Regulation of gene expression

Premedical - Biology

Regulation of gene expression

in prokaryotic cell

- **Operon** units with genes for enzymes, proteins of one metabolic pathway
- system of negative feedback
- positive and negative regulation

in eukaryotic cell

- at any stage of gene expression and proteosynthesis.
- Non-coding RNAs play roles in the regulation.

Transcription and translation

In bacterial cells:

Transcription and translation is **coupled.**

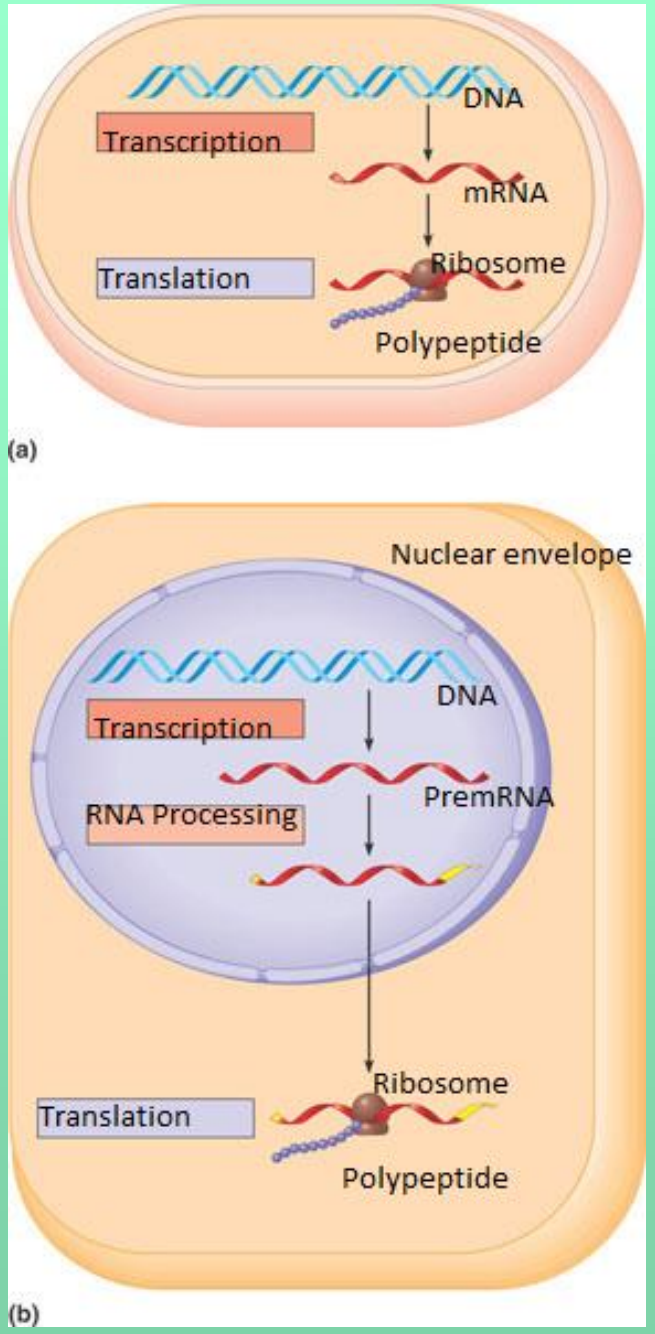
mRNA is immediately translated without processing.

In Eukaryotic cell

The nucleus provides a separate compartment for a transcription.

The pre-mRNA is processed in various ways before leaving nucleus as mRNA

Translation of eukaryotes occurs in **cytoplasm.**



Regulation of gene expression

in Prokaryotes = Operon model

Operon = **functional, transcription and regulatory unit**

- contains **cluster of genes (**for **enzymes of the particular metabolic pathway)**, which are transcribed into **one mRNA *(= polycistronic*** *transcript)*
- They are regulated by **common promotor**
- Prokaryotic genes have no introns (non-coding parts)

Escherichia coli

Lac operon, Trp operon – model systems = metabolic pathways of

- **utilization of lactose** gen lacZ, lacY, lacA**,**

catabolic pathway with negative and positive regulation

- **enzymes for TRP synthesis,** anabolic pathway with negative regulation

Operon

Gene expression – activation or inhibition of transcription is regulated in respond to conditions in environment: presence of substrates or products of metabolic pathways = **effector molecules**

Effector molecules react with regulatory proteins (regulatory gene products) and their complexes activate or repress the transcription.

Regulatory proteins: Activators – positive control

Repressor – negative control

Effector molecules: inducers (induction)

corepressors (repression)

Positive and negative regulation of gene expression

Positive = product of regulatory gene, activator, activates transcription of structural genes protein

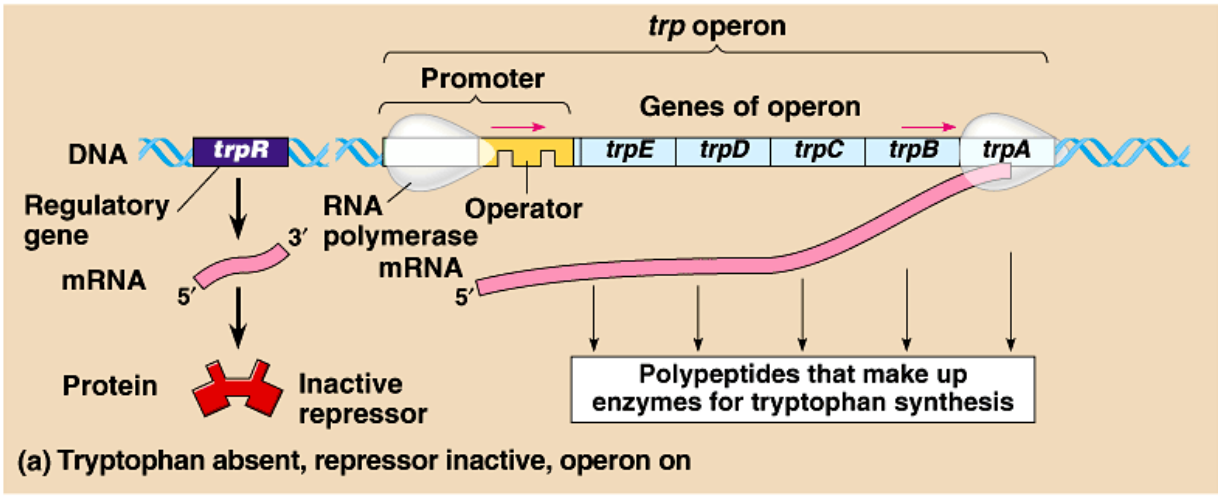
Negative = product of regulatory gene, repressor, suppresses expression of structural genes

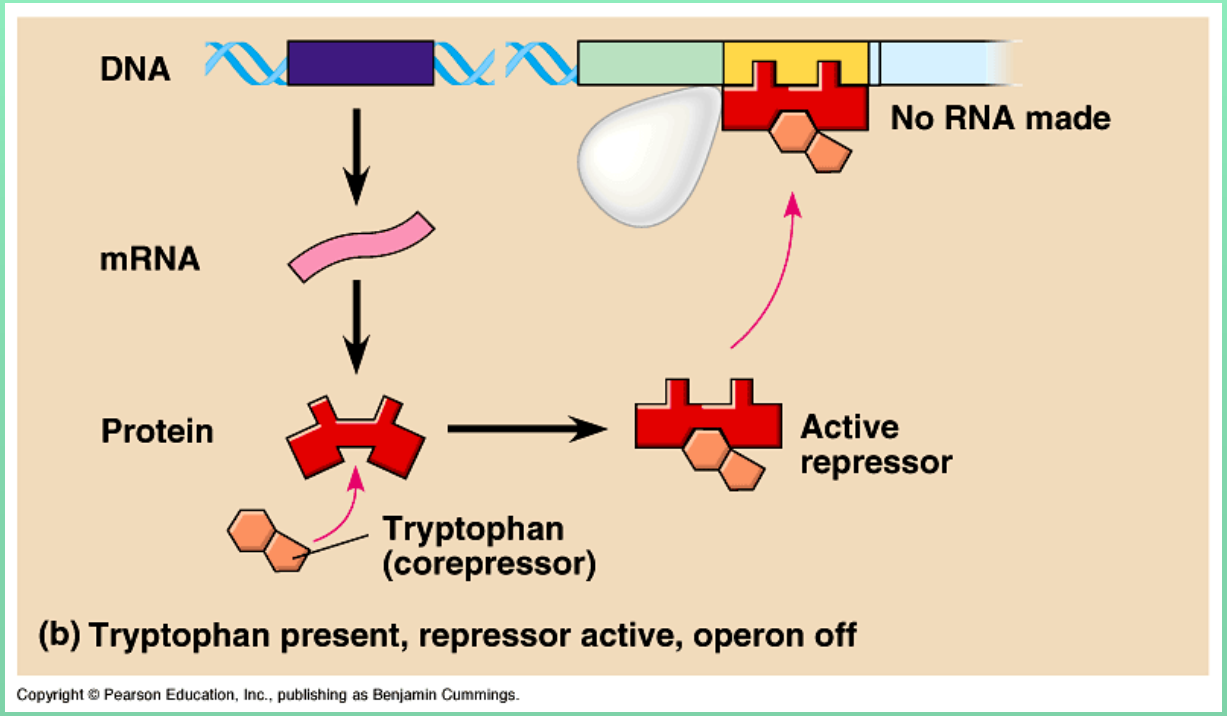
Effector molecules: the bond between them and regulatory proteins changes their ability to bind to the operator

inducer = **molecule of substrate, which** binds to active repressor to block it up = transcription is possible

corepressor = **the bond between corepressor and product of metabolic reaction** inhibits the transcription *(inactive repressor alone is not able to suppres transcription)*

Tryptophan operon





Repressor

Tryptofan -

corepressor

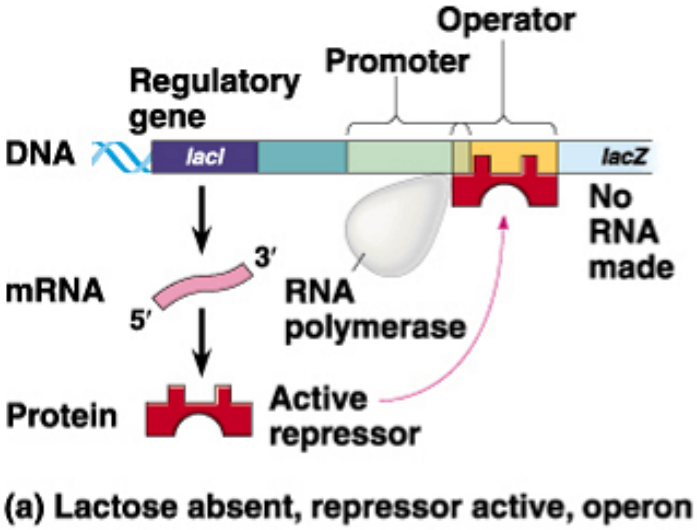
RNAP

Lac operon - negative regulation

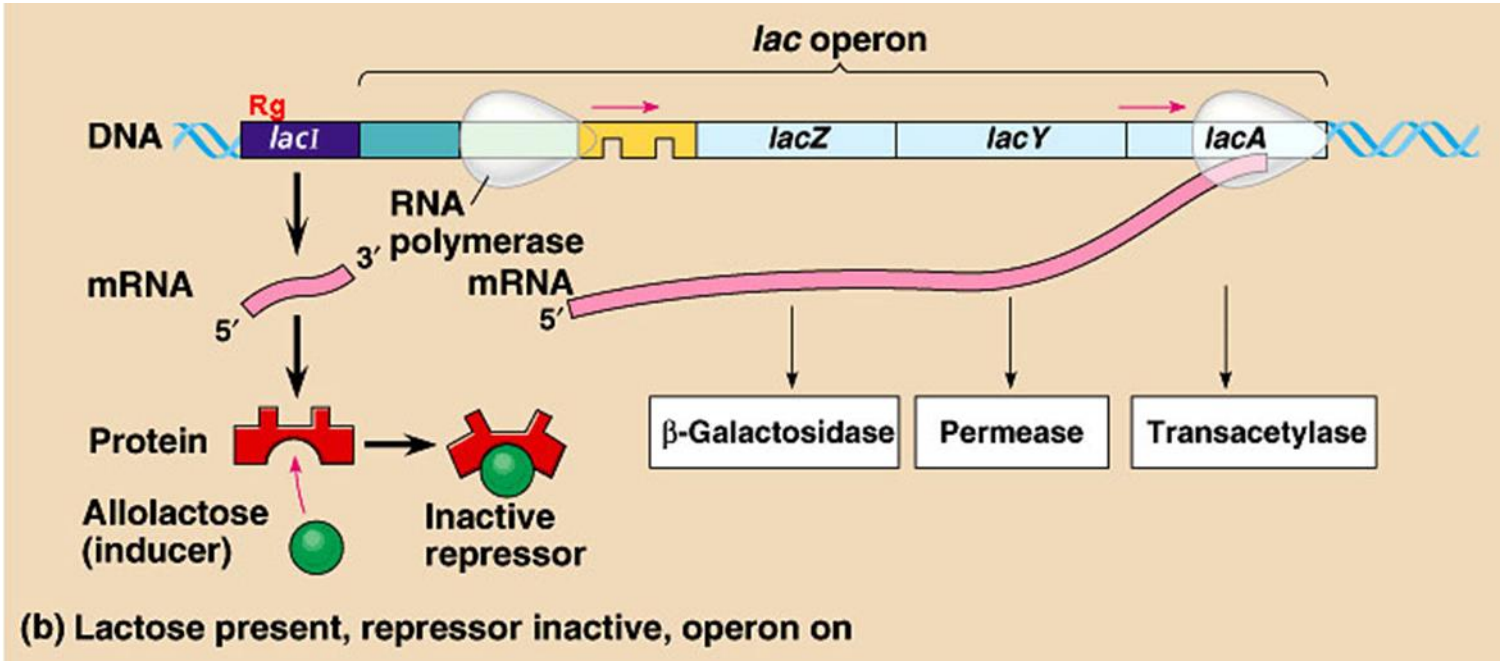
Repressor

Lactose - inducer

RNAP



(a) Lactose absent, repressor active, operon off



(b) Lactose present, repressor inactive, operon on

Lac operon - negative regulation

means the presence of **repressor, which is in active state and binds operator and** causes that **RNAP** is not able to **initialize transcription.**

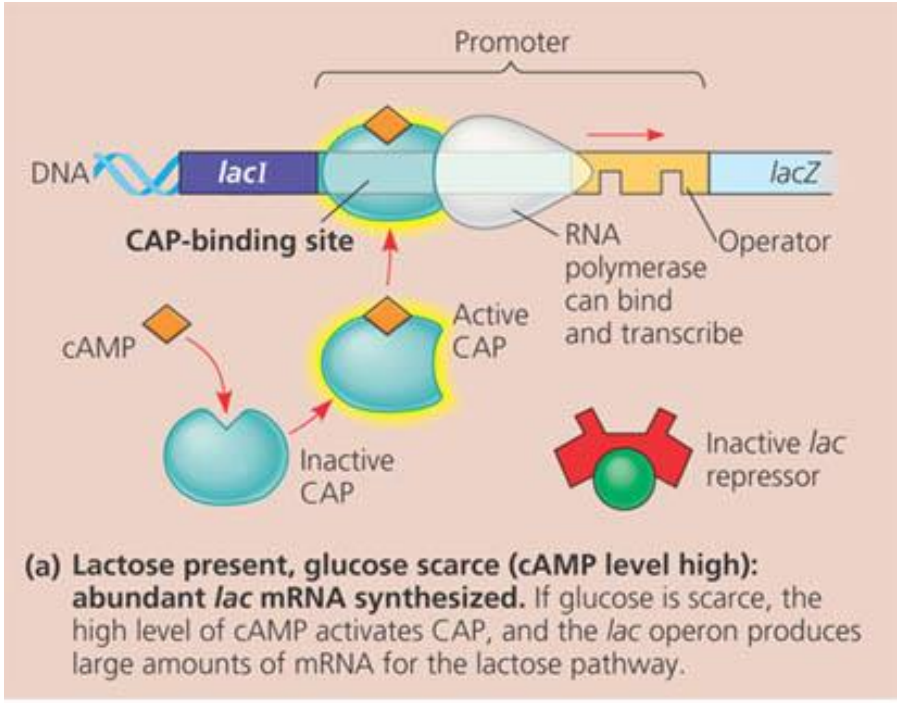
Effector molecule is **lactose** (allolactose), which works as **an inducer.**

It binds a repressor and turns it into inactive state.

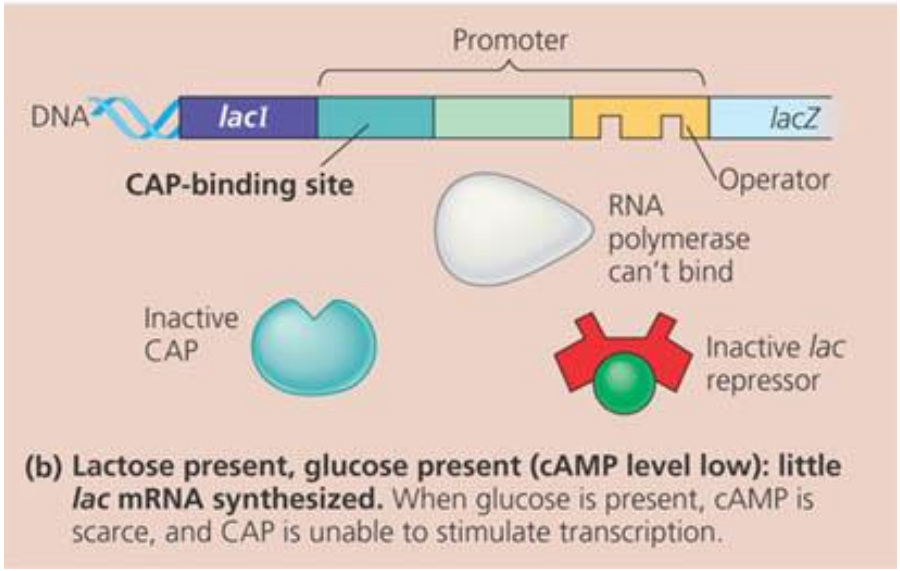
Lactose (allolactose) cause induction (activation) of transcription.

RNA polymerase starts the transcription. In 2-3 minutes the amount of enzymes is increased 1000x

Lac operon - positive regulation



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized. If glucose is scarce, the high level of cAMP activates CAP, and the *lac* operon produces large amounts of mRNA for the lactose pathway.



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized. When glucose is present, cAMP is scarce, and CAP is unable to stimulate transcription.

CAP – Catabolite activator protein

(cAMP receptor protein-CRP)

cAMP - inducer

RNAP

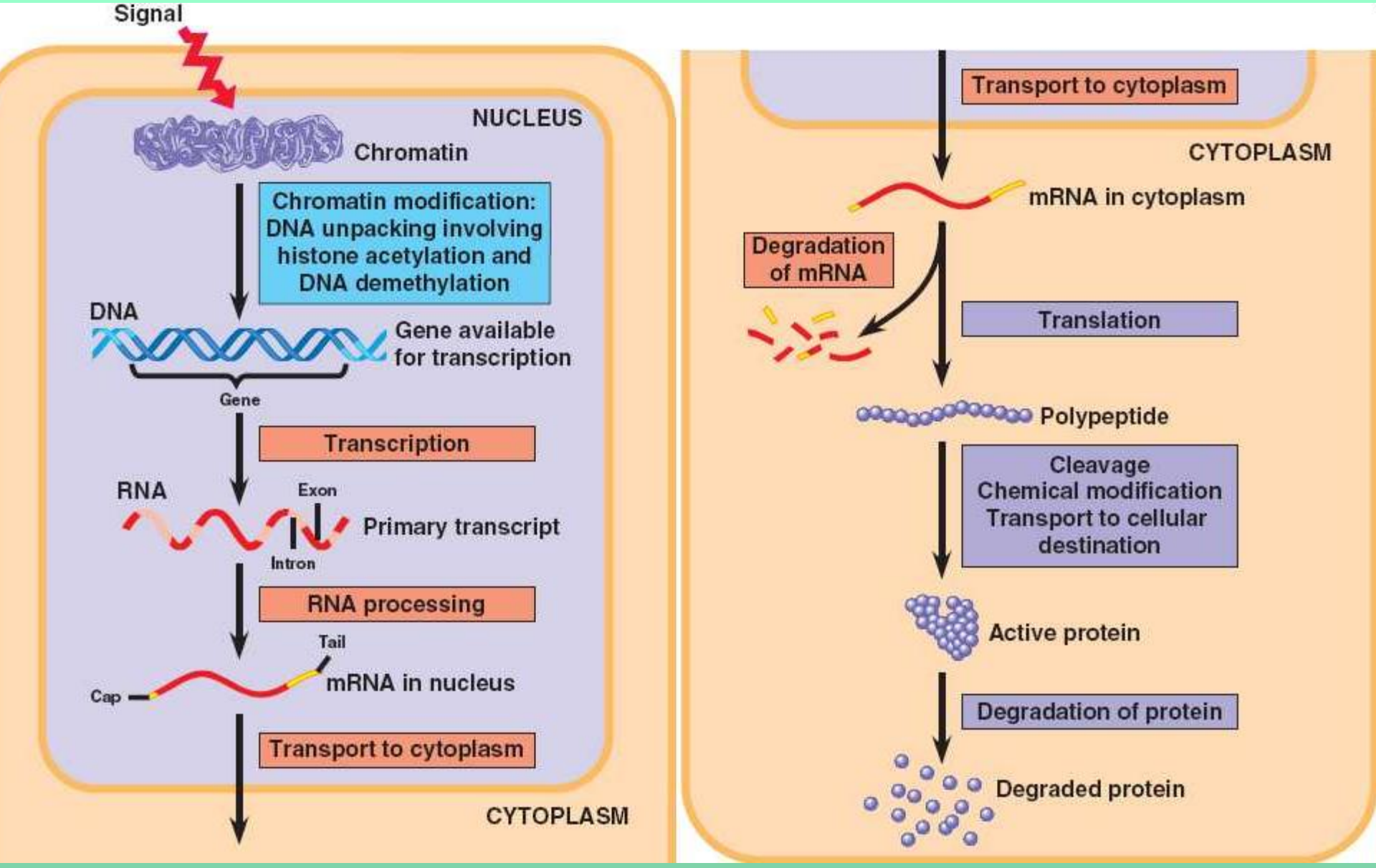
Lac operon - positive regulation

- In the presence of glucose, *E. coli* preferentially uses the glucose for a production of energy.
- If the level of glucose is low, the level of cAMP is high.
- **CAP „Catabolite activator protein“ in the presence of cAMP** attaches the promotor **and allows the RNAP to start transcription.**
- CAP is allosteric regulatory protein;
  it is activated by cAMP = inducer

Gene expression of eukaryotic cells

- **each cell maintains specific program/ differential gene expression**
- **one mRNA carries information for one gene (monocistronic mRNA)**
- **post-transcriptional modifications of RNA, RNA processing and splicing**
- **regulation system is performed at several levels = transcription, translation, protein activation + secretion**

Levels of regulation of gene expression in euk. cell



Disturbance of regulatory system

- chromatin changes
- transcription
- processing RNA
- transport to cytoplasm
- degradation of mRNA
- translation
- cleavage, chemical modification
- protein degradation

1. Chromatin changes

Role of DNA methylation and histone modification:

- **In active chromatin – DNA (promoter) is demethylated, histones are acetylated**
- **In inactive chromatin - DNA is methylated (promoter) and histone de-acetylated**

- **Histone acetylation** removes positive charge of histones – thus reducing the force of attraction with electronegative DNA = open chromatin (active)
- **Deacetylation of histones** restores positive charge of histones leading to close attraction between histones and DNA and to condensed chromatin structure = inactive = i.e. inaccessible to transcription factors
- **First step in gene inactivation = methylation of promoter attracts complex containing histone deacetylase - it starts gene inactivation**

Epigenetic regulation of gene expression

- **DNA methylation, histone modification**

  Heterochromatin (inactive chromatin) is highly methylated

  DNA methylation is essential for long-term inactivation of genes during cell differentiation

  Steps of gene inactivation: methylation of promoter, deacetylation of histones, rearrangement of chromatin to inactive state – inaccessible to transcription factors

- Role of **non-coding RNAs** in posttranscriptional regulation of gene expression (destroying mRNA before translation)

➢ **Gene imprinting** = certain genes are expressed in a parent-of-origin-specific manner - in mammals

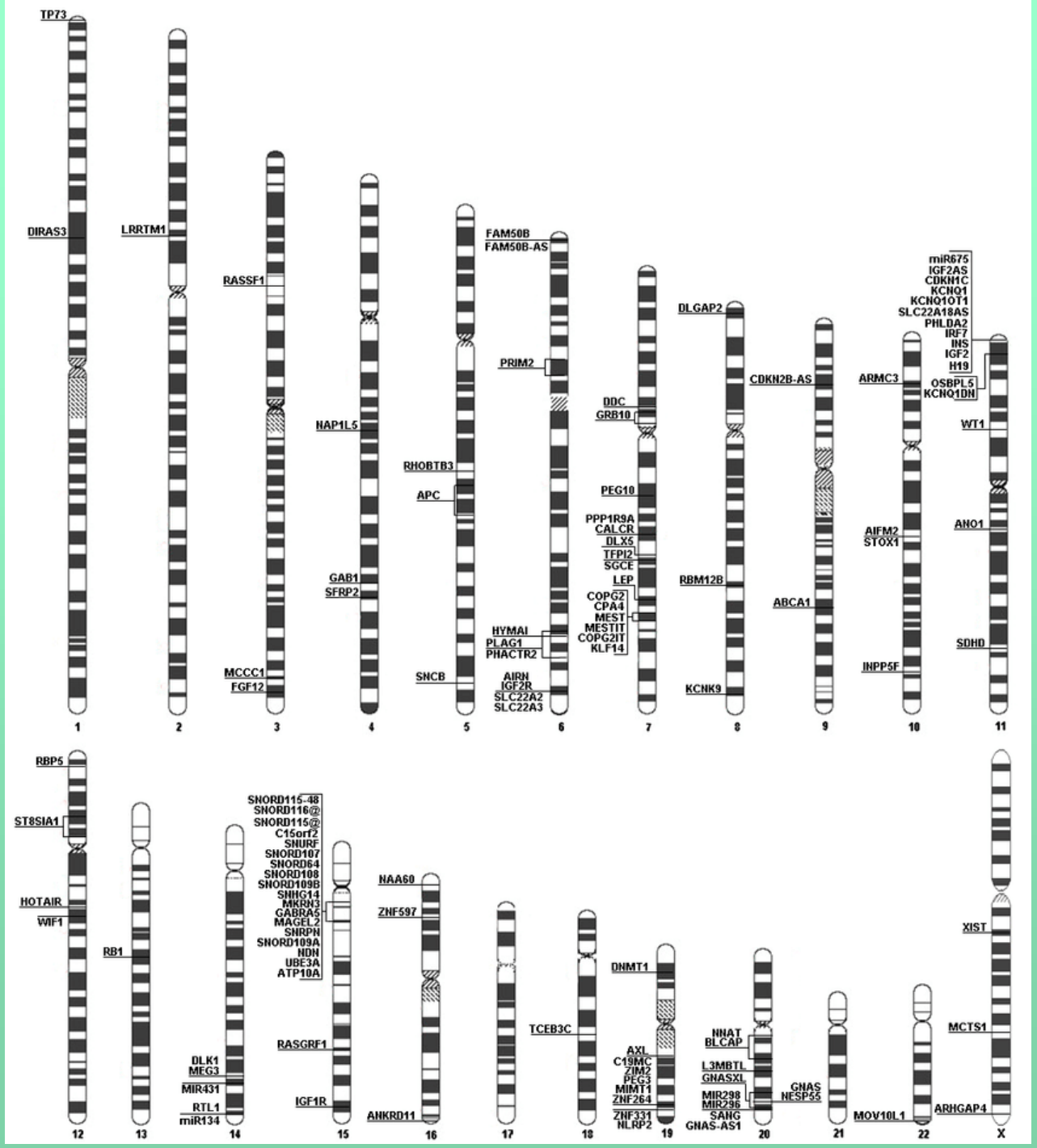
= only one allele of specific parental origin is active, second one is inactive = imprinted

Imprinting is connected with promoter methylation, histone modification and chromatin rearrangement to inactive state

Imprinted genes in early human embryogenesis:

paternally expressed genes *are responsible for placental proliferation and invasiveness*

maternally expressed genes *are responsible for development of embryo*



one hundred and twenty (120) human imprinted genes (confirmed by experimental evidences)

2. Transcription

Transcription factors = **positive and negative** regulation of transcription of eukaryotic genes (to facilitate or inhibit)

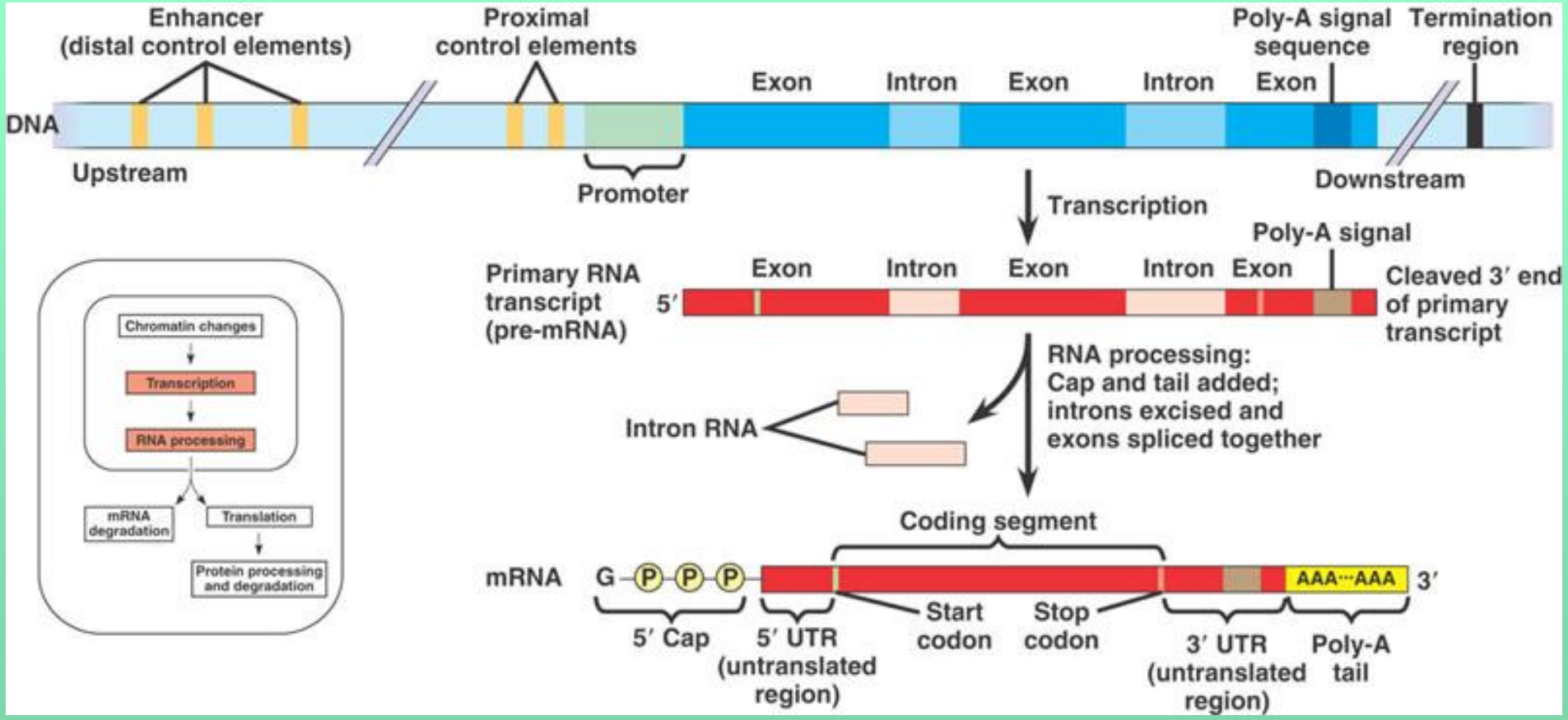
Transcription factors:

general transcription factors for all protein-coding genes

specific transcription factors – transcription of particular genes at specific time and place (in certain cell types or in response to signals)

promoter, **enhancers** and **silencers** **= regulatory DNA elements** *(**outside** or inside gene)*

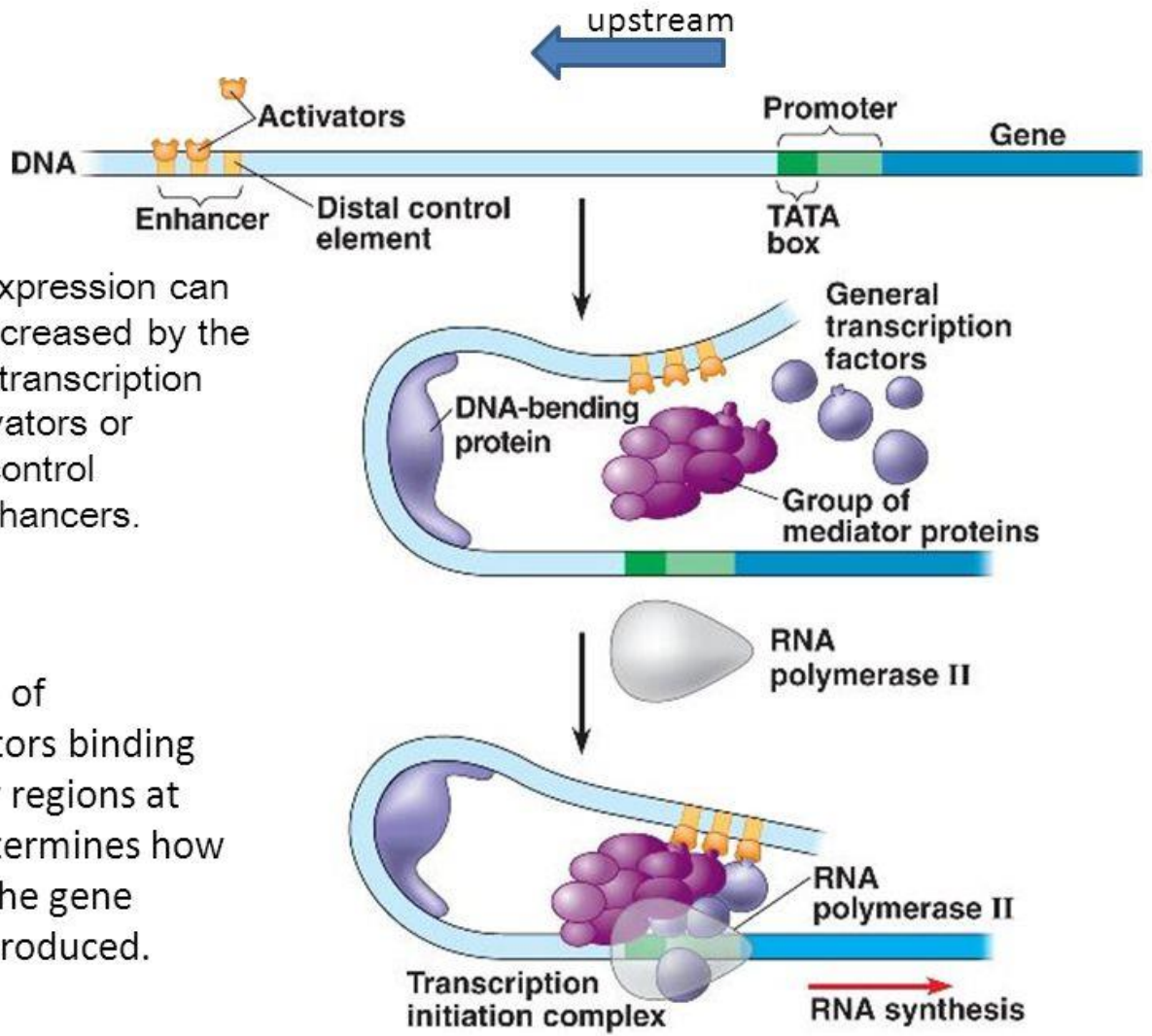
Eukaryotic gene and transcript



Action of enhancers and transcription activators

The rate of gene expression can be increased or decreased by the binding of specific transcription factors, either activators or repressors to the control elements of the enhancers.

The combination of transcription factors binding to the regulatory regions at any one time determines how much, if any, of the gene product will be produced.



Cell-type specific transcription:

Genes encoding enzymes of one metabolic pathway are scattered over the different chromosomes –

- **coordinated control in response of chemical signals** from outside environment.
- The cell accepts signals by receptors.

Signal transduction pathways activate transcription activators or repressors.

3. RNA processing

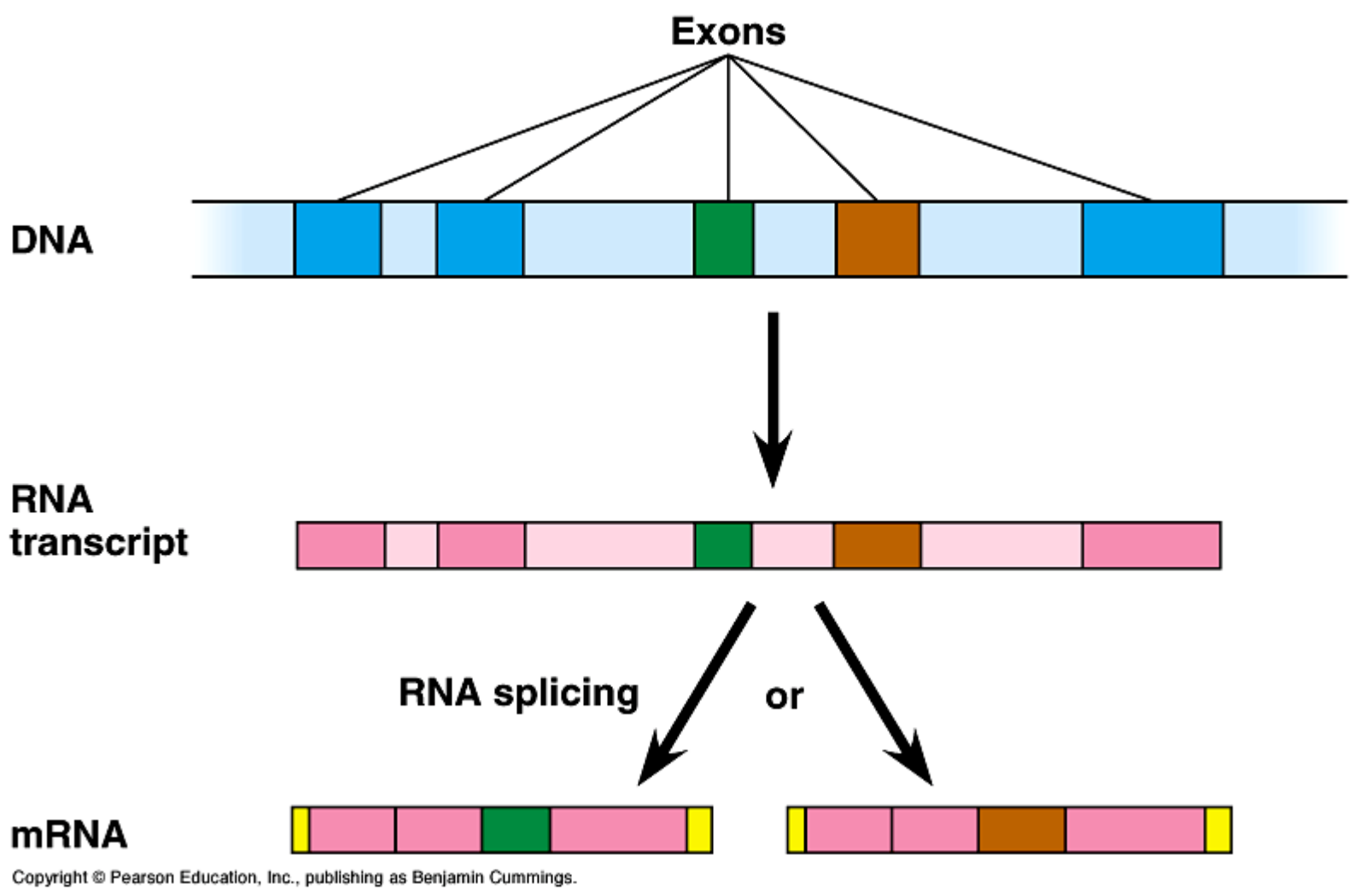
Post-transcriptional modifications:

Splicing and alternative splicing

= the same primary RNA transcript, but different mRNA molecule from it (exons are either retained in the mRNA or targeted for removal in different combinations to create a diverse array of mRNAs)

By **alternative splicing** we can make more than one polypeptide from one gene

Alternative RNA splicing



For example, the 5' **AMP-activated protein kinase (AMPK)**, an enzyme, which performs different roles in human cells, has 3 subunits:

α, catalytic domain, has two isoforms: **α1 and α2** which are encoded from PRKAA1 and PRKAA2

β, regulatory domain, has two isoforms: **β1 and β2** which are encoded from PRKAB1 and PRKAB2

γ, regulatory domain, has three isoforms: **γ1, γ2, and γ3** which are encoded from PRKAG1, PRKAG2, and PRKAG3

In human skeletal muscle, the preferred form is α2β2γ1.
But in the human liver, the most abundant form is α1β2γ1.

Posttranscriptional regulation of gene expression:

some **microRNAs =** small non-coding regulatory RNAs that can cut or can block mRNA to be translated

miRNAs interact with specific mRNAs to influence the translation or stability of the target mRNA = **epigenetic mechanism**

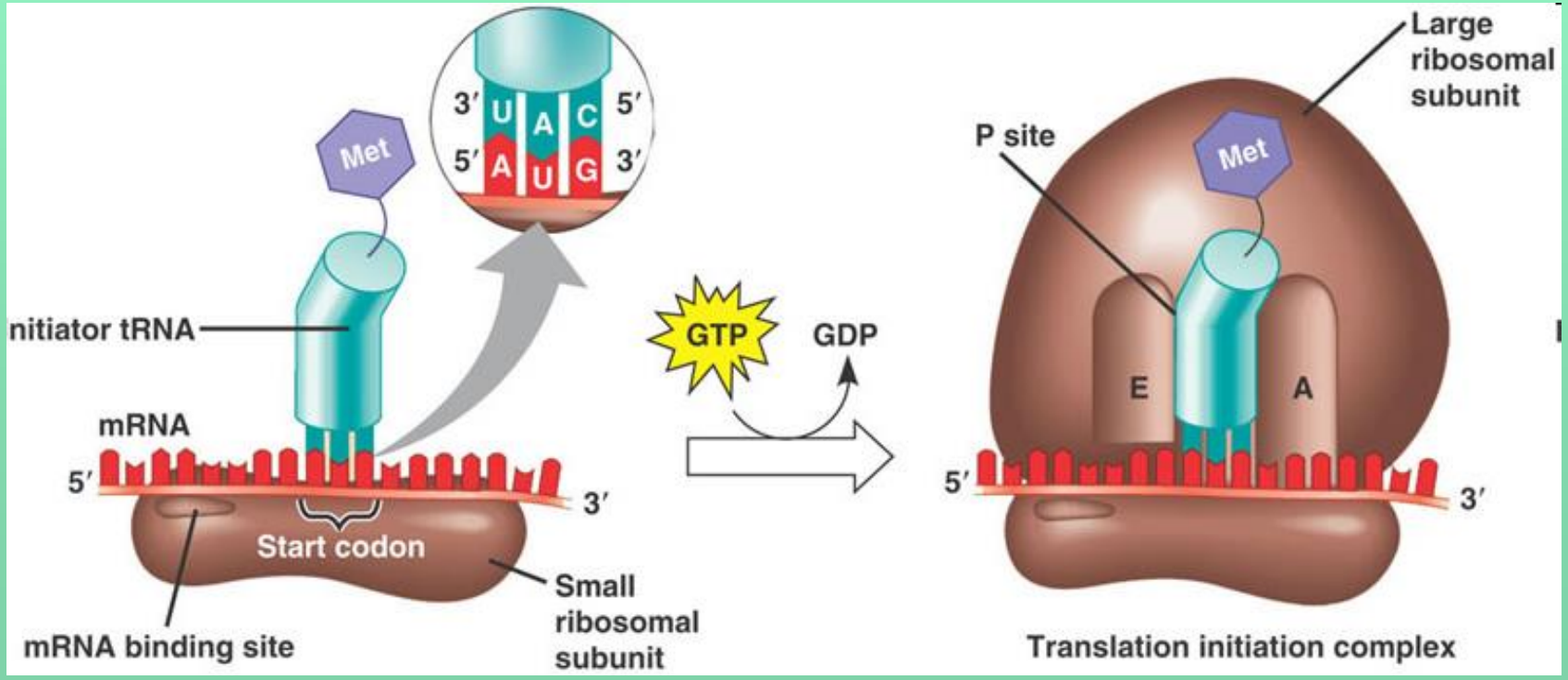
4, 5. transport of mRNA / degradation

A lifespan of mRNA is important for protein synthesis

6. Translation

At the initiation stage – regulatory proteins bind the 5’ end of mRNA with the cap.

Activation or inactivation of protein factors to initiate translation



7. Cleavage, chemical modifications

Post-translational modifications of proteins

= **chemical changes** of proteins after translation,

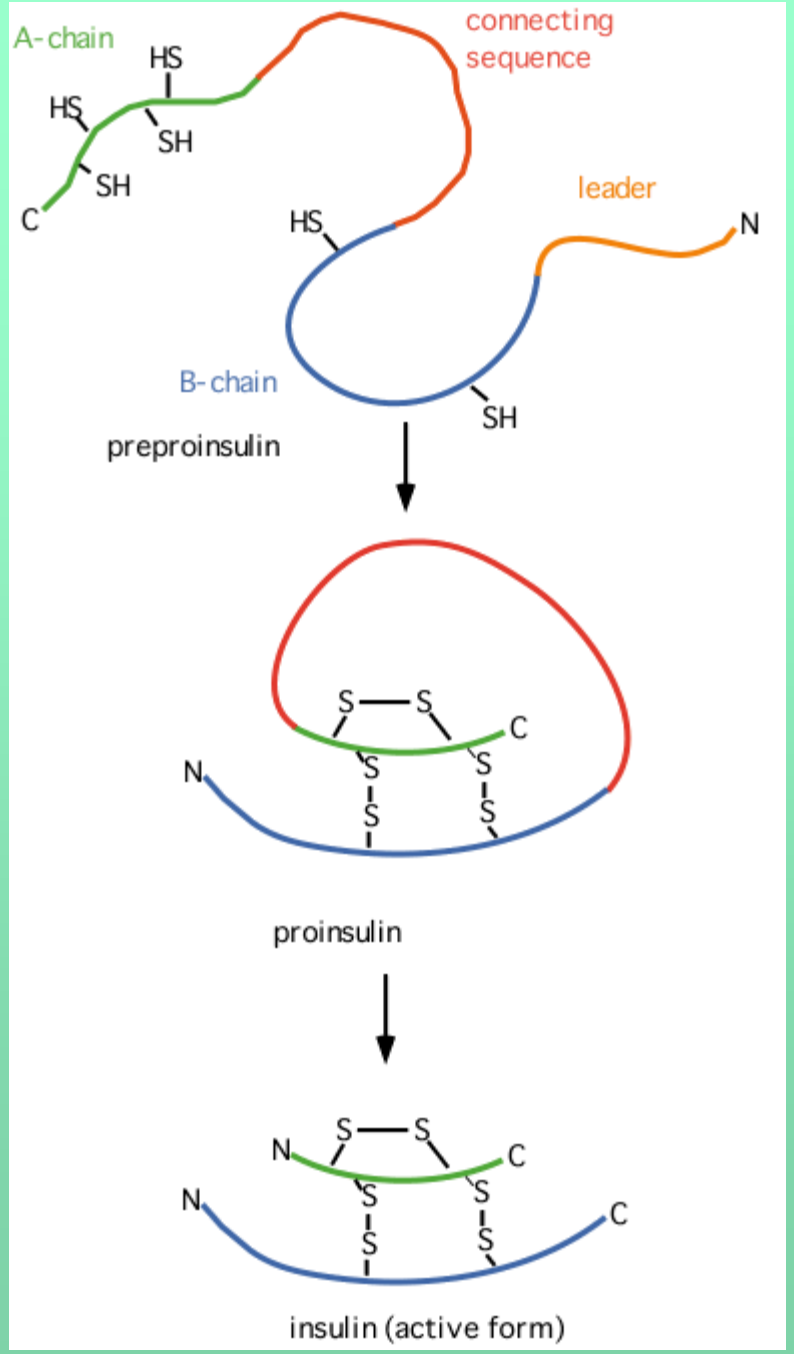
proteolytic **cleavage** of regulatory subunits,

or **degradation** of entire proteins

Chemical modifications:

phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation and proteolysis

→ regulation of activity, localization, targeting and interaction with other cellular molecules such as proteins, nucleic acids, lipids…



Cleavage of polypeptide

- Polypeptide chain may be cleaved into two or three pieces
- Preproinsulin
- Proinsulin - disulfide bridges
- Insulin
- Secretory protein

Post-translational modifications

Acid/base - act/inact

Hydrolysis – localization, act/inact

Acetylation - act/inact

Phosphorylation - act/inact

Prenylation - localization

Glycosylation - targeting

Post-translational modifications

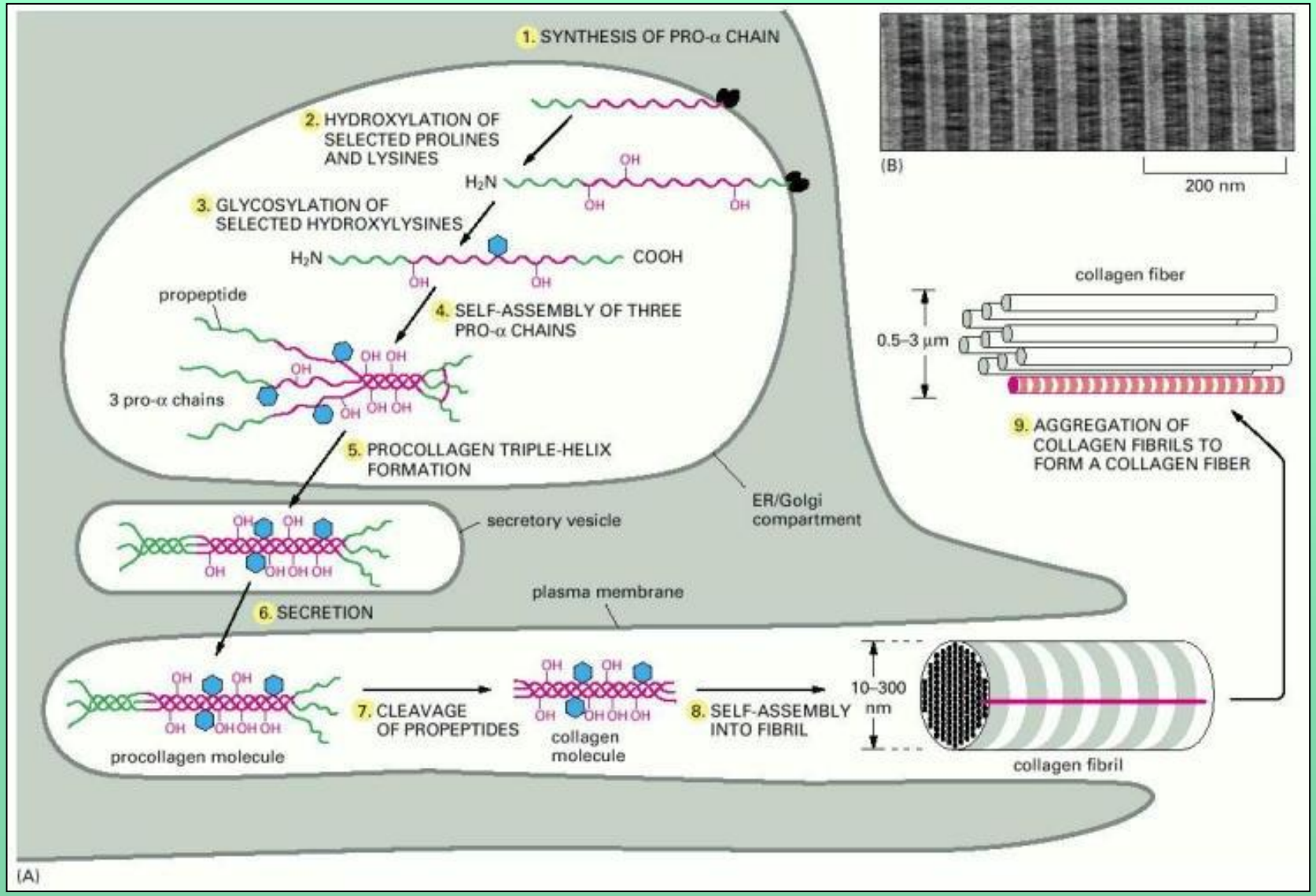
Phosphorylation plays critical roles in the regulation of many cellular processes, including cell cycle, growth, apoptosis and signal transduction pathways

Kinase – phosphorylation = binding of phosphate group to a protein.

Phosphatase removes a phosphate group from a protein. (dephosphorylation)

Both modulate activities of proteins in a cell, often in response to external stimuli.

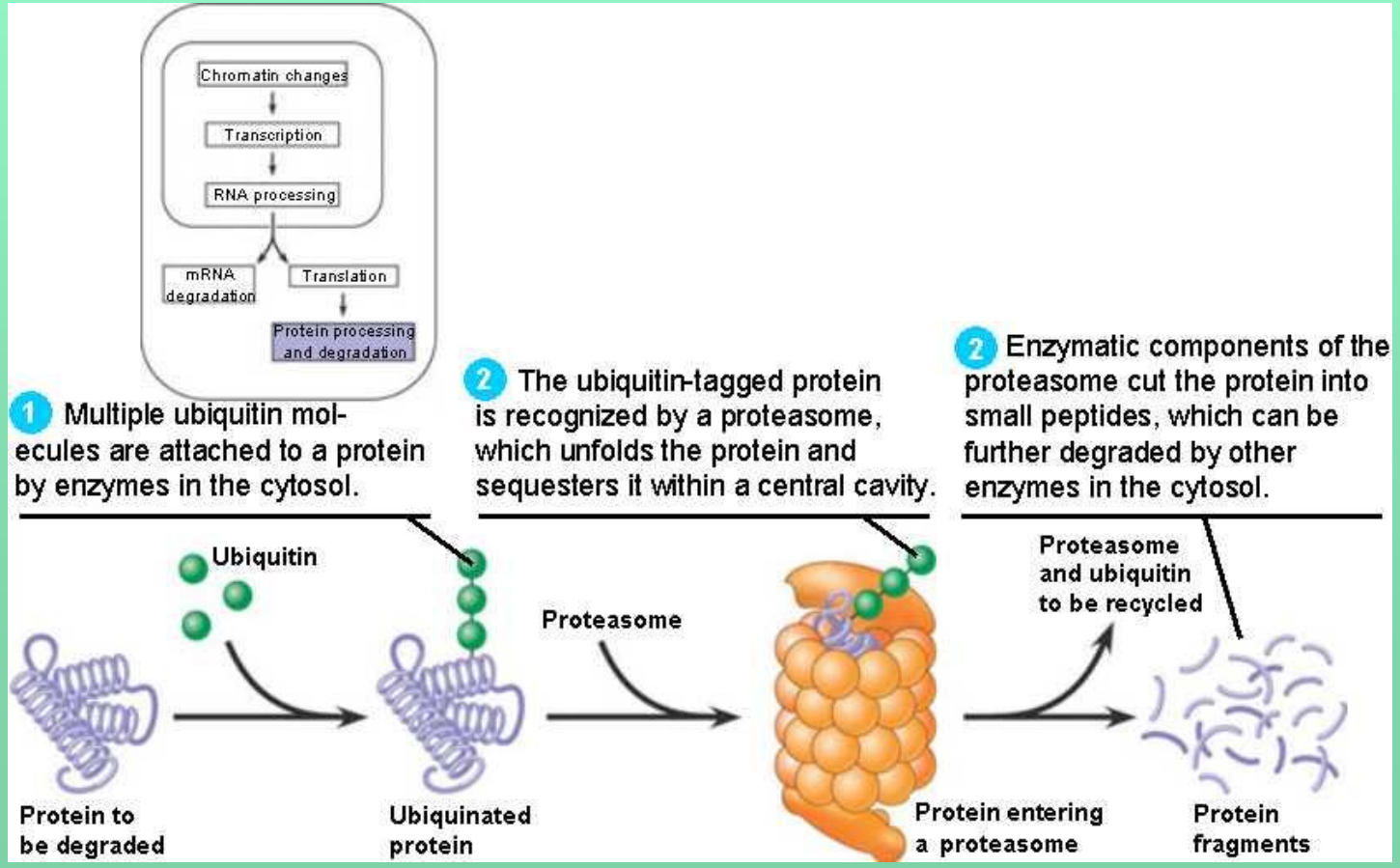
Various steps in the synthesis and assembly of collagen fibrils



8. protein degradation

A lifespan of protein is strictly regulated

Protein for destruction links to a small protein **ubiquitin.** Protein complexes **proteasomes** are places of degradation.



Thank you for your attention

Campbell, Neil A., Reece, Jane B., Cain Michael L., Jackson, Robert B., Minorsky, Peter V.**, Biology**, Benjamin-Cummings Publishing Company, 1996 –2010.