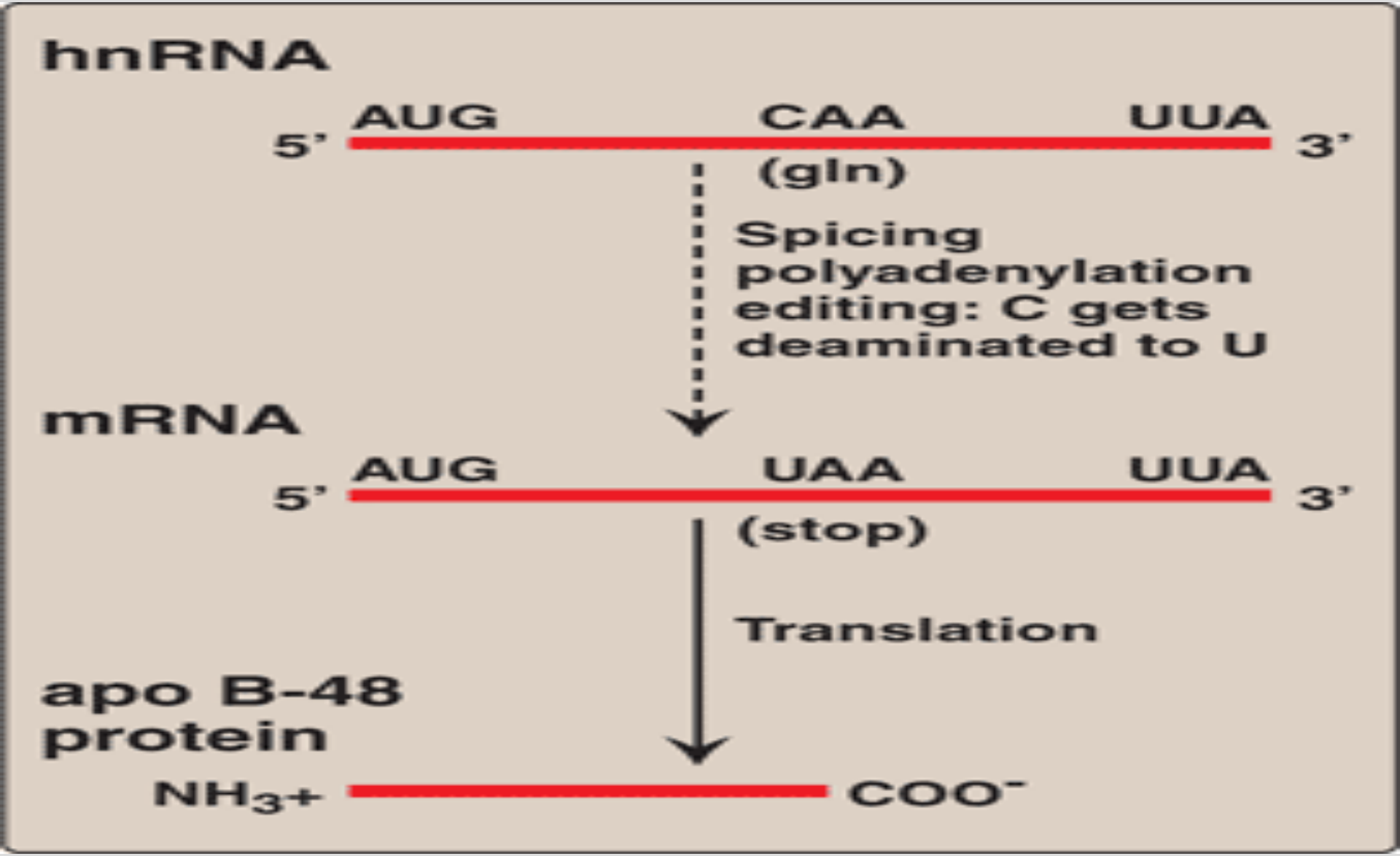
In the liver = Full-length = Apo B-100

In the intestine only, codon (CAA) for glutamine is deaminated to U = Stop codon (UAA)

Regulation by post-transcriptional processes



mRNA Editing



3. mRNA stability:

A. Iron Metabolism

B. RNA interference

C. Translation of RNA

A.Iron Metabolism

Transferrin = Iron Transporter plasma protein

TfR = Transferrin binds to cell-surface receptors.

Internalized

Provide iron to target cell.

mRNA for the TfR has cis-acting iron-responsive elements (IREs)

IREs can be bound by trans-acting iron regulatory proteins

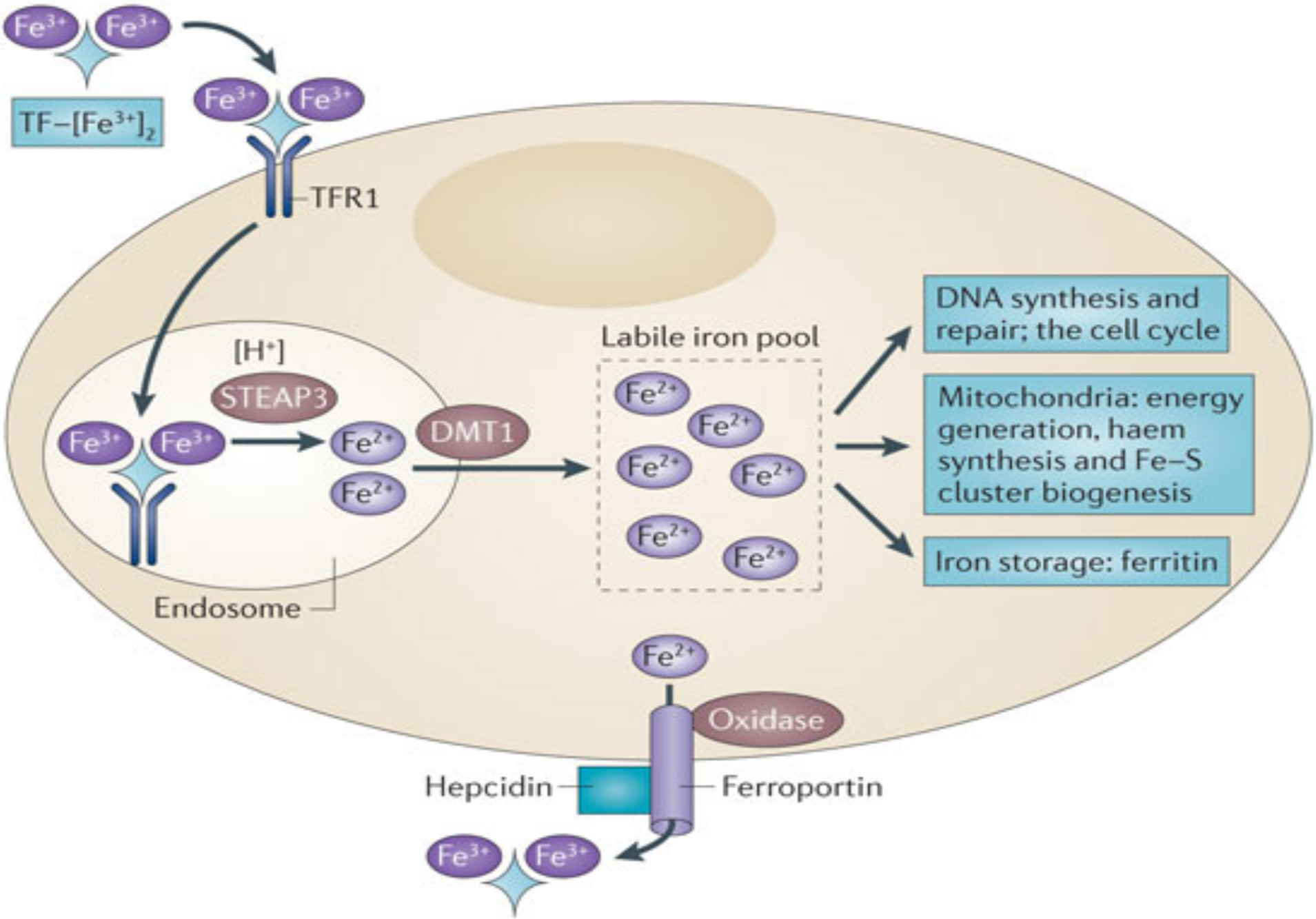
Store the excess iron.

If Iron Low

IRPs bind to the IREs
stabilize the mRNA for TfR
TfR synthesis.

If Iron High

IRPs bind iron instead of IREs
IRPs can not bound to mRNA
mRNA degradation
Decreased TfR synthesis.


Fe3+
Fe3+
TF–[Fe3+]2
Fe3+
Fe3+
TFR1
[H+]
STEAP3
Fe3+
Fe3+
Fe2+
Fe2+
DMT1
Endosome
Labile iron pool
Fe2+
Fe2+
Fe2+
Fe2+
Fe2+
Fe2+
DNA synthesis and repair; the cell cycle
Mitochondria: energy generation, haem synthesis and Fe–S cluster biogenesis
Iron storage: ferritin
Fe2+
Oxidase
Hepcidin
Ferroportin
Fe3+
Fe3+

IRP binding to IRE

- If At 5' end of Transcription unit
  - decreases rate of synthesis;
  - e.g. Ferritin

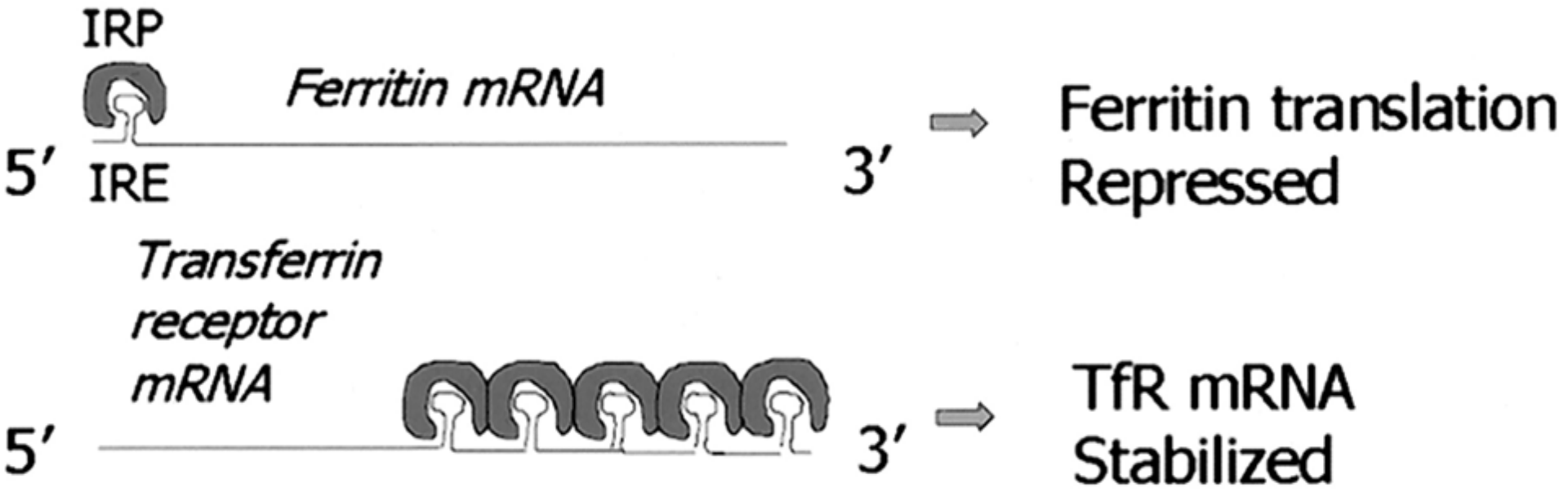
- If At 3' end of Transcription unit
  - mRNA half life prolonged
  - increased synthesis.
  - e.g. Transferin receptor

In low Iron concentration

Less Ferritin require to store iron

More Transferin Receptor require to absorb iron

Low Iron: active IRP1 and IRP2

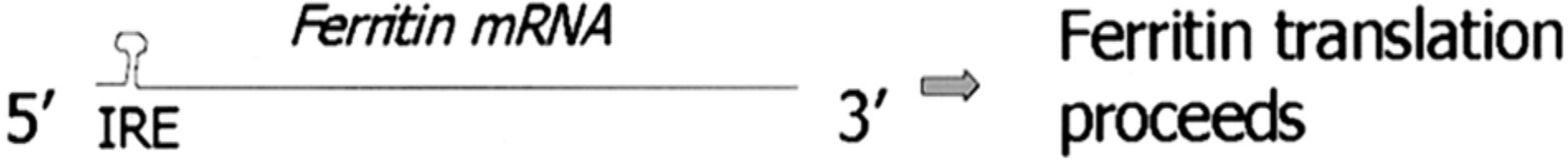


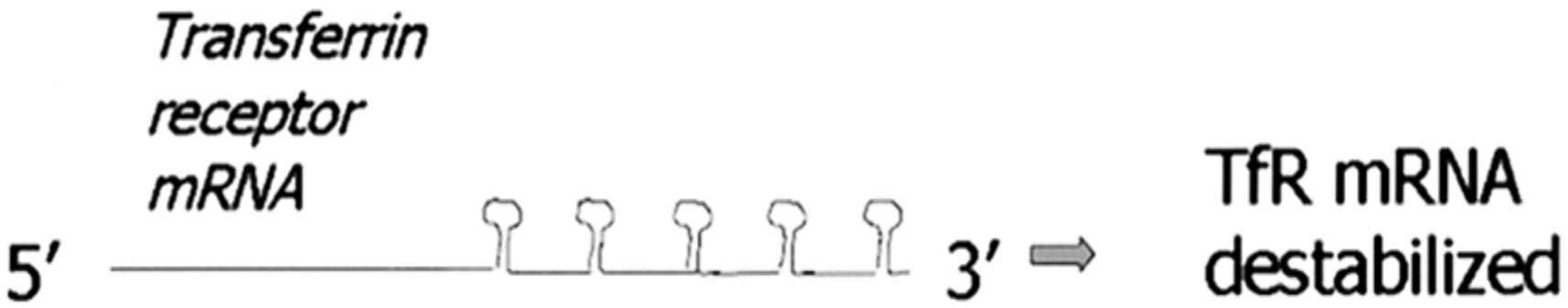
In High Iron concentration

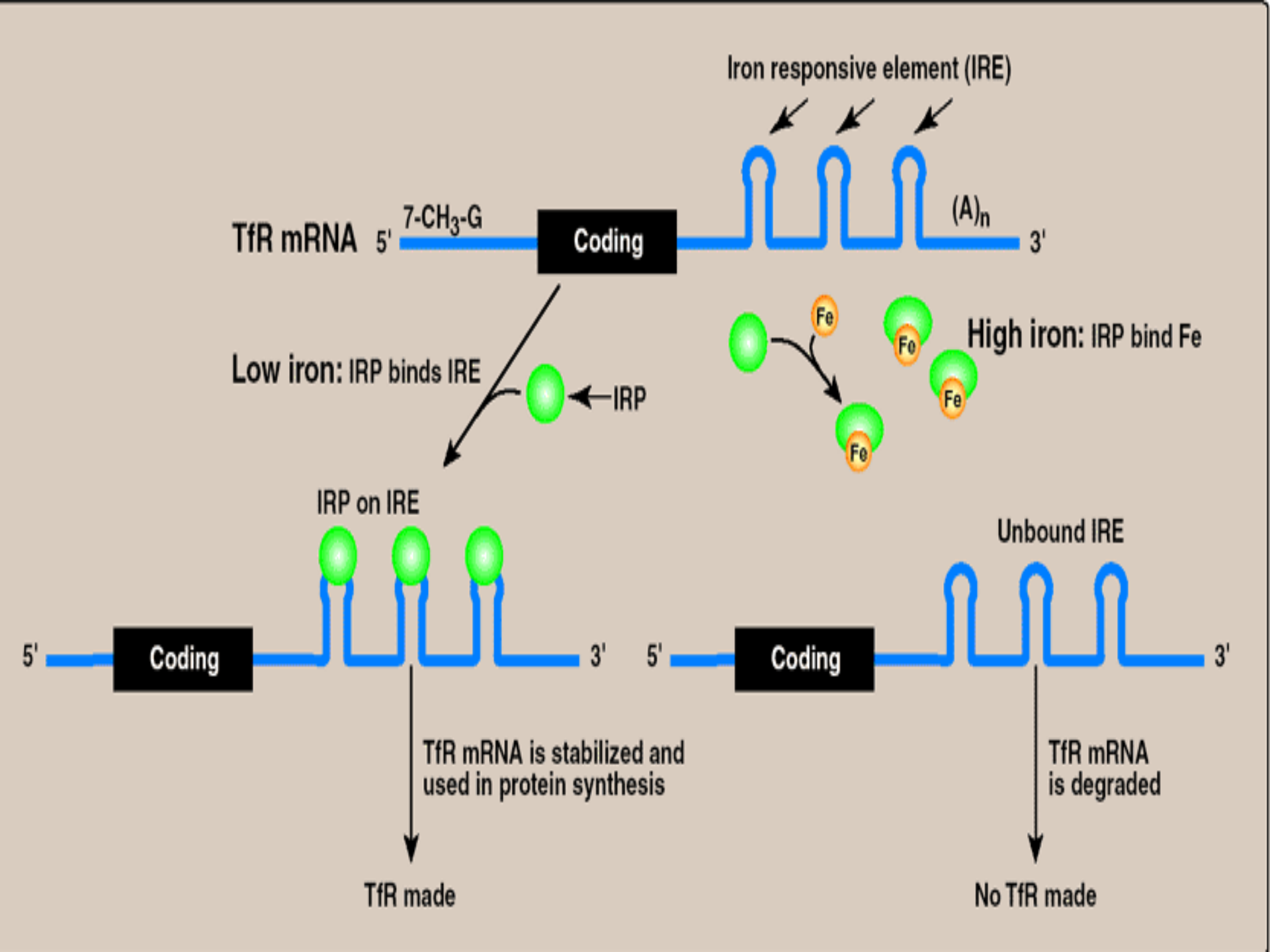
More Ferritin require to store iron

Less Transferin Receptor require to absorb iron

<u>High iron: IRP1 converted to c-acon; IRP2 degraded</u>





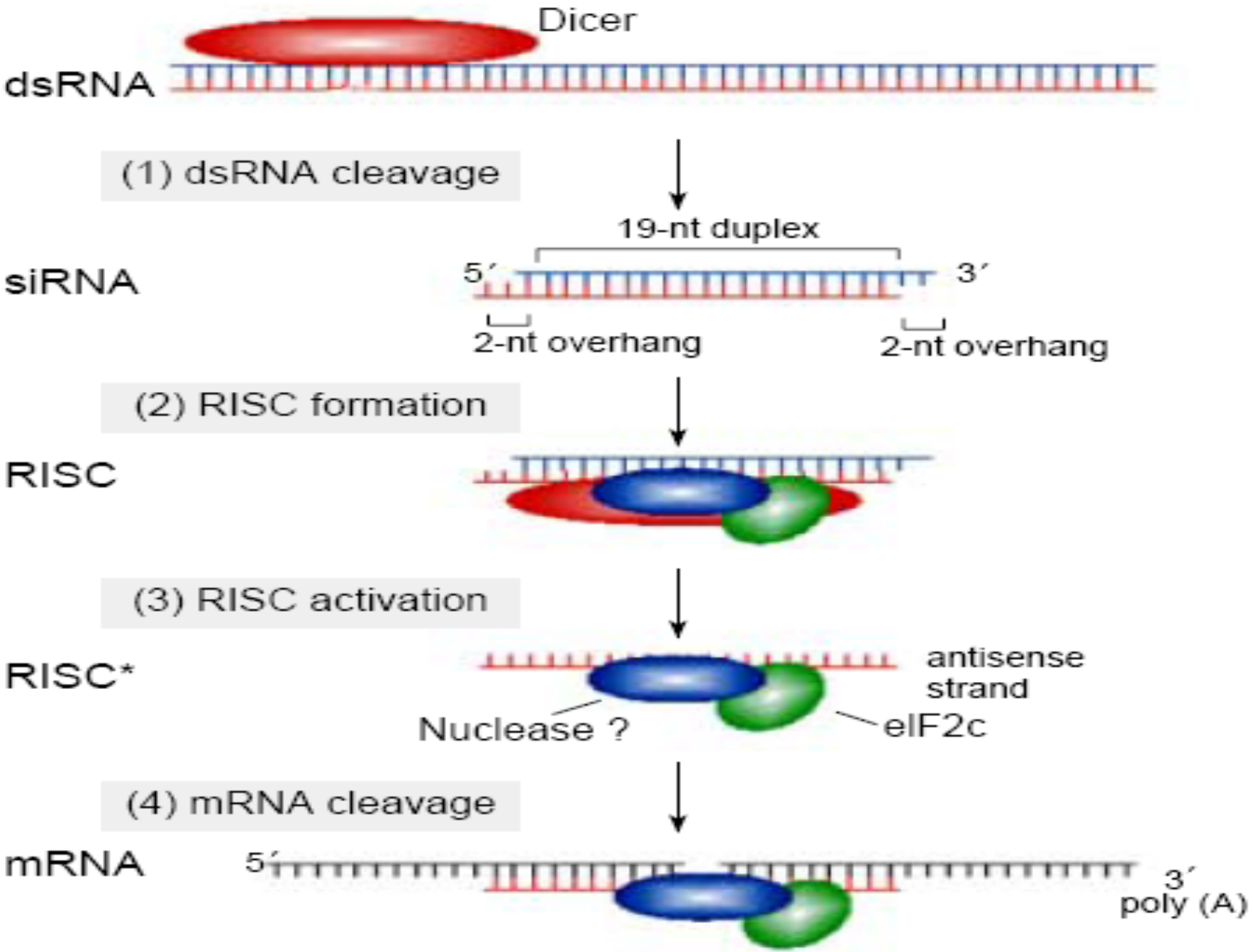

Iron responsive element (IRE)
TfR mRNA
5'
7-CH3-G
Coding
(A)n
3'
Low iron: IRP binds IRE
IRP
Fe
High iron: IRP bind Fe
IRP on IRE
Unbound IRE
5'
Coding
3'
TfR mRNA is stabilized and used in protein synthesis
TfR made
TfR mRNA is degraded
No TfR made

B. RNA interference

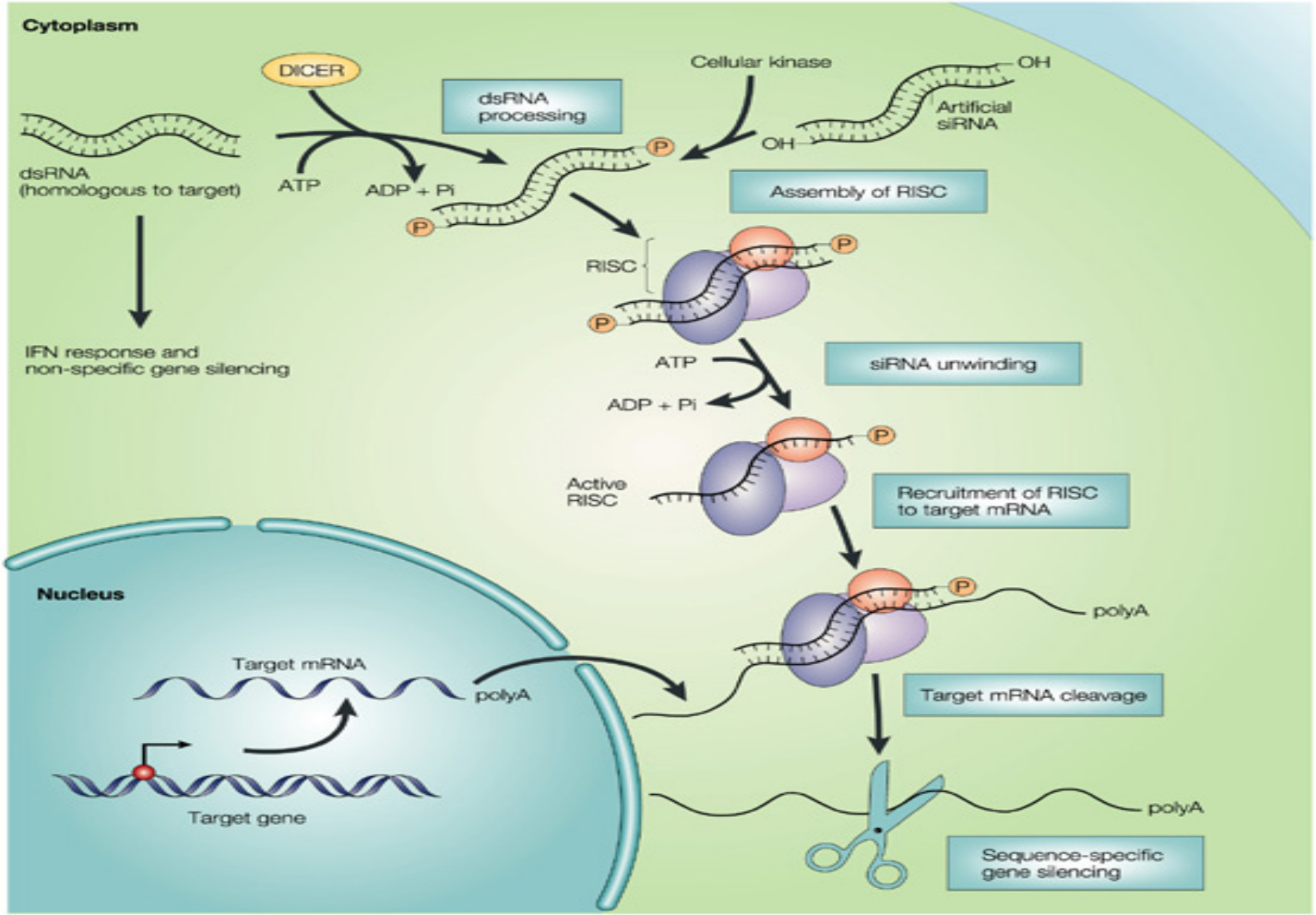
ØRNAi = RNA silencing or RNA inactivation

ØdsRNA is recognized by endonuclease (Dicer)
Øcleaved into smaller molecules of 21–24 nucleotides
ØCalled short interfering RNA (siRNA).

ØsiRNA associates with proteins
ØForm RNA-induced silencing complex or RISC.
ØRISC make complex target mRNA.
ØEndonuclease (Slicer) in the RISC degrades the target mRNA

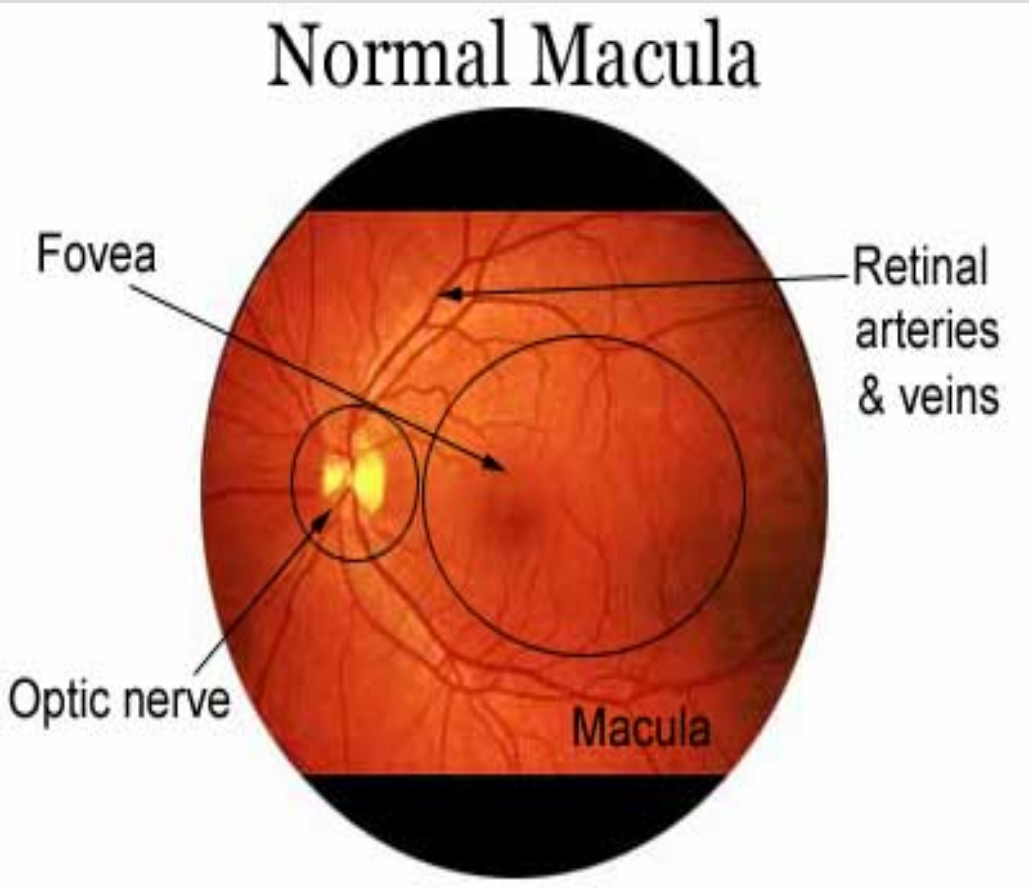

Dicer
dsRNA
(1) dsRNA cleavage
19-nt duplex
siRNA
5′
3′
2-nt overhang
2-nt overhang
(2) RISC formation
RISC
(3) RISC activation
RISC*
antisense strand
Nuclease ?
eIF2c
(4) mRNA cleavage
mRNA
5′
3′
poly (A)

- A part of the body's natural immune system evolved as a defense against retroviruses, such as HIV, that store their genetic information in dsRNA.


Cytoplasm
DICER
dsRNA processing
Cellular kinase
OH
Artificial siRNA
OH
dsRNA (homologous to target)
ATP
ADP + Pi
P
P
Assembly of RISC
RISC
P
P
IFN response and non-specific gene silencing
ATP
siRNA unwinding
ADP + Pi
P
Active RISC
Recruitment of RISC to target mRNA
Nucleus
P
polyA
Target mRNA
polyA
Target mRNA cleavage
Target gene
polyA
Sequence-specific gene silencing

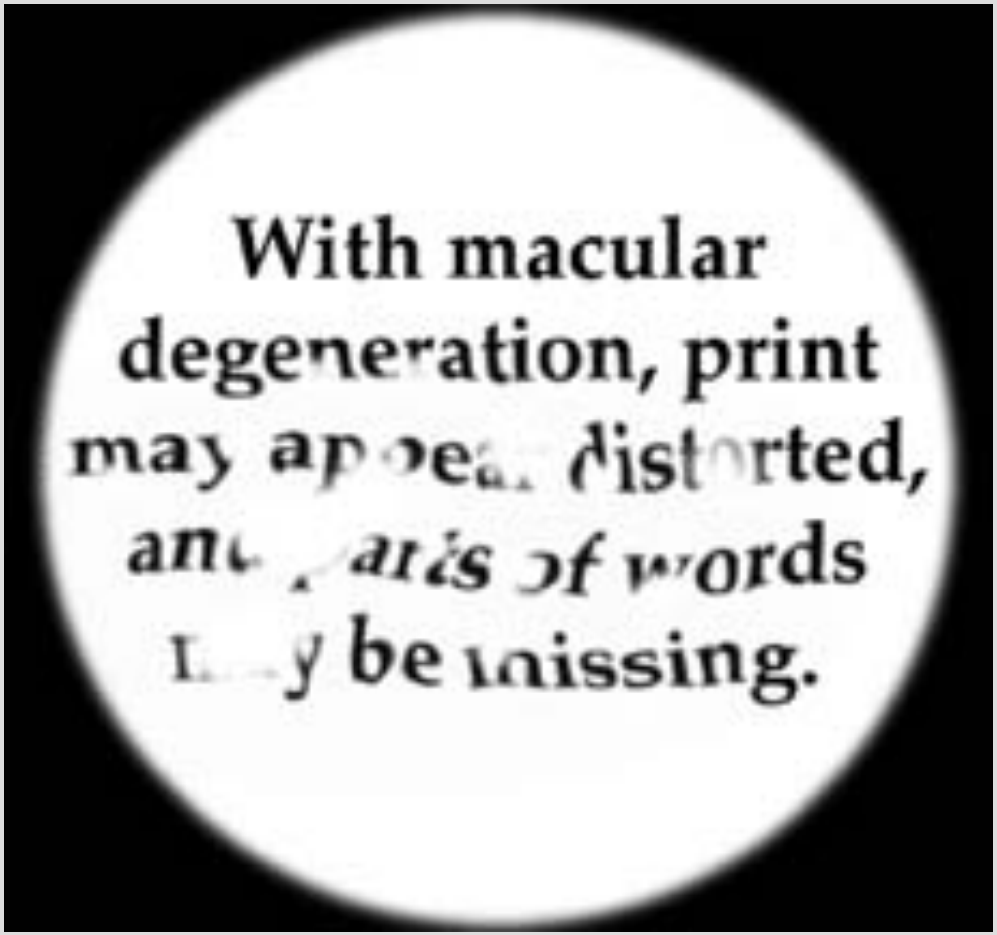
Age related Macular Degeration (AMD)

Blindness



Age Related Macular Degeneration

- Visual blurring



RNA i in AMD therapy

- Due to excess of vascular endothelial growth factor (VEGF),
- VEGF promotes blood vessel growth.
- Excess blood vessels behind the retina.

- siRNA drug—a 21-nucleotide dsRNA (injected into the eye)
- specifically targets the mRNA of VEGF.

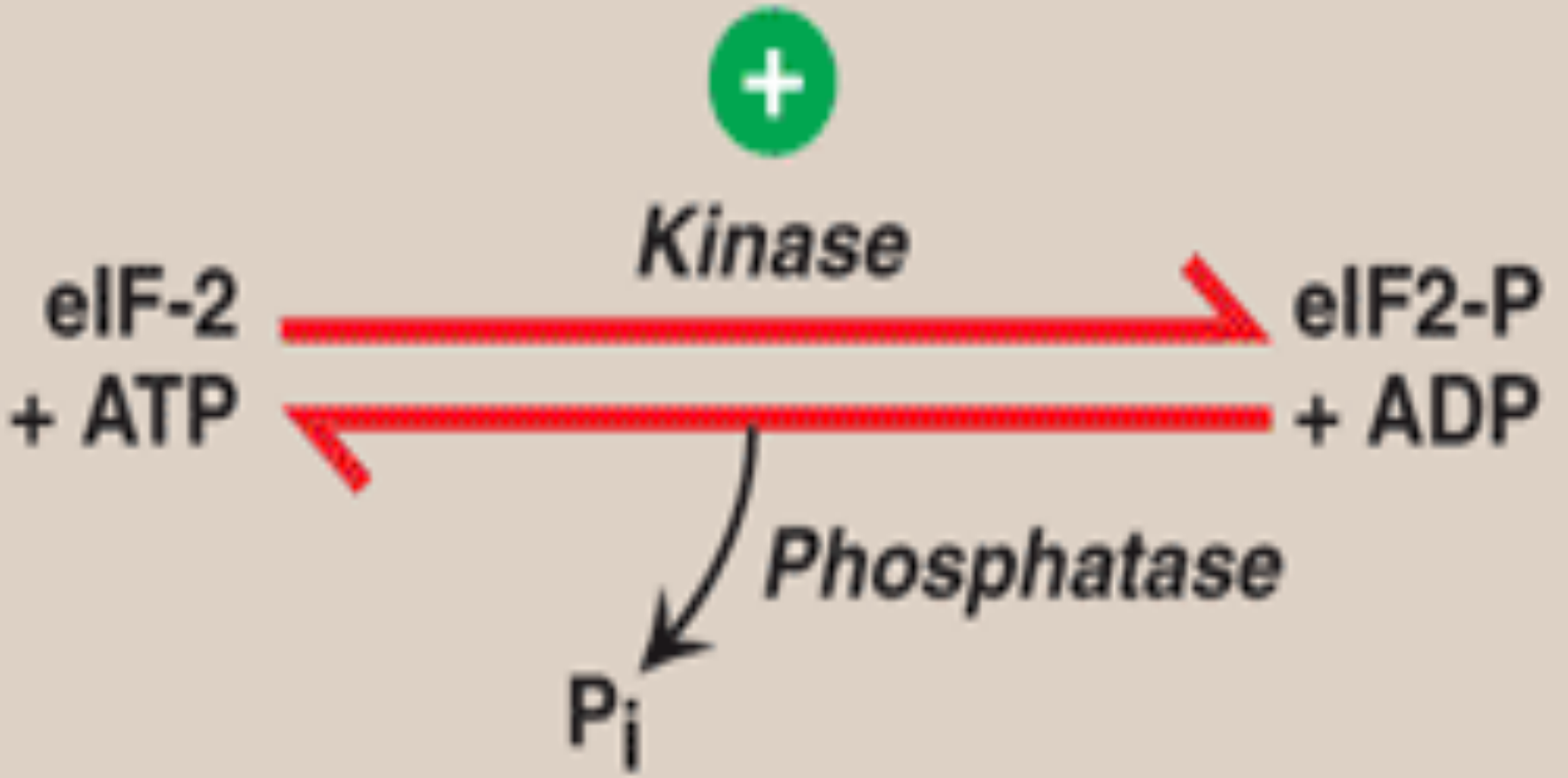
- One siRNA molecule can destroy hundreds of mRNA,
- So suppression of thousands of VEGF

C. Translation of mRNA

Translation regulated is through phosphorylation of eIF-2.
Phosphorylation of eIF-2 inhibits its function and so inhibits translation.
Phosphorylation is catalyzed by kinases
Kinase activated in response to environmental conditions

- amino acid starvation
- heme deficiency
- presence of dsRNA
- accumulation of misfolded proteins

Amino acid starvation, heme deficiency, accumulation of misfolded proteins in RER, dsRNA



D. Regulation through modifications to DNA

1. Access to DNA
2. Amount of DNA
3. Arrangement of DNA
4. Mobile DNA elements

1. Access to DNA

Euchromatin = Active =less condensed
Heterochromatin = Inactive = more condensed

In Euchromatin

Histone are Acetylation or Phosphorylation

Decrease positive charge

Decreases their association with negatively charged DNA.
Relaxes the nucleosome
Allowing transcription factors to access a specific regions of DNA

1. Access to DNA

More Methylated Cytosine at Transcriptionally inactive site

Cytosine bases in CG-rich regions

CG = upstream to many genes.

Transcriptionally active genes are less methylated (hypomethylated) .

2. Amount of DNA

More Number of copies of a gene
Increase in copy number
Increase Gene amplification
Increase Protein synthesis

Dihydrofolate reductase (DHFR)
= Synthesis of TTP.
= Pyrimidine biosynthetic pathway
Methotrexxate = inhibitor of DHFR
TTP is essential for DNA synthesis. Of DNA

DHFR Gene amplification in response to Methotrexate.

DHFR Gene amplification
Increase in number of DHFR gene
Increase concentration of DHFR
Resistance to the drug
Pyrimidine & DNA synthesis does not get inhibited
Chemotherapy FAIL.

3. Arrangement of DNA:

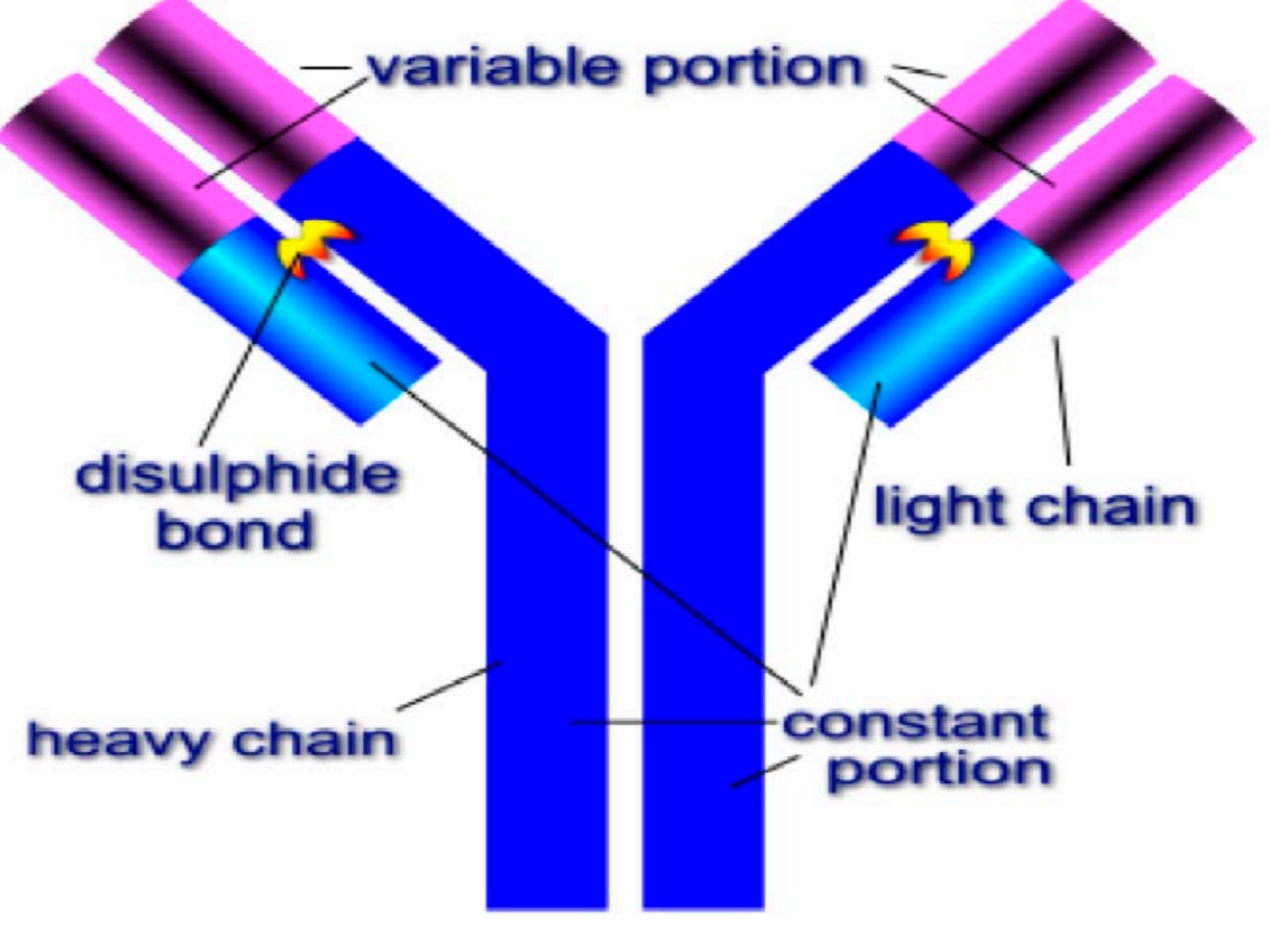
IgG = 2 Light & 2 heavy chains

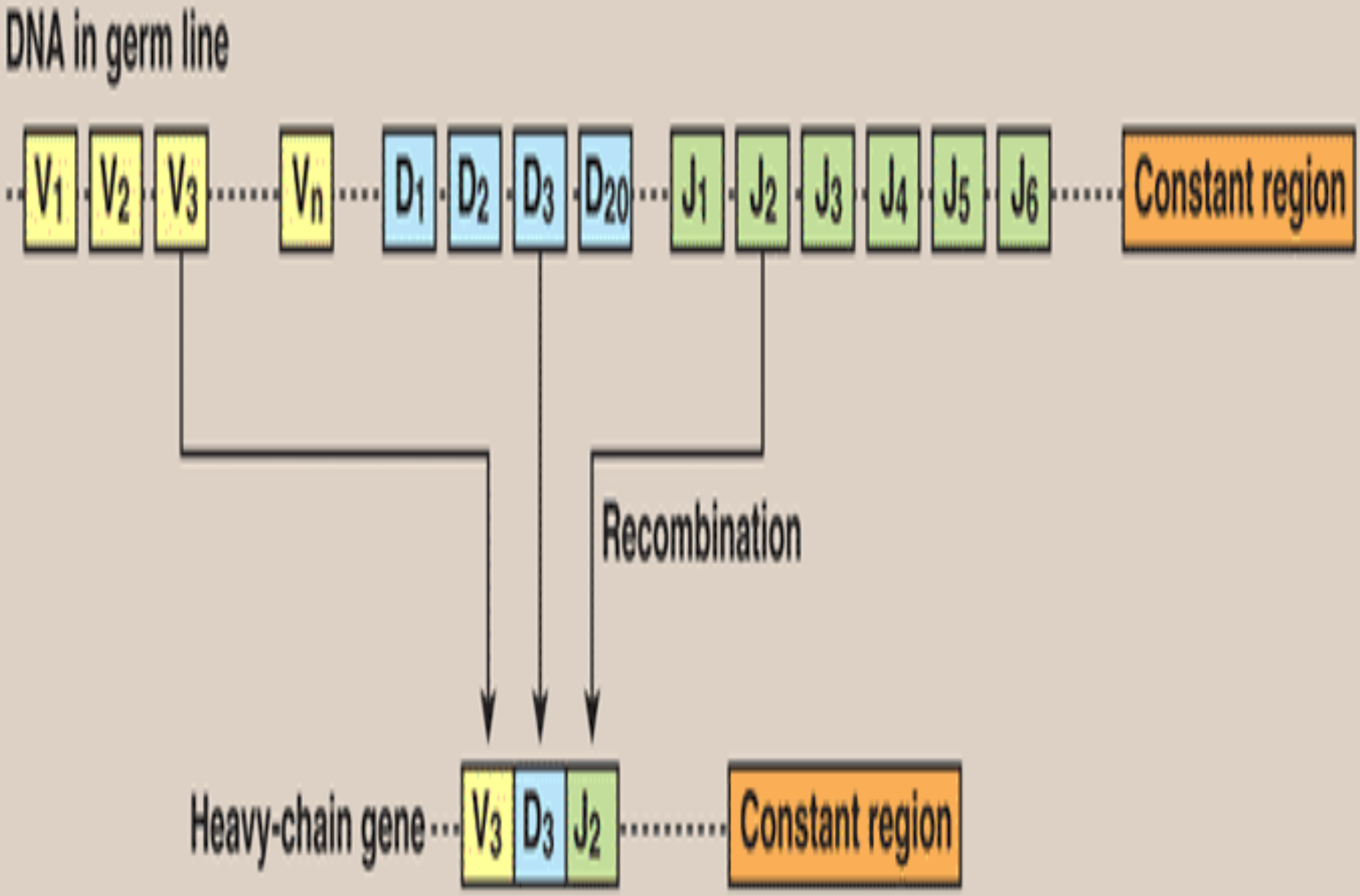
= Variable & Constant amino acid sequence.

B-lymphocyte development recombination of gene

- **Variable (V) gene**
- **Diversity (D) gene**
- **joining (J) gene**

Providing diversity needed for the recognition of different antigens.


variable portion
disulphide bond
light chain
heavy chain
constant portion


DNA in germ line
V1
V2
V3
Vn
D1
D2
D3
D20
J1
J2
J3
J4
J5
J6
Constant region
Recombination
Heavy-chain gene
V3
D3
J2
Constant region

4. Mobile DNA elements

Transposons (Tn) = Mobile segments of DNA

Move Randomly from one site to another on the same or a different chromosome.

Transposase cuts out and then inserts the Tn at a new site.

Some time Tn is replicative

Transposon is copied

And copy inserted elsewhere

while the original remains in place.

If RNA involve in = Retrotransposon.

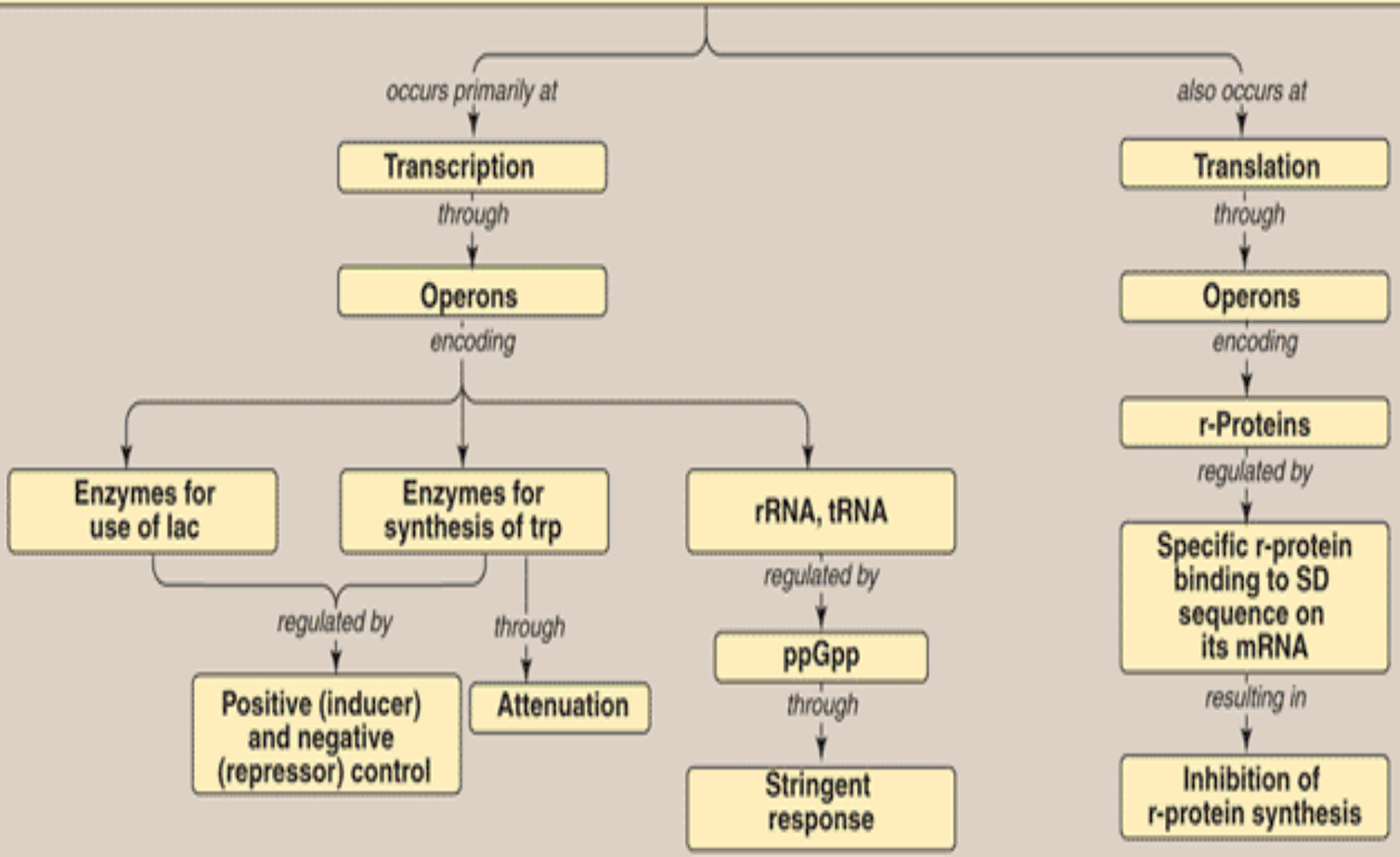
Expanded genome = Alter gene expression and even to cause disease.

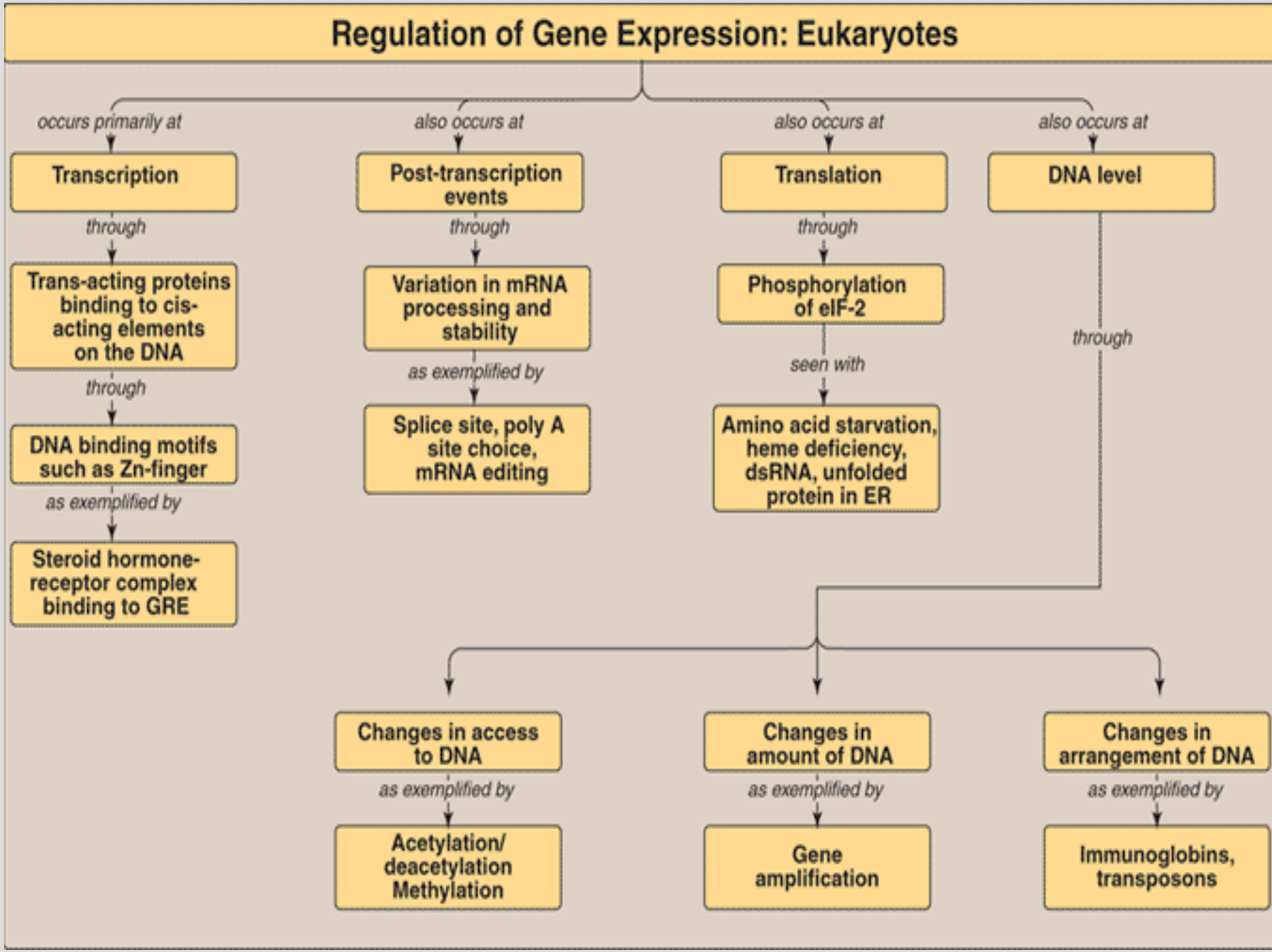
ast majority of retrotransposons in the human genome have lost the ability to move, a small percentage is still active.

4. Mobile DNA elements Basis in

Hemophilia A
Duchenne muscular dystrophy.
Antibiotic-resistant bacteria , of the exchange of plasmids among bacterial cells.
If the plasmids contain transposons carrying antibiotic resistance genes, the recipient bacteria gain resistance to one or more antimicrobial drugs.

Regulation of Gene Expression: Prokaryotes




Regulation of Gene Expression: Eukaryotes
occurs primarily at
Transcription
through
Trans-acting proteins binding to cis-acting elements on the DNA
through
DNA binding motifs such as Zn-finger
as exemplified by
Steroid hormone-receptor complex binding to GRE
also occurs at
Post-transcription events
through
Variation in mRNA processing and stability
as exemplified by
Splice site, poly A site choice, mRNA editing
also occurs at
Translation
through
Phosphorylation of eIF-2
seen with
Amino acid starvation, heme deficiency, dsRNA, unfolded protein in ER
also occurs at
DNA level
through
Changes in access to DNA
as exemplified by
Acetylation/ deacetylation Methylation
Changes in amount of DNA
as exemplified by
Gene amplification
Changes in arrangement of DNA
as exemplified by
Immunoglobins, transposons