Basics of gene expression regulation in humans

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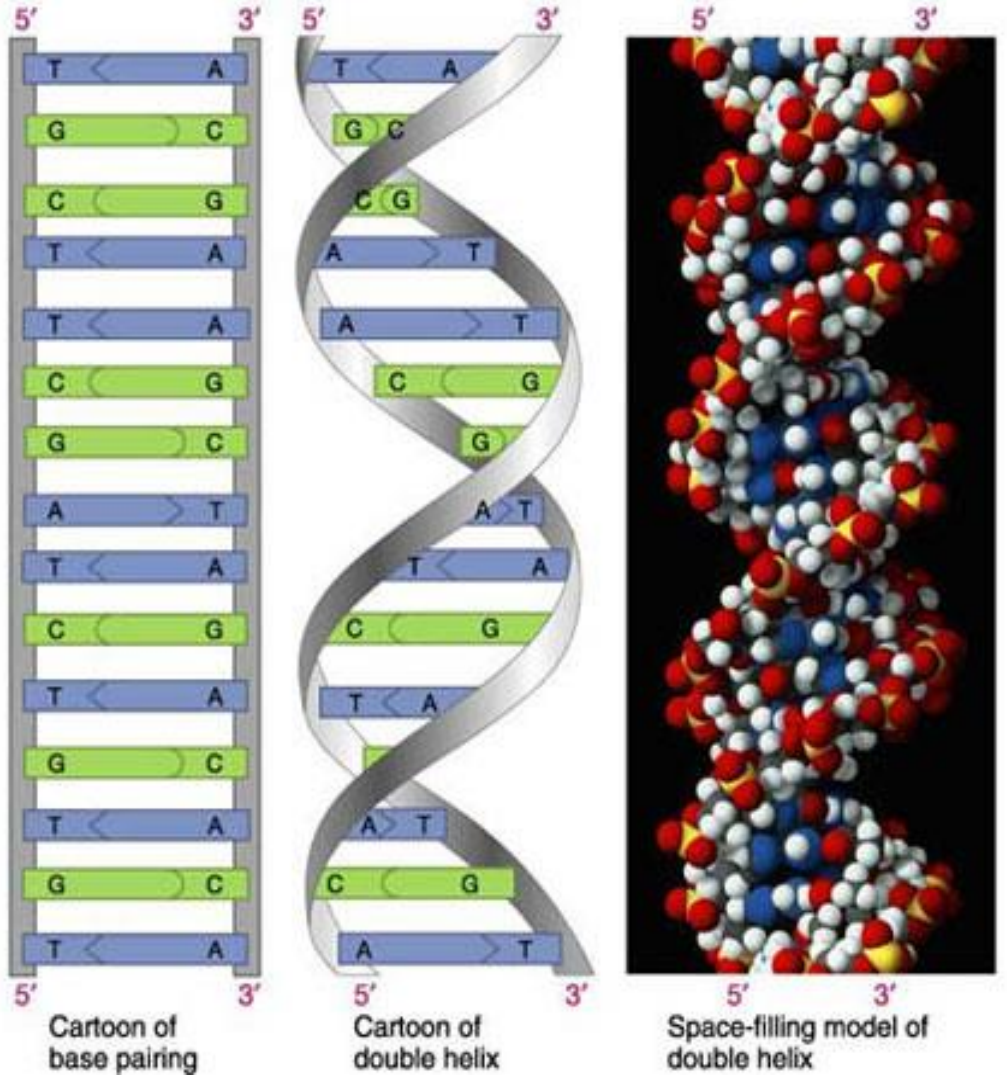
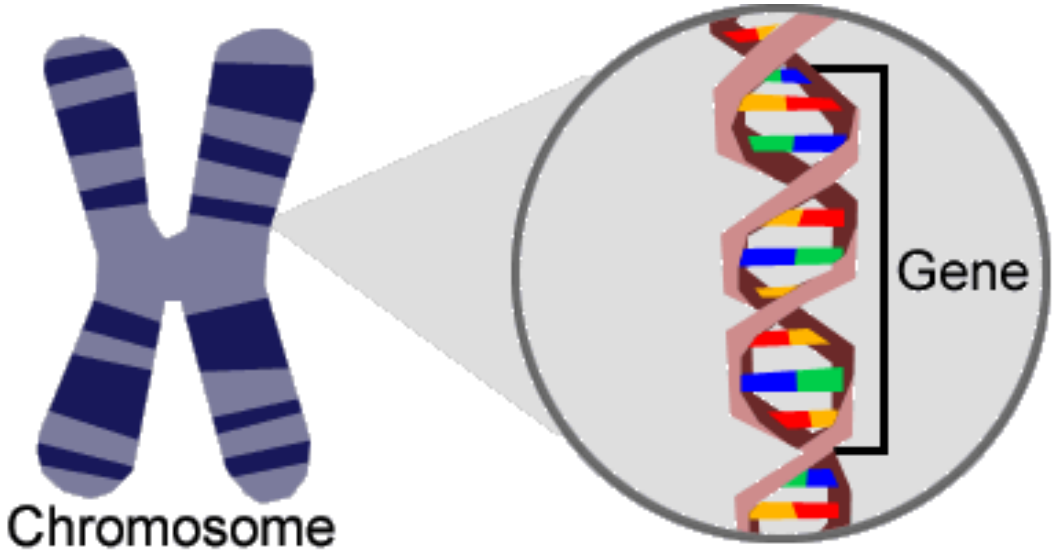
- What is a gene ?
- What is gene expression ?
- How is gene expression regulated in humans ?

What is a gene ?

- Genes are consisted of a polynucleotide chain called **DNA** that generally exists as a double helix.

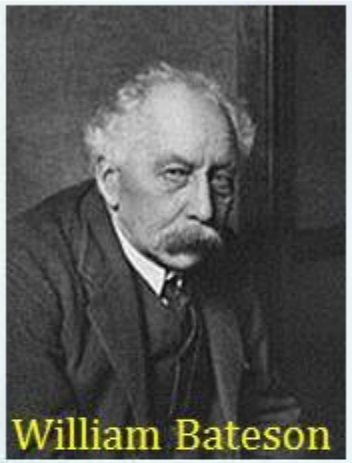




- **<u>Genes</u>** constitute distinct regions on the chromosome
- Each gene codes for a protein product
- DNA -> RNA -> protein
- Differences in proteins brings about differences between individuals and species

Definitions

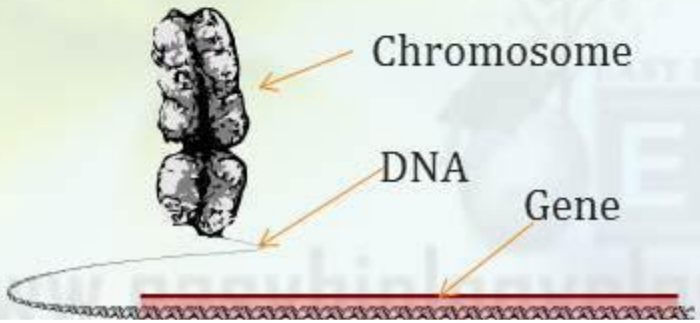
Terminologies in Genetics



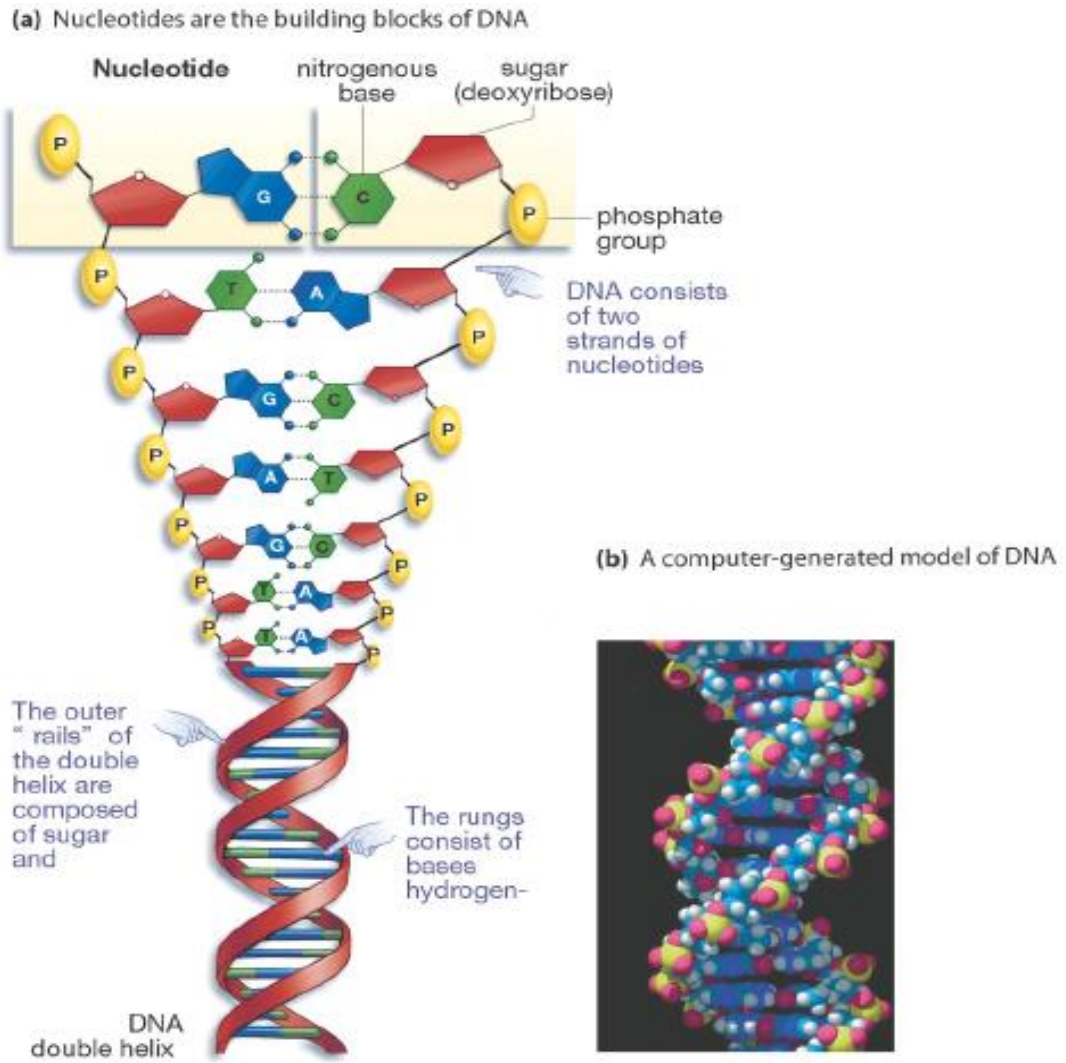
- The term '**Genetics**' was coined by **William Bateson** in 1905

(1). Gene

- The term '**Gene**' was coined by **Johanson** in 1909
- **Definition**: *Gene is the hereditary determining factor*
- Gene consists of a **continuous segment of DNA**
- In eukaryotes, the gene occupies in specific position on the chromosome called ***locus (loci)***

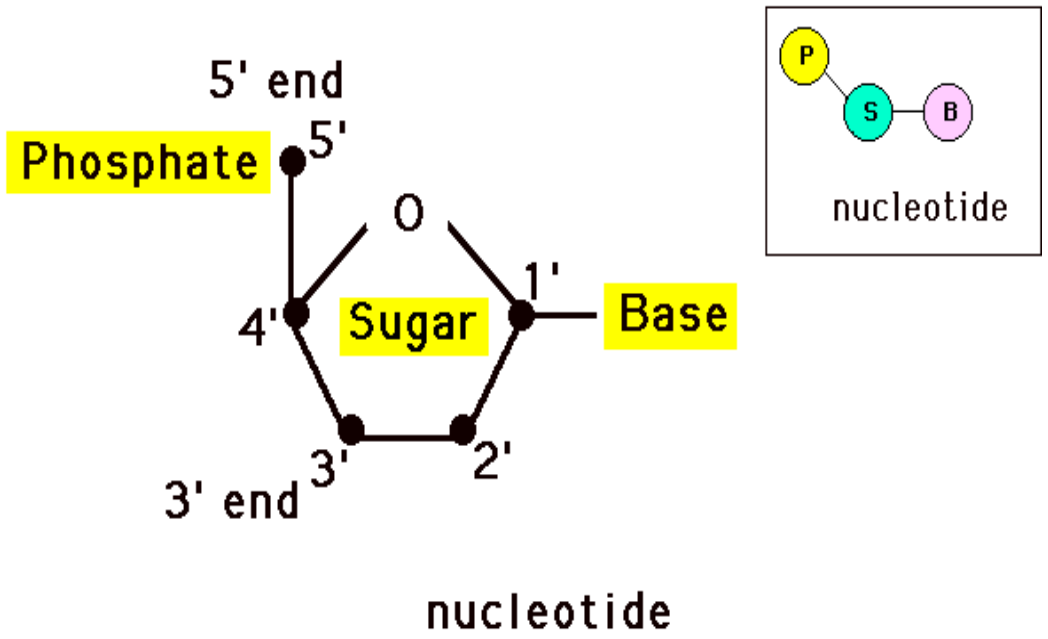


The DNA molecule



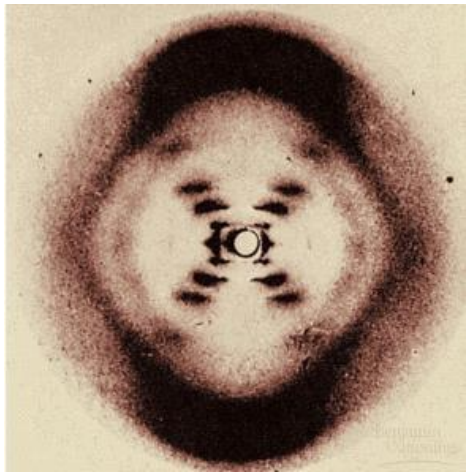
- Composed of 2 polymers of nucleotides
- Polymers are oriented in antiparallel
- Molecule resembles a spiral staircase of complementary base pairs

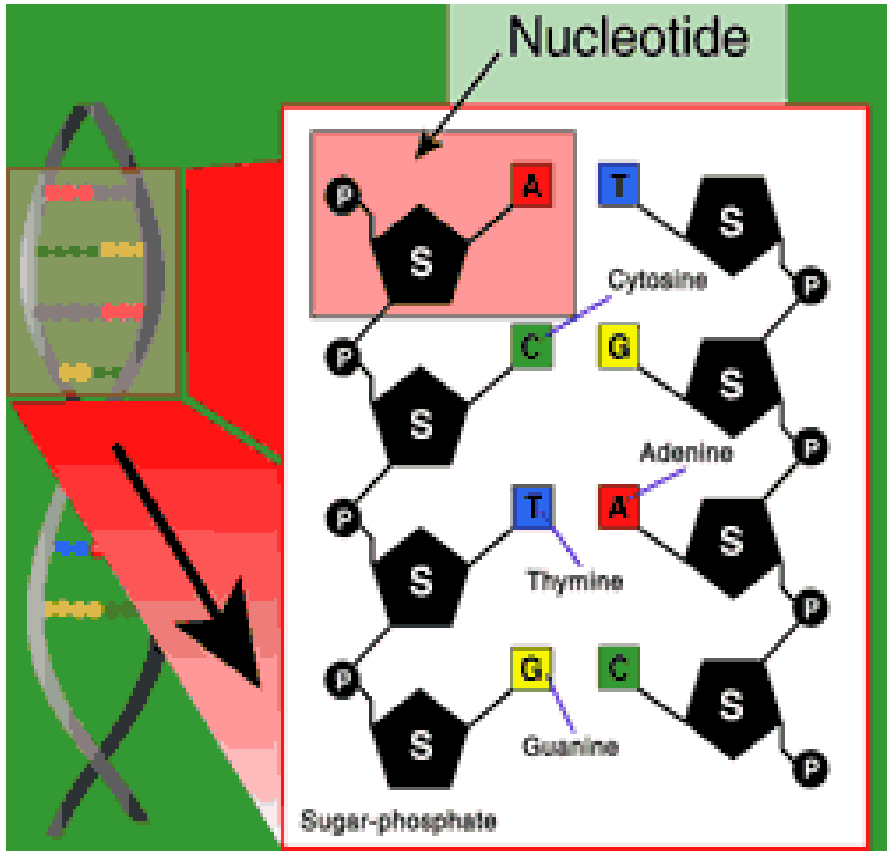
Nucleotide structure of DNA



- Each nucleotide of DNA contains:
  - Deoxyribose
  - Phosphate
  - Nitrogen base (either A, G, C, T)

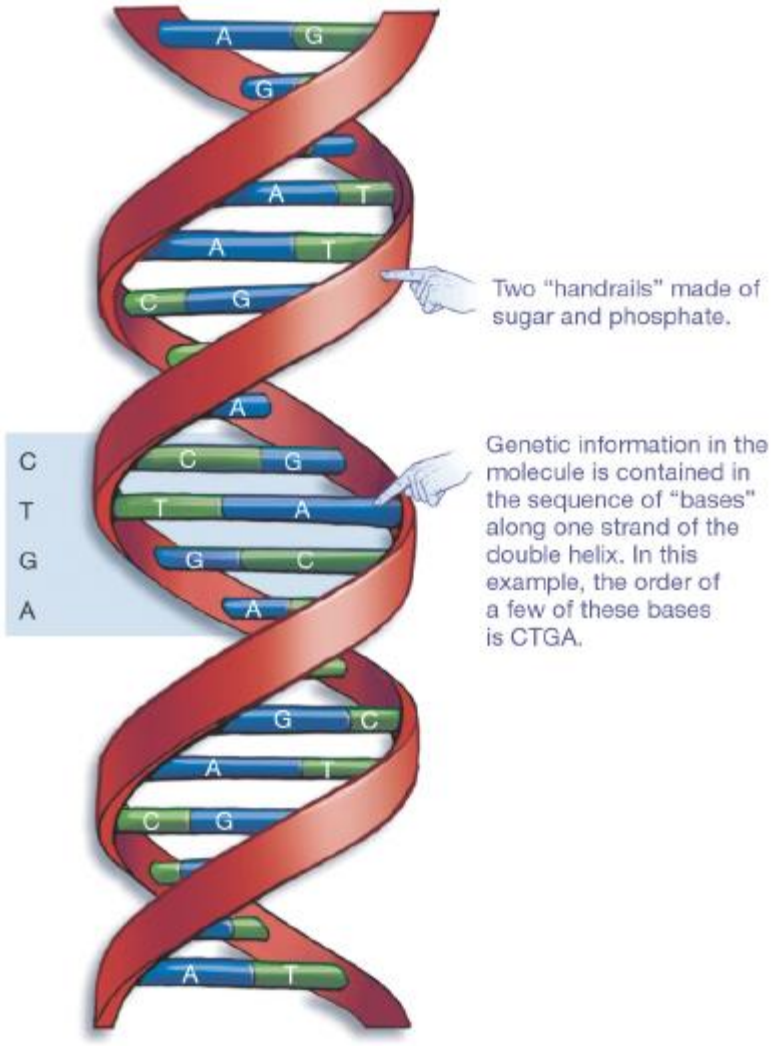
DNA structure





- "Double helix" proposed by Watson and Crick (1953) with the huge contribution of Rosalind Franklin
- Antiparallel backbones
- Complementary base pairing:
  - Adenine to Thymine
  - Cytosine to Guanine

DNA structure



The DNA

DNA = deoxyribonucleic acid

It is a macromolecule constituted of a nucleotide chain (sequence of elementary units : the deoxyribonucleotides)

It is the storage form of genetic information represented by a linear sequence of genes

The DNA molecule is constituted of 2 complementary strand wrap in double helix which allow the replication in 2 identical molecules

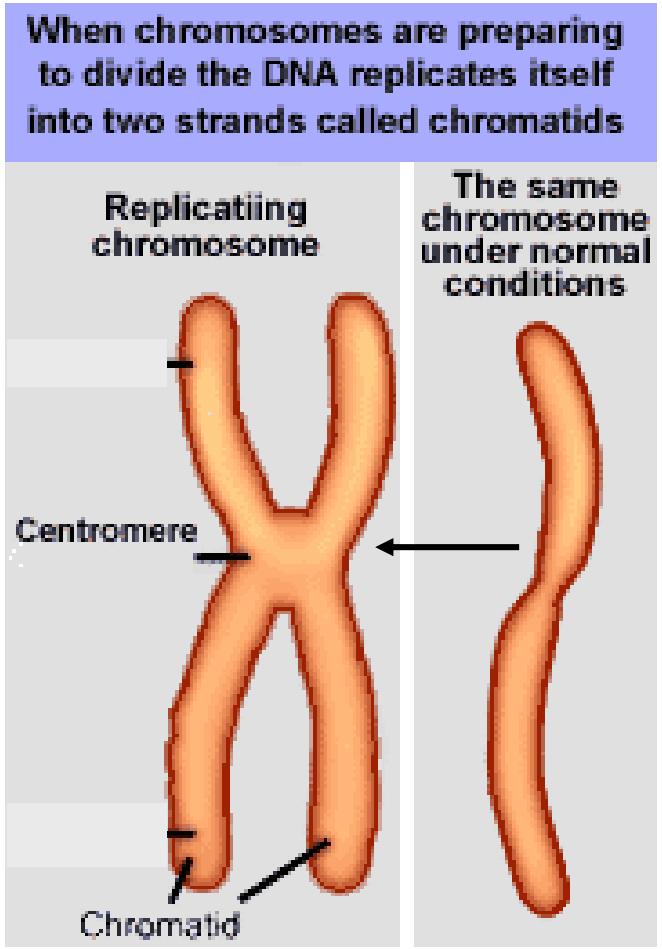
In mammals, there is 2 types of DNA:

- Genomic DNA
- Mitochondrial DNA

DNA replication

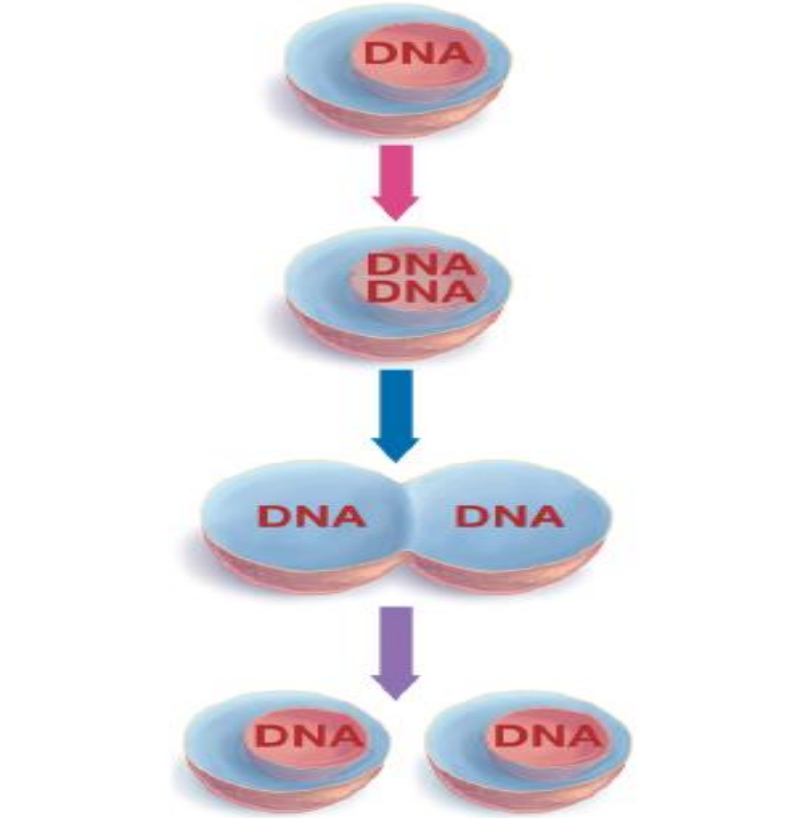
- DNA molecule splits, each half gets copied
- Why is DNA double stranded?
  - More stable
    - Replication consists of half-new, half old
  - Allows for error-correction
    - If a base is damaged, it can be corrected

How do chromosomes become double stranded? Answer: DNA replication



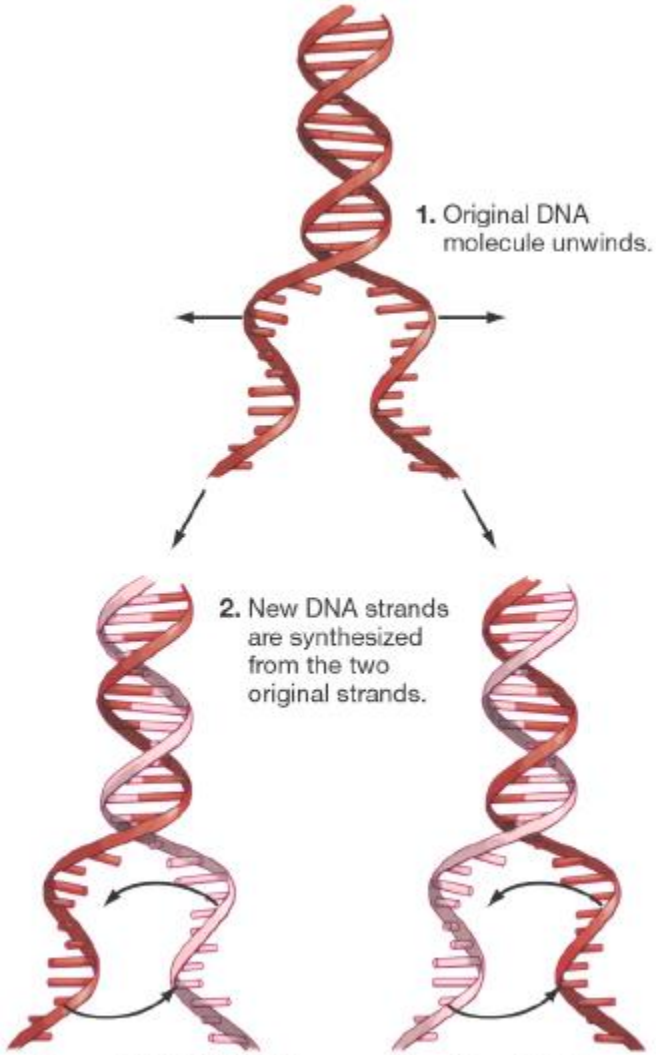
- During the life of the cell, each chromosome of DNA makes a copy of itself
- This must occur prior to cell division to insure each daughter cell gets a complete set

Therefore, prior to dividing, any cell must first replicate DNA



- Each single-stranded (SS) chromosome duplicates to become a double-stranded (DS) chromosome
- Example:
  - A human cell is formed with 46 SS chromosomes
  - Each chromosome replicates to produce 46 DS chromosomes

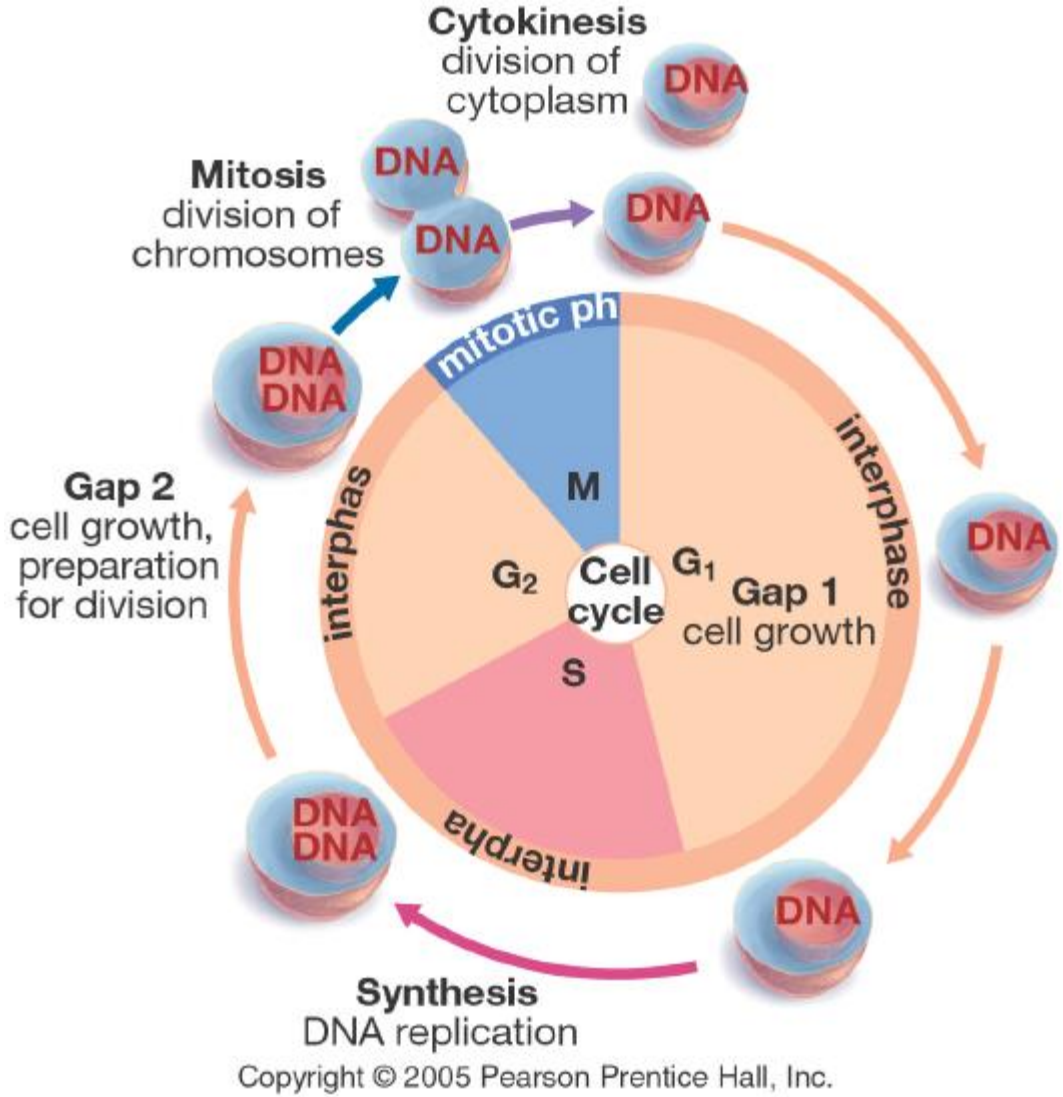
DNA replication



DNA replication occurs during the life of a cell = the **Cell Cycle**

- DNA replicates (makes a copy of itself) to produce DS chromosomes
- During this time, the cytoplasmic contents also duplicate
- Spindle tubules form to aid in the process of cell division
  - Mitosis in body cells
  - Meiosis in sex cells

The cell cycle



The genome

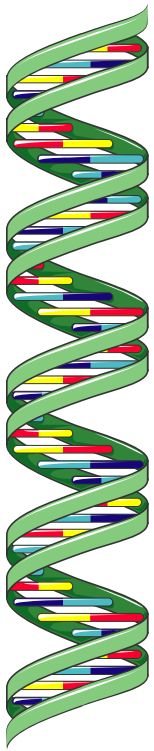
Example Genome Sizes

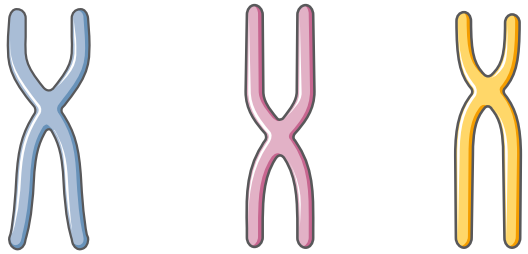
Species	Number of base pairs	Number of genes
E. coli	4,600,000	3200
Fruit fly	180,000,000	13,600
Chicken	1,000,000,000	23,000
Mouse	2,500,000,000	30,000
Corn	2,500,000,000	59,000
Human	3,000,000,000	25,000-30,000
Grasshopper	180,000,000,000	?
Amoeba	670,000,000,000	?

Why so many base pairs?

- “Junk” DNA (or non-coding DNA)
- Portions of DNA sequence for which no function is identified
- 98.5% of human genome
- May serve functions that are not yet understood

What is gene expression ?





gene

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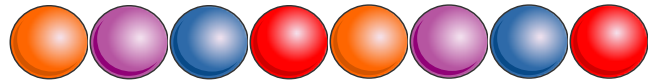
GENE EXPRESSION



protein

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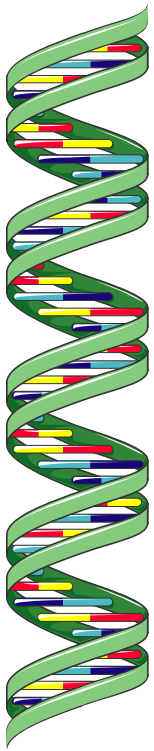

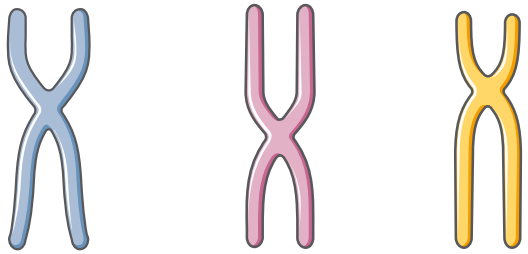

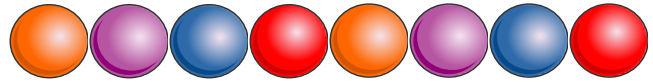

gene

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GENE EXPRESSION

protein

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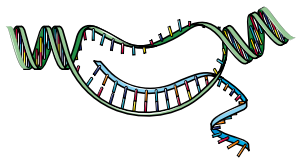
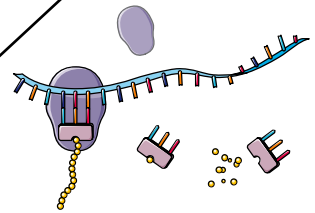


TRANSCRIPTION

RNA



TRANSLATION

DEFINITION OF GENE EXPRESSION

GENE EXPRESSION

- Process by which information from a gene is used in the synthesis of a functional gene product.

- These products are often proteins, but in non-protein coding genes such as transfer RNA (tRNA) or small nuclear RNA (snRNA) genes, the product is a functional RNA.

- The process of gene expression is used by all known eukaryotes (including multicellular organisms), prokaryotes (bacteria and archaea), and utilized by viruses to generate the macromolecular machinery for life.

CLASSIFICATION OF GENES ACCORDING TO THEIR EXPRESSION

- **Constitutive (housekeeping) genes:**

- Are expressed at a fixed rate, irrespective to the cell condition
- Their structure is simpler

- **Controllable genes:**

- Are expressed only as needed. Their amount may increase or decrease with respect to their basal level in various conditions
- Their structure is relatively complicated with some response elements

VARIOUS TYPES OF GENE EXPRESSION REGULATION

- **Positive regulation:**

- When the expression of genetic is quantitatively increased by the presence of specific regulatory element is known as positive regulation
- Element modulating positive regulation is known as activator or positive regulator

- **Negative regulation:**

- When the expression of genetic information diminished by the presence of specific regulatory element
- The element or molecule mediating the negative regulation is said to be repressor

PURPOSES OF GENE EXPRESSION REGULATION

Regulated expression of genes is required for:

- **Adaptation**

Cells of multicellular organisms respond to varying conditions.
Such cells exposed to hormones and growth factors change substantially in:
o shape
o growth rate
o other characteristics

- **Tissue specific differentiation and development**

The genetic information present in each somatic cell of a organism is practically identical.
Cells from muscle and nerve tissue show strikingly different morphologies and other properties, yet they contain exactly the same DNA.
These diverse properties are the result of differences in gene expression.
Expression of the genetic information is regulated during ontogeny and differentiation of the organism and its cellular components.

PURPOSES OF GENE EXPRESSION REGULATION

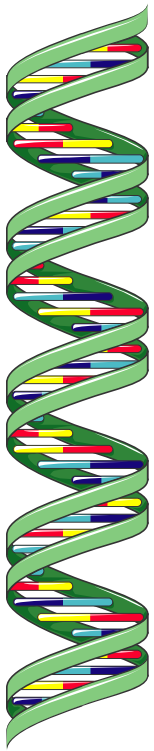
Different cell types are present in most eukaryotes.

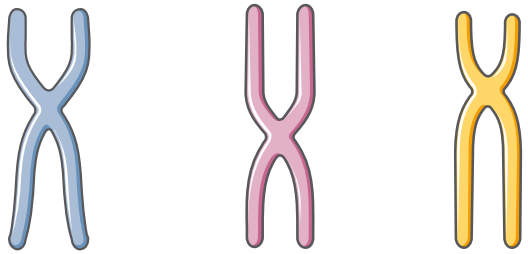
- Liver and pancreatic cells, for example, differ dramatically in the genes that are highly expressed
- Different mechanisms are involved in the regulation of such genes

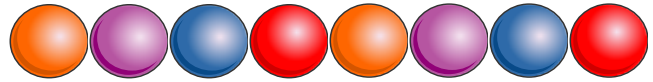
The DNA in eukaryotic cells is extensively folded and packed into the protein-DNA complex called chromatin.

- Histones are an important part of this complex since they both form the structures known as nucleosomes and also contribute significantly into gene regulatory mechanisms

How is gene expression regulated ?







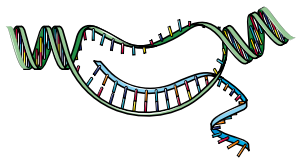
gene

« INFORMATION »

GENE EXPRESSION

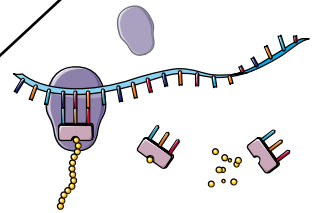
protein

« PRODUCT »



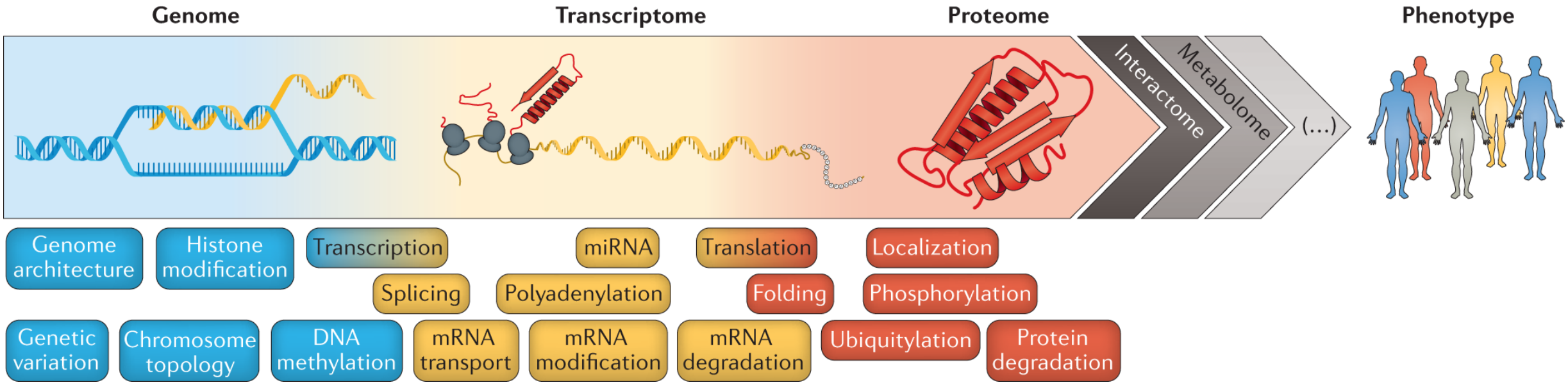
TRANSCRIPTION

RNA



TRANSLATION

GENE EXPRESSION REGULATION



Buccitelli&Selbach, Nature Review Genetics, 2020

- Gene amplification and rearrangements influence gene expression.
- Gene expression can be controlled at multiple levels by chromatin modifications, changes in transcription, RNA processing, localization, and stability, protein formation and degradation.

CONTROL OF GENE EXPRESSION

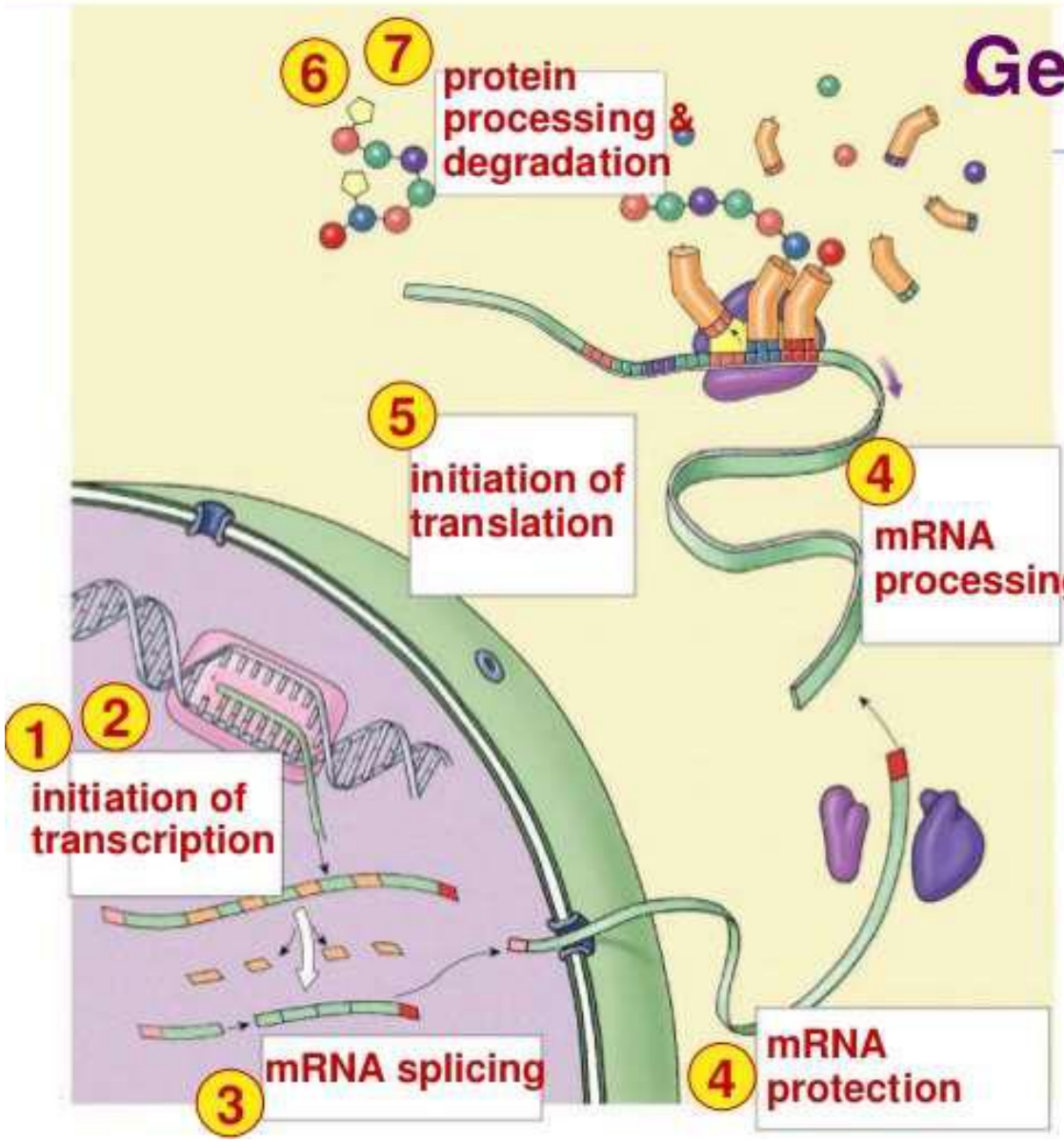
Gene activity is controlled first and foremost at the level of transcription.

- Much of this control is achieved through the interplay between proteins that bind to specific DNA sequences (transcription factors) and their DNA binding sites
- This can have a positive or negative effect on transcription

In addition to transcription level controls, gene expression can also be modulated by:

- Gene rearrangement
- Gene amplification
- Post-transcriptional modifications

LEVELS OF GENE EXPRESSION REGULATION



Gene Regulation

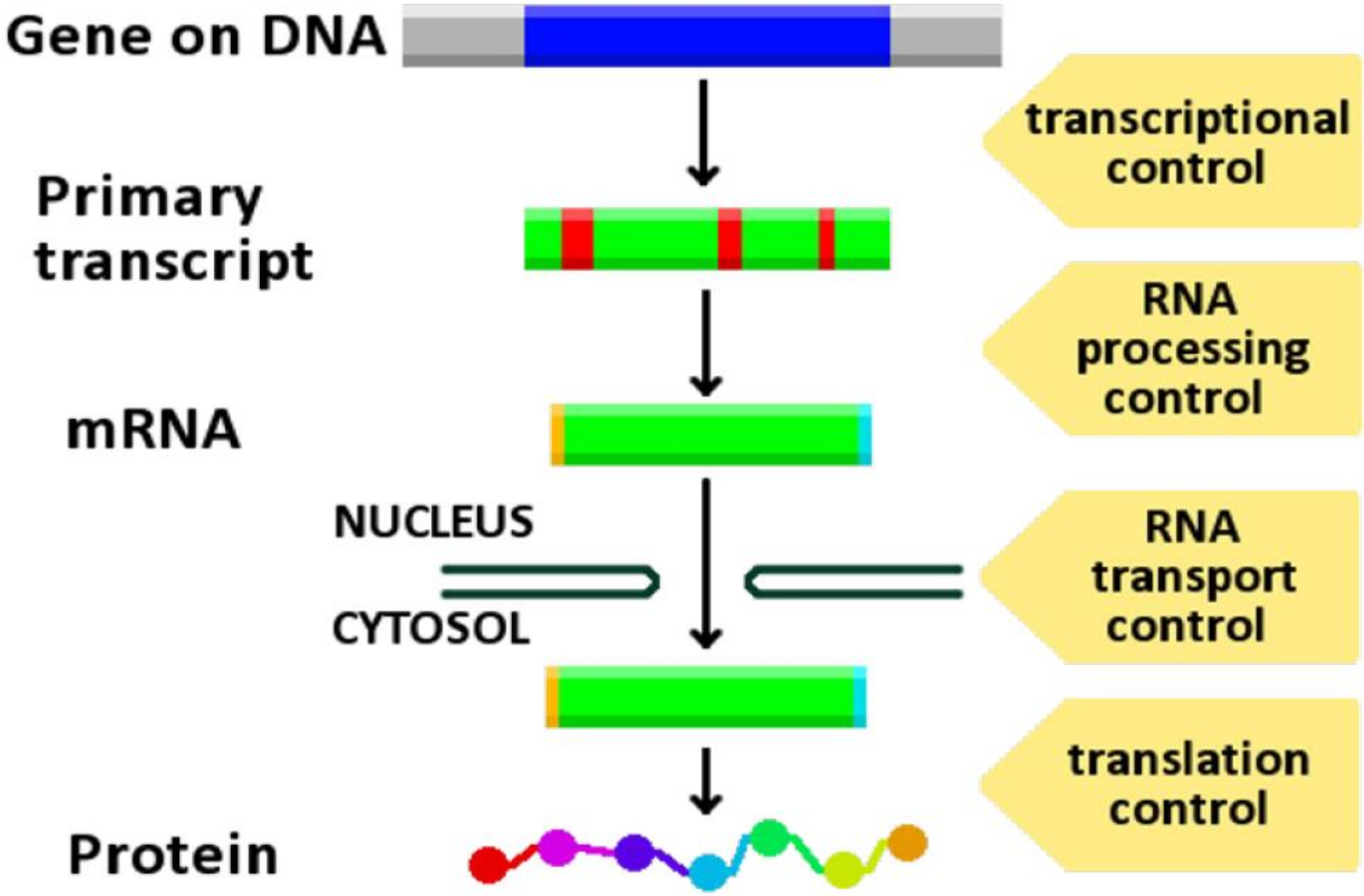
1 & 2. transcription
- DNA packing
- transcription factors

3 & 4. post-transcription
- mRNA processing
- splicing
- 5' cap & poly-A tail
- breakdown by siRNA

5. translation
- block start of translation

6 & 7. post-translation
- protein processing
- protein degradation

LEVELS OF GENE EXPRESSION REGULATION



- **DNA MODIFICATIONS**
- **TRANSCRIPTIONAL REGULATION**
- **POST-TRANSCRIPTIONAL REGULATION**
- **TRANSLATIONAL REGULATION**
- **POST-TRANSLATIONAL REGULATION**

- **DNA MODIFICATIONS**
- TRANSCRIPTIONAL REGULATION
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- POST-TRANSLATIONAL REGULATION

DNA modifications

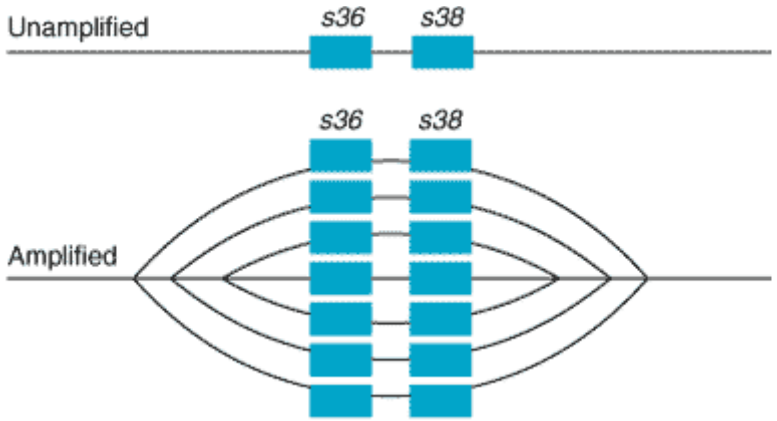
Structural: copy number variants, gene rearrangement

Epigenetics: DNA methylation and histone modifications

DNA MODIFICATIONS: GENE AMPLIFICATION

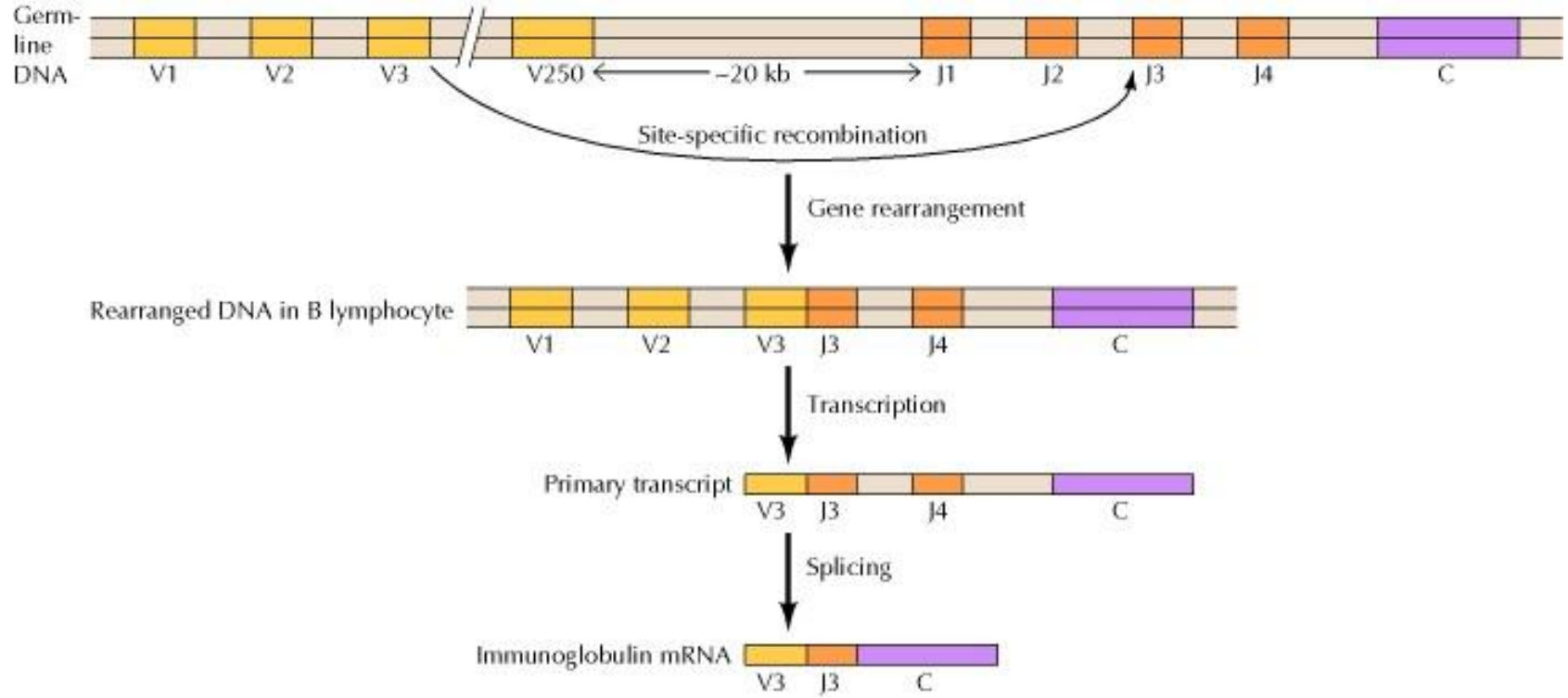
• The gene product can be increased by increasing the number of genes available for transcription of specific molecules
• Among the repetitive DNA sequences are hundreds of copies of ribosomal RNA genes and tRNA genes

• Subsequently, these amplified genes, presumably generated by a process of repeated initiations during DNA synthesis, provide multiple sites for gene transcription.



DNA MODIFICATIONS: GENE REARRANGEMENT

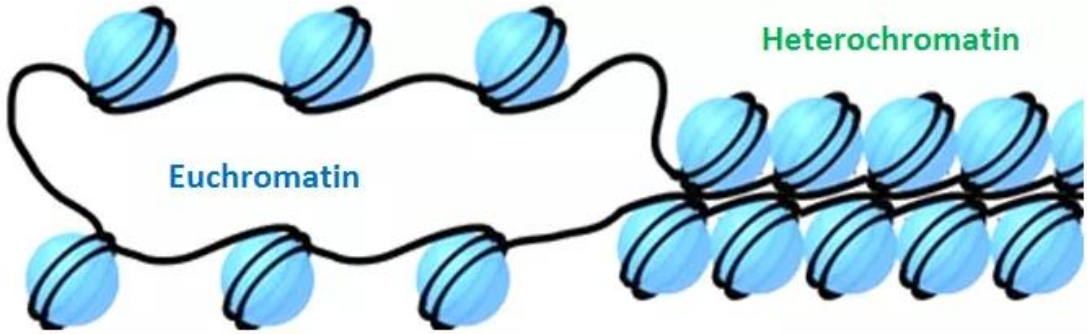
Example: Gene rearrangement is observed during immunoglobulin synthesis



The segment of DNA move from one location to another in the genome. These DNA coding changes are needed for generating the required recognition diversity central to appropriate immune function.

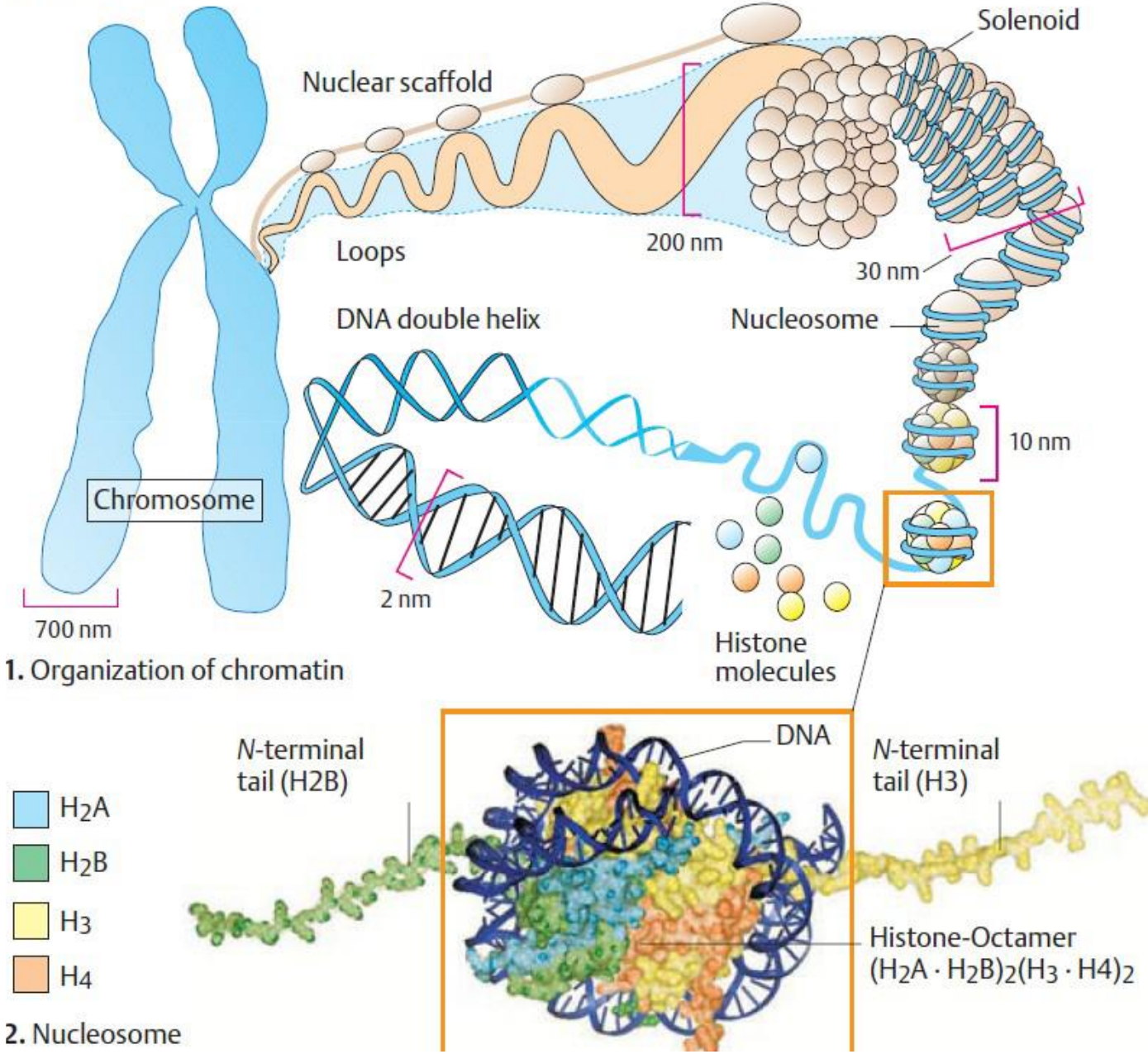
DNA MODIFICATIONS: CHROMATIN CONFORMATION

- Heterochromatin = inactive ; euchromatin = active
- The chromatin conformation can be different amongst cells leading to various expression in specialized cells
- For example, the DNA containing the globin gene cluster is in "active" chromatin in the reticulocytes but in "inactive" chromatin in muscle cells.



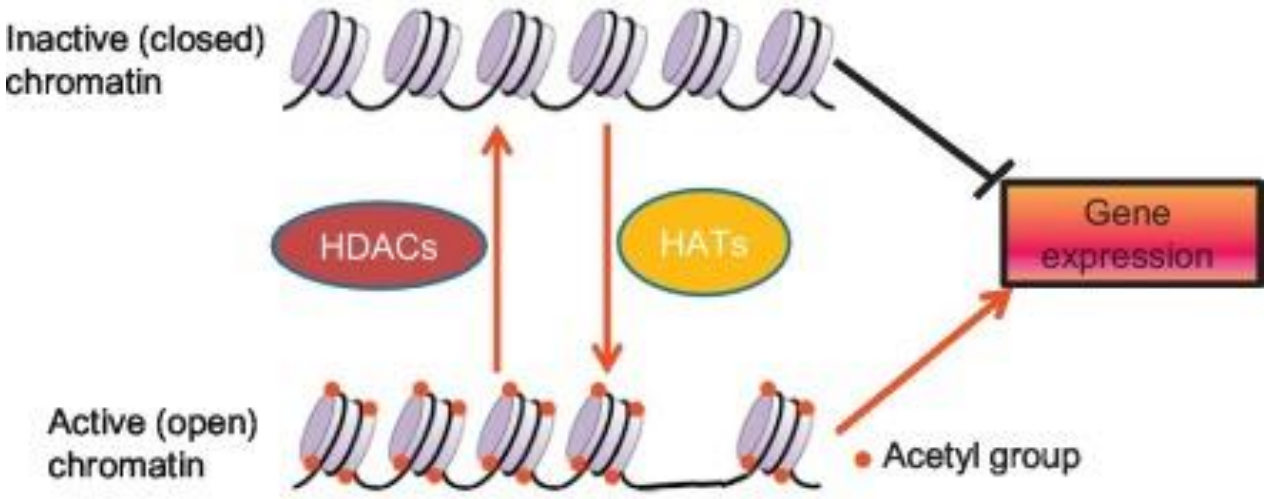
DNA MODIFICATIONS: CHROMATIN CONFORMATION

- Nucleosomes are portions of double-stranded DNA (dsDNA) that are wrapped around protein complexes called histone cores.
- These histone cores are composed of 8 subunits, two each of H2A, H2B, H3 and H4 histones. This protein complex forms a cylindrical shape that dsDNA wraps around with approximately 147 base pairs.
- Nucleosomes are formed as a beginning step for DNA compaction that also contributes to structural support as well as serves functional roles.



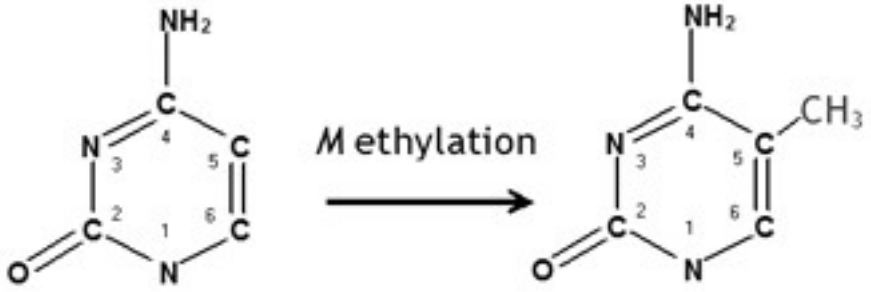
DNA MODIFICATIONS: HISTONE ACETYLATION AND DEACETYLATION

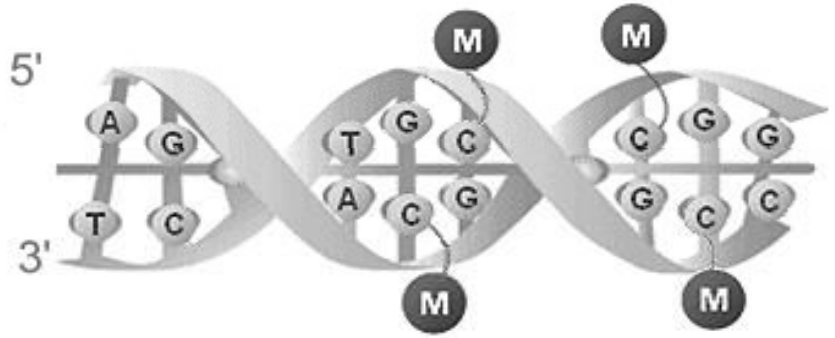
- Lysine residues in the amino terminal tails of histone molecules can be acetylated or deacetylated by histone acetyltransferases (HAT)/histone deacetylases (HDAC).
- This mechanism is an essential part of gene regulation.
- This modification reduces the positive charge of these tails and decreases the binding affinity of histone for the negatively charged DNA.
- Accordingly, the acetylation of histones could result in disruption of nucleosomal structure and allow readier access of transcription factors to the regulatory DNA elements (cis regulation).



DNA MODIFICATIONS: DNA METHYLATION

- DNA methylation is the addition or removal of a methyl group by a DNA methyl-transferase, mostly in C.
- When the promoter is methylated, the transcription is repressed.
- DNA methylation is dependent of developmental and environmental mechanisms.



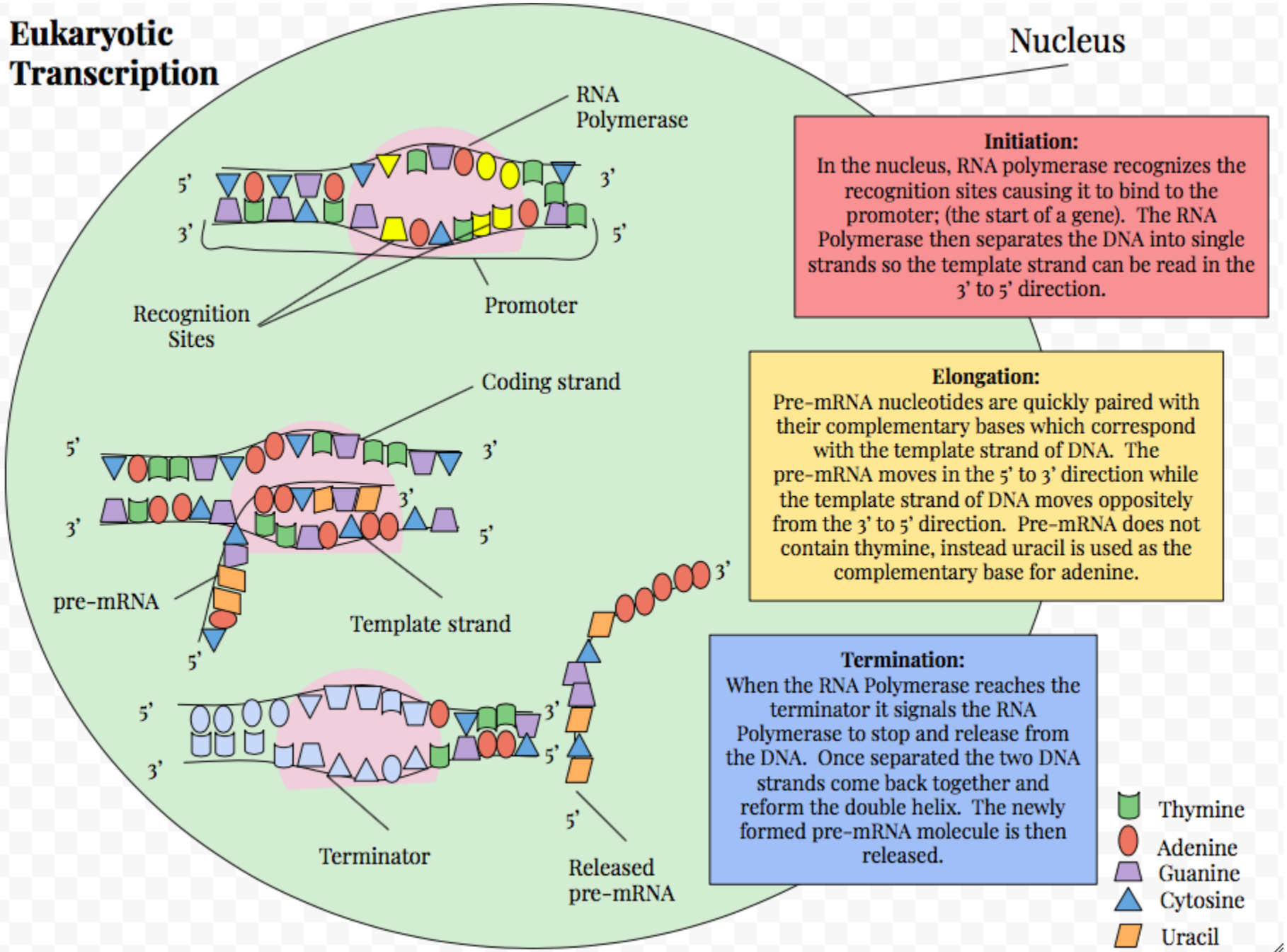


- DNA MODIFICATIONS
- **TRANSCRIPTIONAL REGULATION**
- POST-TRANSCRIPTIONAL REGULATION
- TRANSLATIONAL REGULATION
- POST-TRANSLATIONAL REGULATION

GENERAL MECHANISMS OF TRANSCRIPTION

- Transcription = first step of gene expression, in which a specific fragment of DNA is copied in RNA
- This mechanism occurs in cell nucleus
- The RNA produced can be « coding » (mRNA) or « non coding » and the enzymes (RNA polymerases) leading to their formation are different
- 3 steps : initiation, elongation, termination

Eukaryotic Transcription



TRANSCRIPTION REGULATION: CIS REGULATORS

- Promoter: DNA region near the gene, constituted of conserved regions including generally the initiation transcription point (adenin framed by 2 pyrimidins C or T), TATA box (-25), CAAT box (-75) which increases promoter activity, CG box (-90, GGGCGG) which regulates transcription

- Enhancer: DNA region (50-1500 bp) binding the TF to activate transcription.

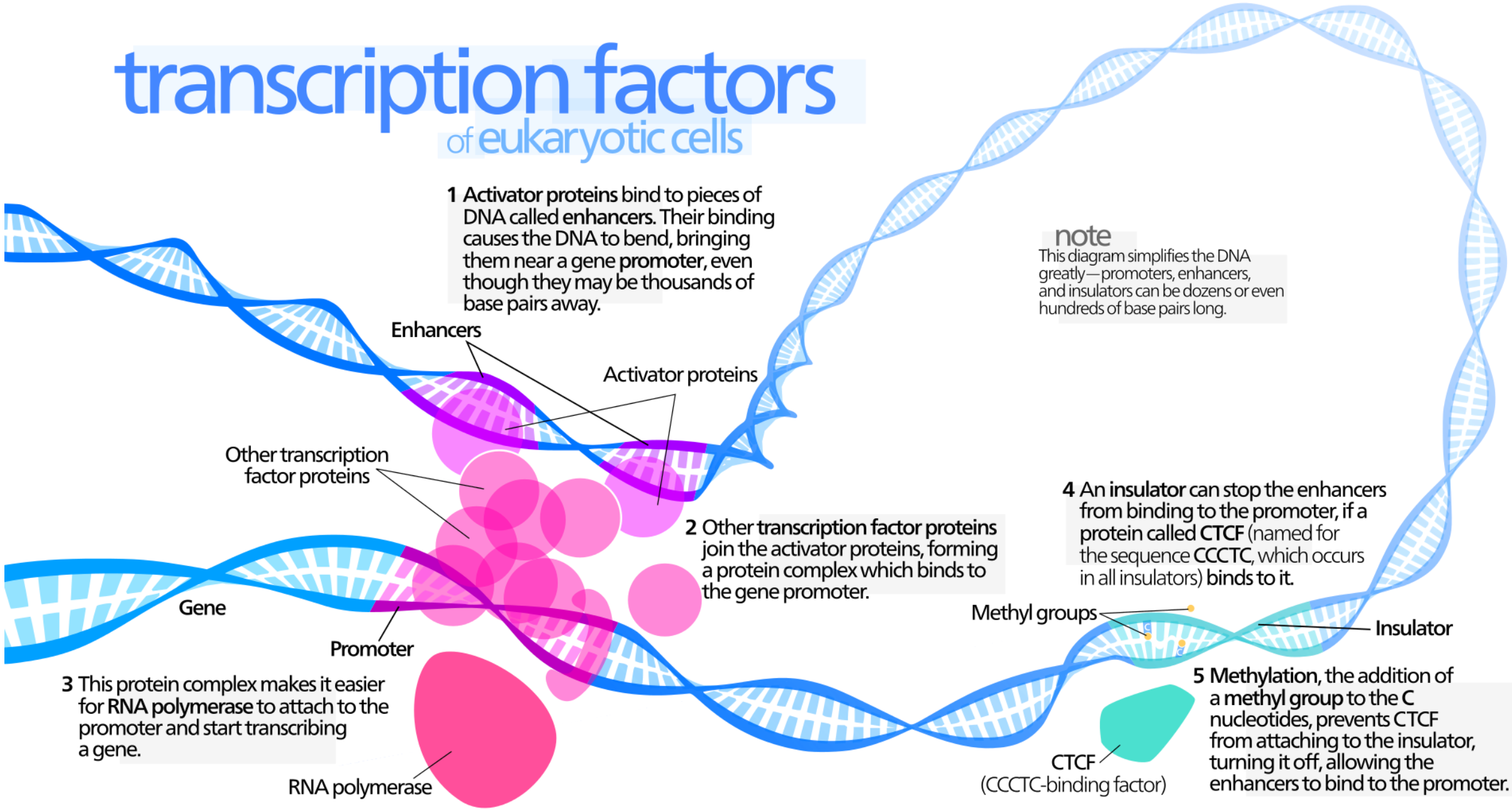
- Silencer: opposite to enhancer

- Insulator: impairs enhancer or silencer activity

Use of alternative promoter

Some genes has series of promoters showing tissue specific expression so in different tissues different transcript and different proteins are produced

transcription factors
of eukaryotic cells



TRANSCRIPTIONAL REGULATION: TRANS FACTORS

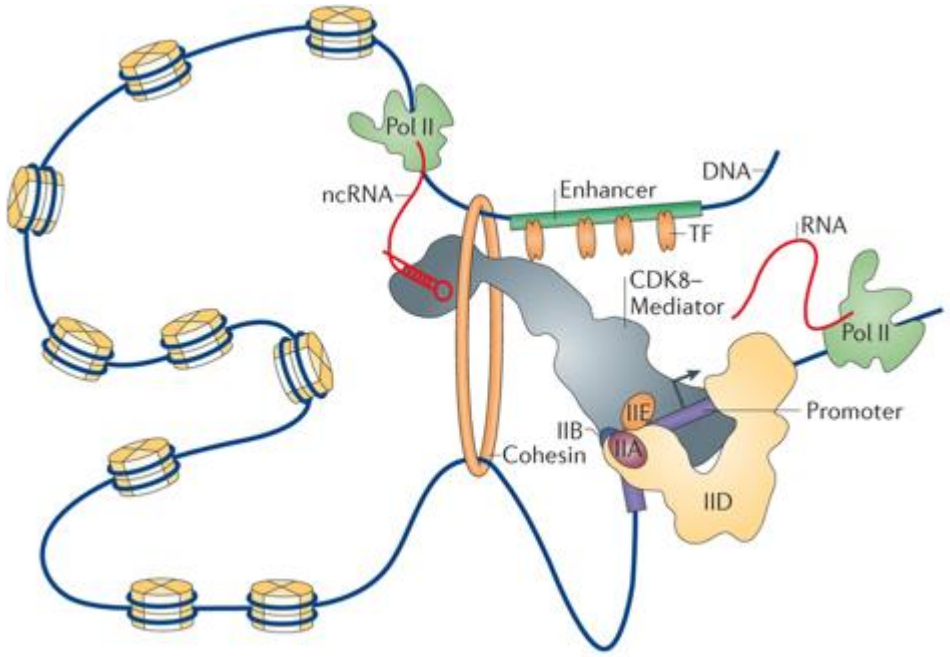
= proteins which bind to cis sequences to activate or repress transcription

Activators, enzymes, repressors

Transcription factors

TRANSCRIPTIONAL REGULATION: TRANS FACTORS

Mediator = 31 subunits in humans
Act as a coactivator (binds to the transcription preinitiation complex) to regulate PolII activity by communicating regulation signals from TF
Involved also in other transcription processes (initiation, elongation, chromatin architecture and promoter/activator loop formation)



TRANSCRIPTIONAL REGULATION: TRANS FACTORS

Transcription factor (TFs) = protein which binds on specific DNA sequence

The TFs regulate transcription alone or in combination

They can activate or inhibit RNApolymerase recruitment via different mechanisms:

- Stabilization or impairment of RNA pol binding on DNA
- Histone acetylation or deacetylation:
  - Histone acetyltransferase activity: ↓ DNA association with histone (↑ DNA accessibility and consequently transcription activation)
  - Histone desacetylase activity: transcription repression

- Recruitment of co-activators or co-repressors

TFs act in combination and various combinations trigger different regulatory effects on transcription initiation. Each cell type possess the specific combinations leading to various phenotypes.

BASAL TRANSCRIPTIONAL REGULATION: GENERAL TRANSCRIPTION FACTORS

- Five « General Transcription Factors » (TFII-B, TFII-D, TFII-E, TFII-F et TFII-H) have to mediate first RNA pol binding and transcription initiation.

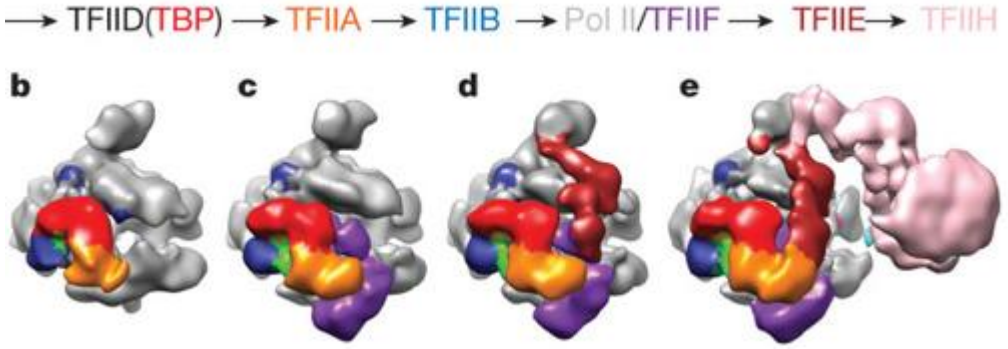
TRANSCRIPTION INITIATION WITH GENERAL TRANSCRIPTION FACTORS

The complex [RNA pol – TF – DNA] is called transcription pre-initiation complex.

This complex allows:

- The specific RNApolII binding on initiation transcription site
- The DNA opening
- The release of DNApolII

However, some mechanisms and functions of this complex are still unknown, notably because of a lack of structural informations due to the huge size of the complex (2 millions Da).



Various steps of pre-initiation complex assembling [TBP - TFIIA - TFIIB - ADN - Pol II] (b) then addition of TFIIF (c), TFIIE (d), TFIIH (e).

Source : He *et al*. (2013)

TRANSCRIPTIONAL REGULATION: OTHER TRANSCRIPTION FACTORS

Transregulatory TF have some common structural characteristics with at least two domains:

- The DNA binding domain with specific structural motifs
- The transcription domain (glutamin rich, prolin rich, alpha helix acid domain)
- Some TF have a third domain allowing to bind an external cellular element (ligand) as nuclear receptors

TF families can be defined according to the DNA binding domains

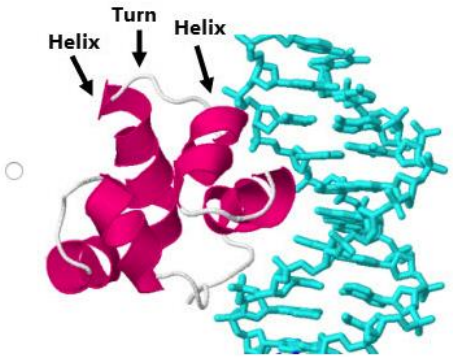
TRANSCRIPTIONAL REGULATION: SPECIFIC REGULATORY DOMAINS

• Certain DNA binding proteins having specific motifs bind certain region of DNA to influence the rate of transcription.
• The specificity involved in the control of transcription requires that regulatory proteins bind with high affinity to the correct region of DNA.

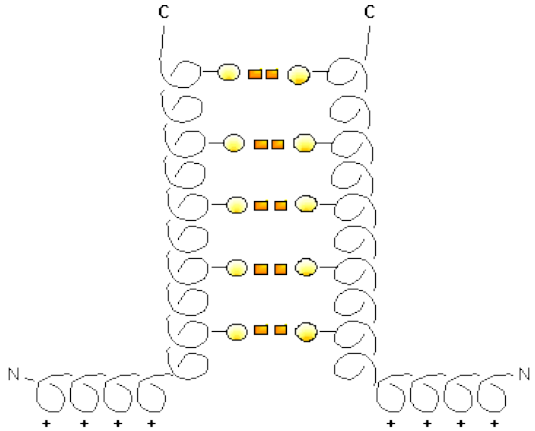
• Three unique motifs—the **helix-turn-helix,** the **zinc finger,** and the **leucine zipper**—account for many of these specific protein-DNA interactions.
• The motifs found in these proteins are unique; their presence in a protein of unknown function suggests that the protein may bind to DNA.
• The protein-DNA interactions are maintained by hydrogen bonds and van der Waals forces.

TRANSCRIPTIONAL REGULATION: DNA BINDING DOMAINS

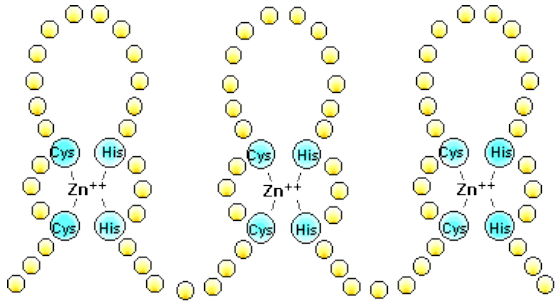
Helix-Turn-Helix



Leucine Zipper

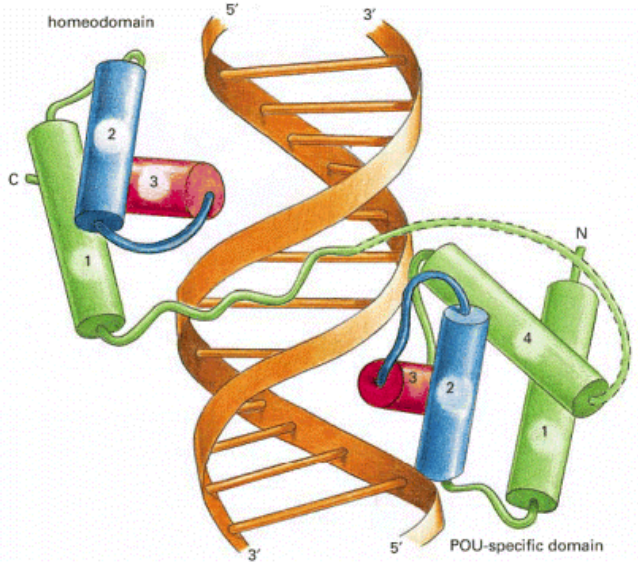


Zinc Finger proteins

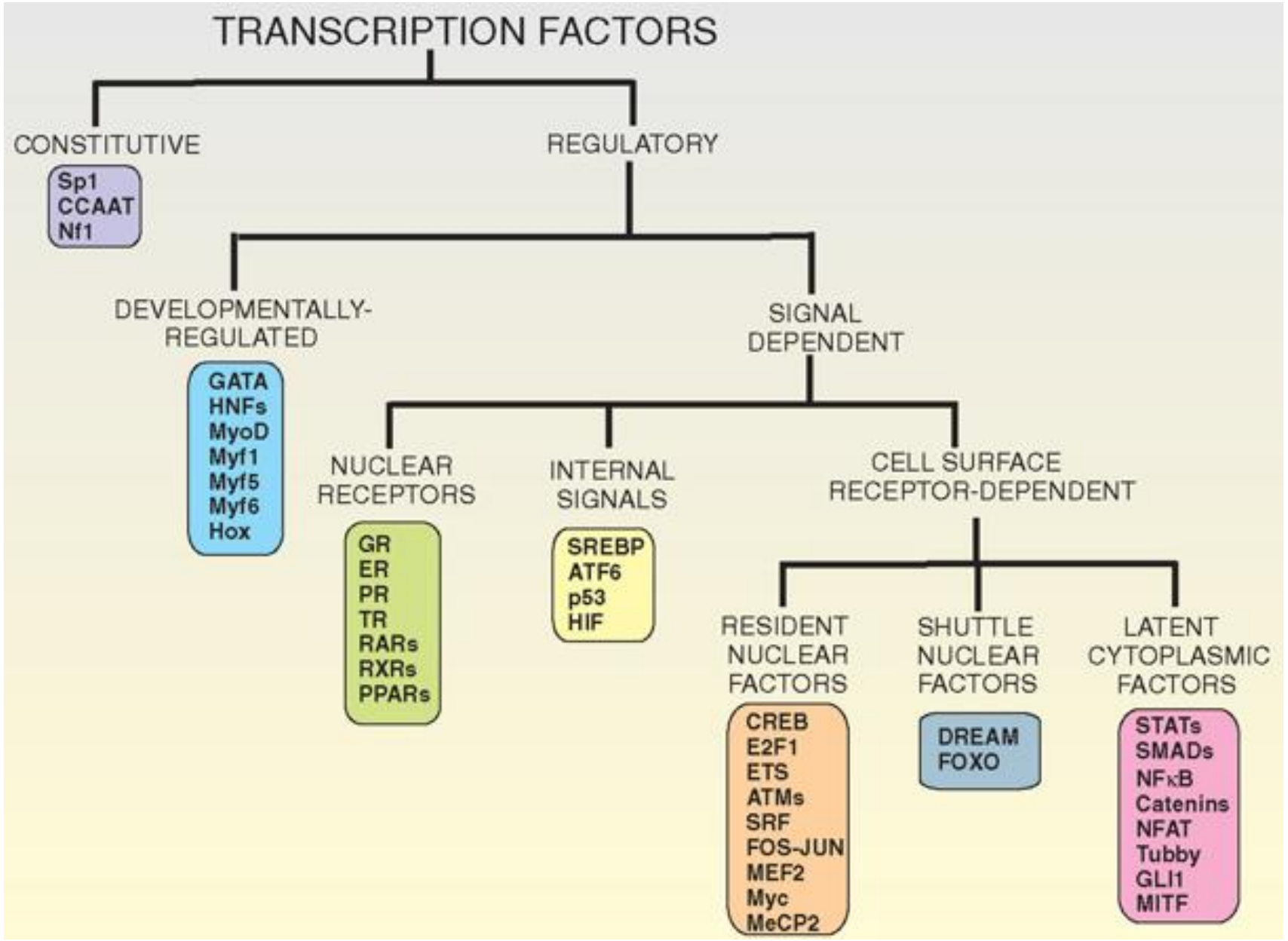


Basic Helix-Loop-Helix

Homeodomain



TRANSCRIPTIONAL REGULATION: TRANSCRIPTION FACTORS CLASSIFICATION



Source : "Cell signaling biology" - Module 4

TRANSCRIPTIONAL REGULATION: TRANSCRIPTION FACTOR RESPONSE ELEMENTS

Elements de réponse ("*Response element*" - RE)

Agent régulateur	Module	Séquence consensus	paires de bases d'ADN fixées	Facteur	Masse molaire (Da)
Choc thermique	HSE	CNNGAANNTCCNNG	27 bp	HSTF	93,000
Glucocorticoide	GRE	TGGTACAAATGTTCT	20 bp	Receptor	94,000
Cadmium	MRE	CGNCCCGGNCNC	--------	?	--------
Ester de phorbol	TRE	TGACTCA	22 bp	AP1	39,000
Sérum	SRE	CCATATTAGG	20 bp	SRF	52,000
Anti-oxydant	ARE	GTGACTCAGC	--------	--------	--------
Phéromone		ACAAAGGGA	--------	--------	--------
Hypoxie	HRE	CCACAGTGCATACGT GGGCTCCAACAGGTC CTCTCCCTCCCATGCA	--------	"Hypoxia Inducible Factor"	826 aa
"Peroxisome Proliferator Activated Receptor" (PPAR)	PPRE	aGG_CAAAGGT(CG)A	--------	PPAR	59,000
Stéroides (progèsterone, androgène, minéralcorticoides, glucocorticoides)		AGAACAxxxACAAGA (séquence répétée inversée)	--------	--------	--------

TRANSCRIPTIONAL REGULATION: NUCLEAR RECEPTORS

Nuclear receptors can be divided in 2 main classes according to their action mechanism and their subcellular localization (in the absence or their ligand)

- Type I nuclear receptors located in cytosol and then transported in the nucleus

Ligand binding on this receptor induces:

- Thermal shock protein dissociation
- Nuclear receptor homodimerization
- Nuclear receptor translocation (via active transport)
- Nuclear receptor binding on DNA HRE (Hormone Responsive Element).

Ex: androgen receptors, estrogen receptor, glucocorticoid receptor, progesterone receptor

- Type II nuclear receptors always located in the nucleus

They link to DNA as heterodimers (for example with retinoid X receptors)

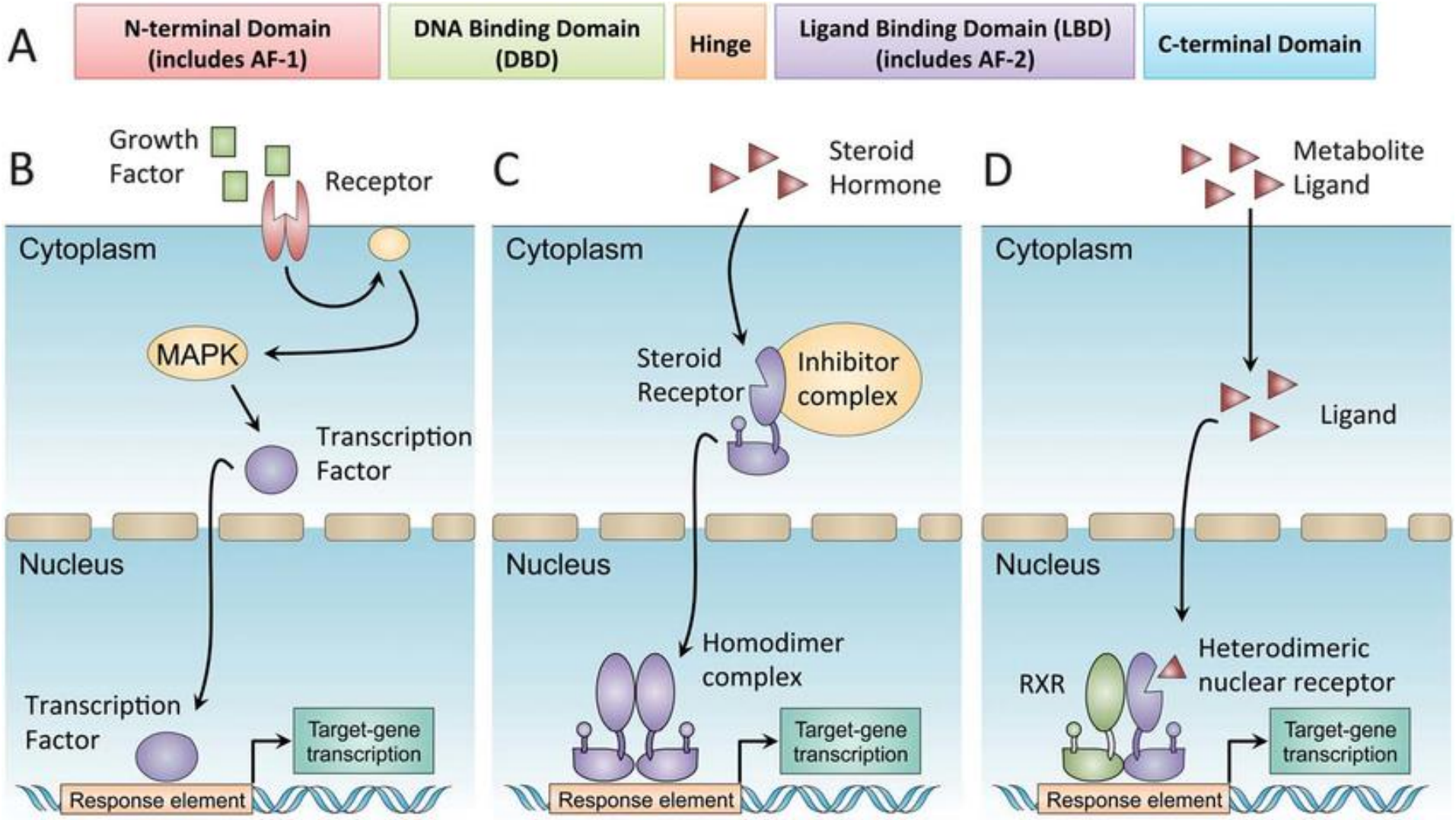
Without ligand, they are often linked to corepressors.

Ligand binding on this receptor induces:

- Co-repressor dissociation and co-activator recruitment
- RNApol recruitment by DNA/protein complex

Ex: Retinoid acid receptor, Retinoid X receptor, Thyroid hormone receptor

TRANSCRIPTIONAL REGULATION: NUCLEAR RECEPTORS



- DNA MODIFICATIONS
- TRANSCRIPTIONAL REGULATION
- **POST-TRANSCRIPTIONAL REGULATION**
- TRANSLATIONAL REGULATION
- POST-TRANSLATIONAL REGULATION

POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION

- Alternative RNA processing (splicing)
- Class switching
- Regulation of RNA stability
- RNA editing

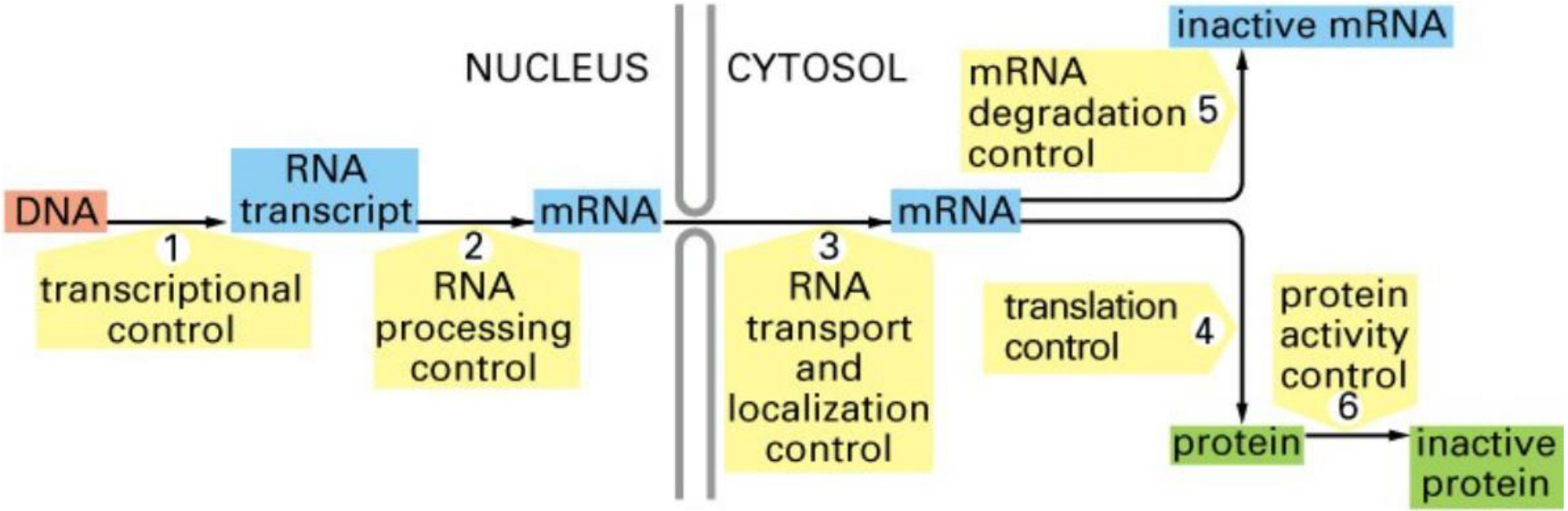
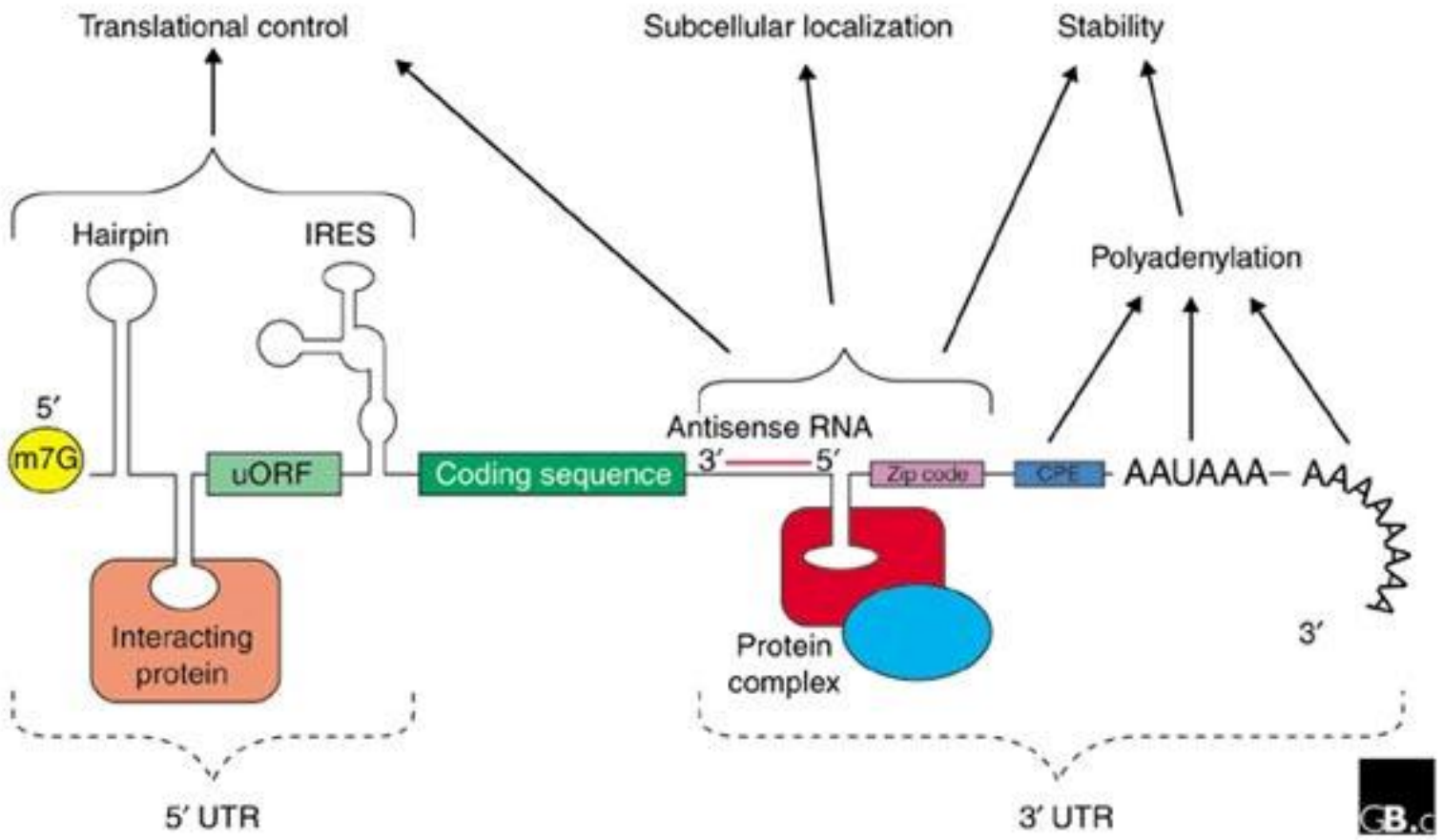


Figure 7–5. Molecular Biology of the Cell, 4th Edition.

POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION



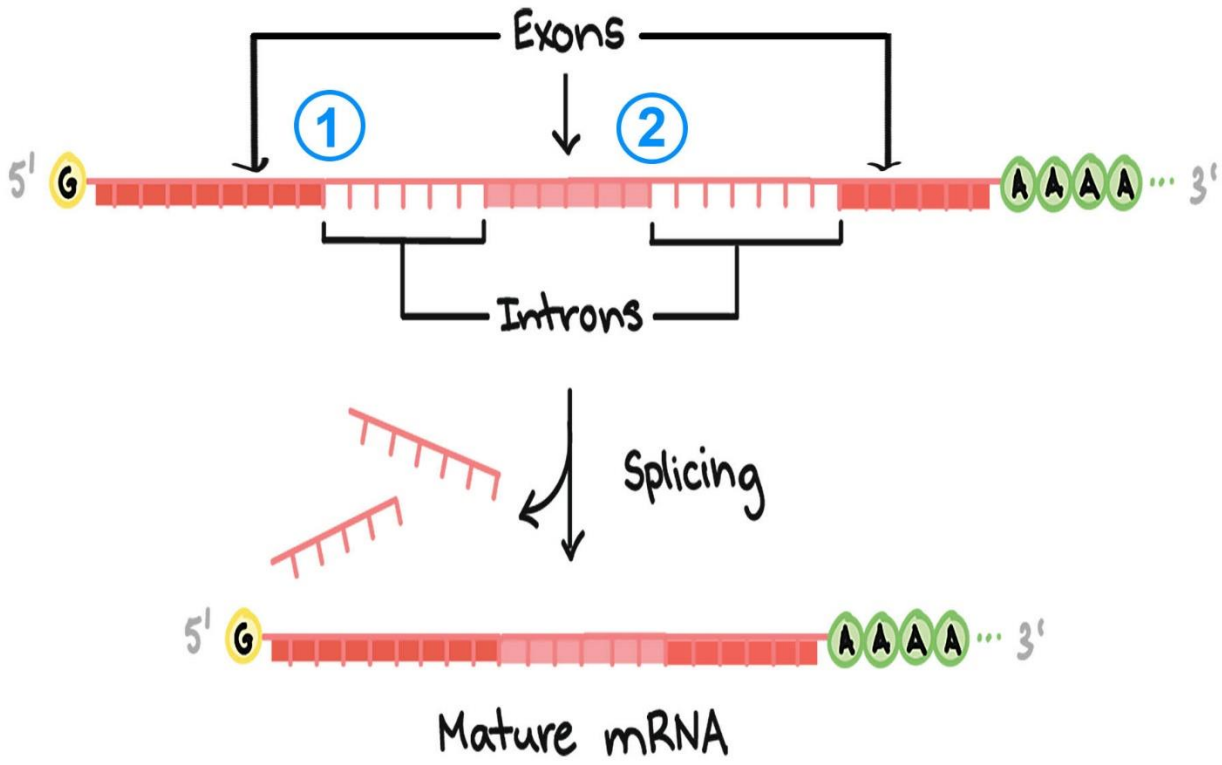
The generic structure of a eukaryotic mRNA, illustrating some posttranscriptional regulatory elements that affect gene expression. Abbreviations (from 5 to 3): UTR, untranslated region; m7G, 7-methyl-guanosine cap; hairpin, hairpin-like secondary structures; uORF, upstream open reading frame; IRES, internal ribosome entry site; CPE, cytoplasmic polyadenylation element; AAUAAA, polyadenylation signal.

POST-TRANSCRIPTIONAL REGULATION: RNA SPLICING

Definitions:

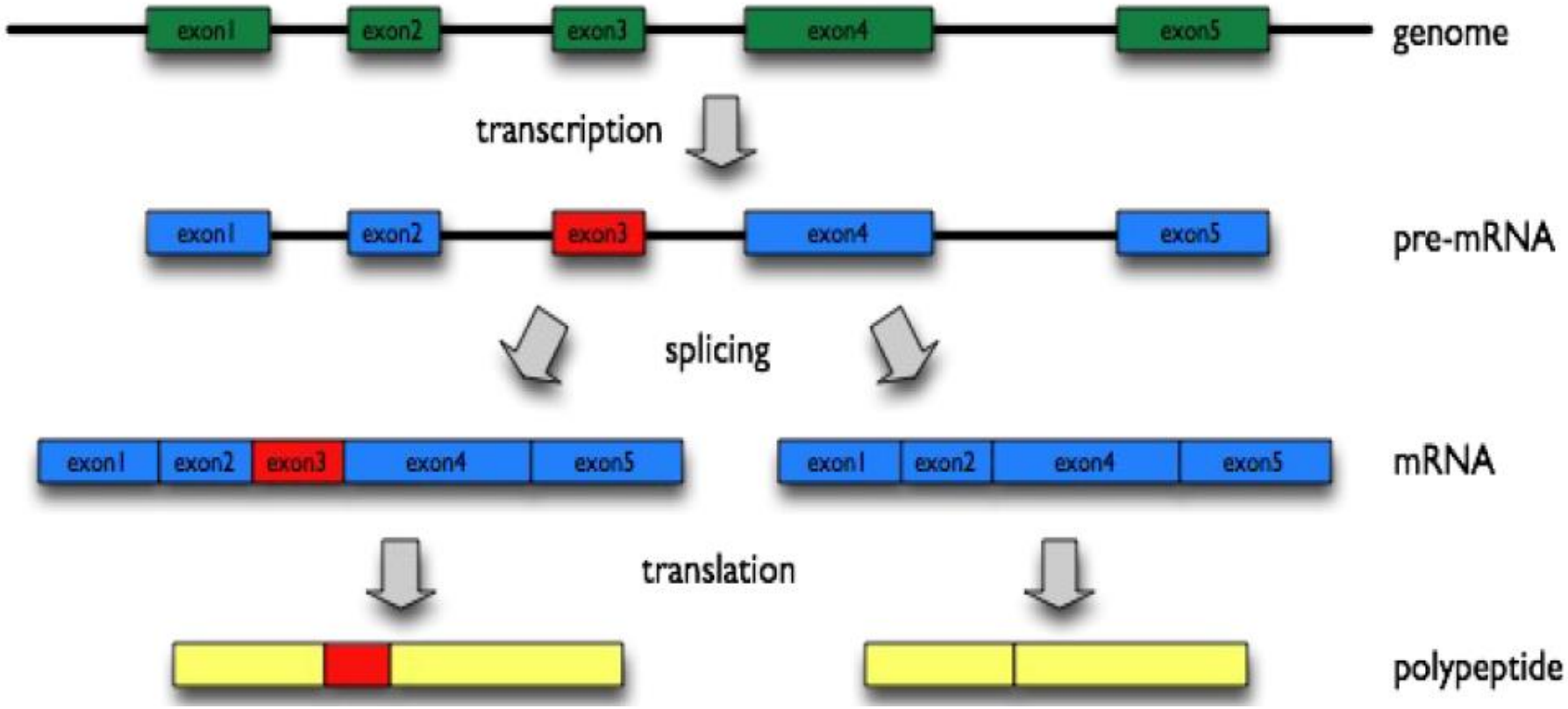
- Exons = coding sequences involved in translation
- Introns = noncoding sequences

RNA splicing is the process by which introns, regions of RNA that do not code for proteins, are removed from the pre-mRNA and the remaining exons connected to re-form a single continuous molecule.



POST-TRANSCRIPTIONAL REGULATION: ALTERNATIVE RNA SPLICING

- Eukaryotic cells employ alternative RNA processing to control gene expression.
- This can result when alternative promoters, intron-exon splice sites, or polyadenylation sites are used.
- Occasionally, heterogeneity within a cell results, but more commonly the same primary transcript is processed differently in different tissues.



POST-TRANSCRIPTIONAL REGULATION: mRNA STABILITY

- Different mRNAs within the same cell have distinct lifetimes (stabilities).
- In mammalian cells, mRNA lifetimes range from several minutes to days.
- The greater the stability of an mRNA the more protein may be produced from that mRNA.
- The limited lifetime of mRNA enables a cell to alter protein synthesis rapidly in response to its changing needs.

- Changes in the stability of a specific mRNA can therefore have major effects on biologic processes.
- The stability of the mRNA can be influenced by hormones and certain other effectors.
- The ends of mRNA molecules are involved in mRNA stability.
- The 5' cap structure in eukaryotic mRNA prevents attack by 5' exonucleases, and the poly(A) tail prohibits the action of 3' exonucleases.

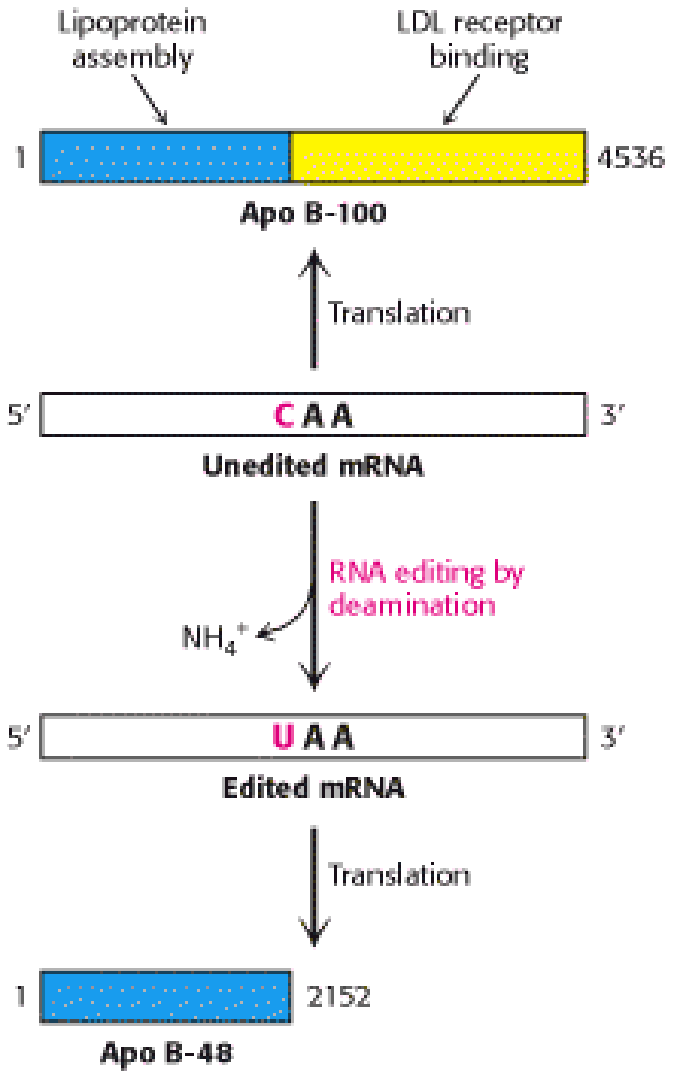
- Capping of the pre-mRNA involves the addition of 7-methylguanosine (m7G) to the 5' end.
- To achieve this, the terminal 5' phosphate requires removal, which is done with the aid of a phosphatase enzyme.
- The pre-mRNA processing at the 3' end of the RNA molecule involves cleavage of its 3' end and then the addition of about 250 adenine residues to form a poly(A) tail.

POST-TRANSCRIPTIONAL REGULATION: mRNA EDITING

RNA editing (also **RNA modification**) is a molecular process through which some cells can make discrete changes to specific nucleotide sequences within an RNA molecule after it has been generated by RNA polymerase

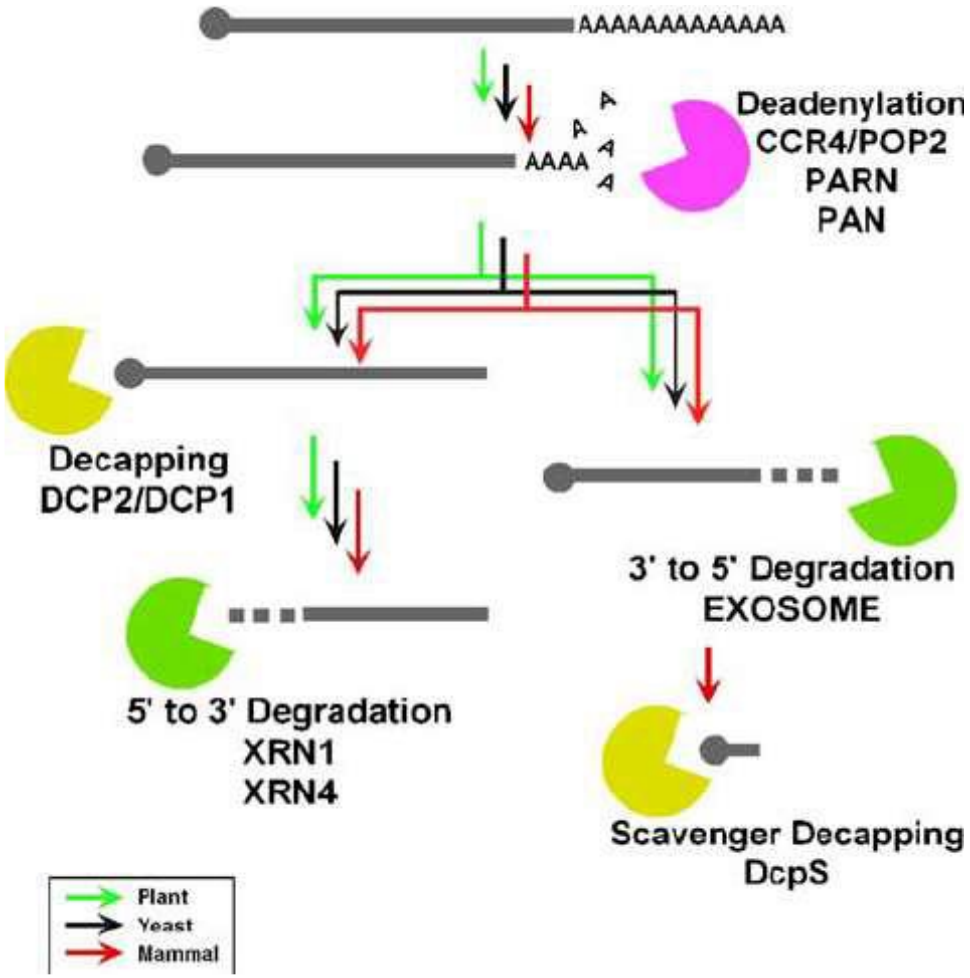
It can be:

- insertion or deletion
- deamination (C-to-U, A-to-I)



POST-TRANSCRIPTIONAL REGULATION: mRNA DEGRADATION

Inside eukaryotic cells, there is a balance between the processes of translation and mRNA decay.



AU-rich element decay

Eukaryotic messages are subject to surveillance by nonsense mediated decay (NMD), which checks for the presence of premature stop codons (nonsense codons) in the message.

Small interfering RNA (siRNA)

Small interfering RNAs (siRNAs) processed by Dicer are incorporated into a complex known as the RNA-induced silencing complex or RISC.
This complex contains an endonuclease that cleaves perfectly complementary messages to which the siRNA binds. The resulting mRNA fragments are then destroyed by exonucleases

MicroRNA (miRNA)

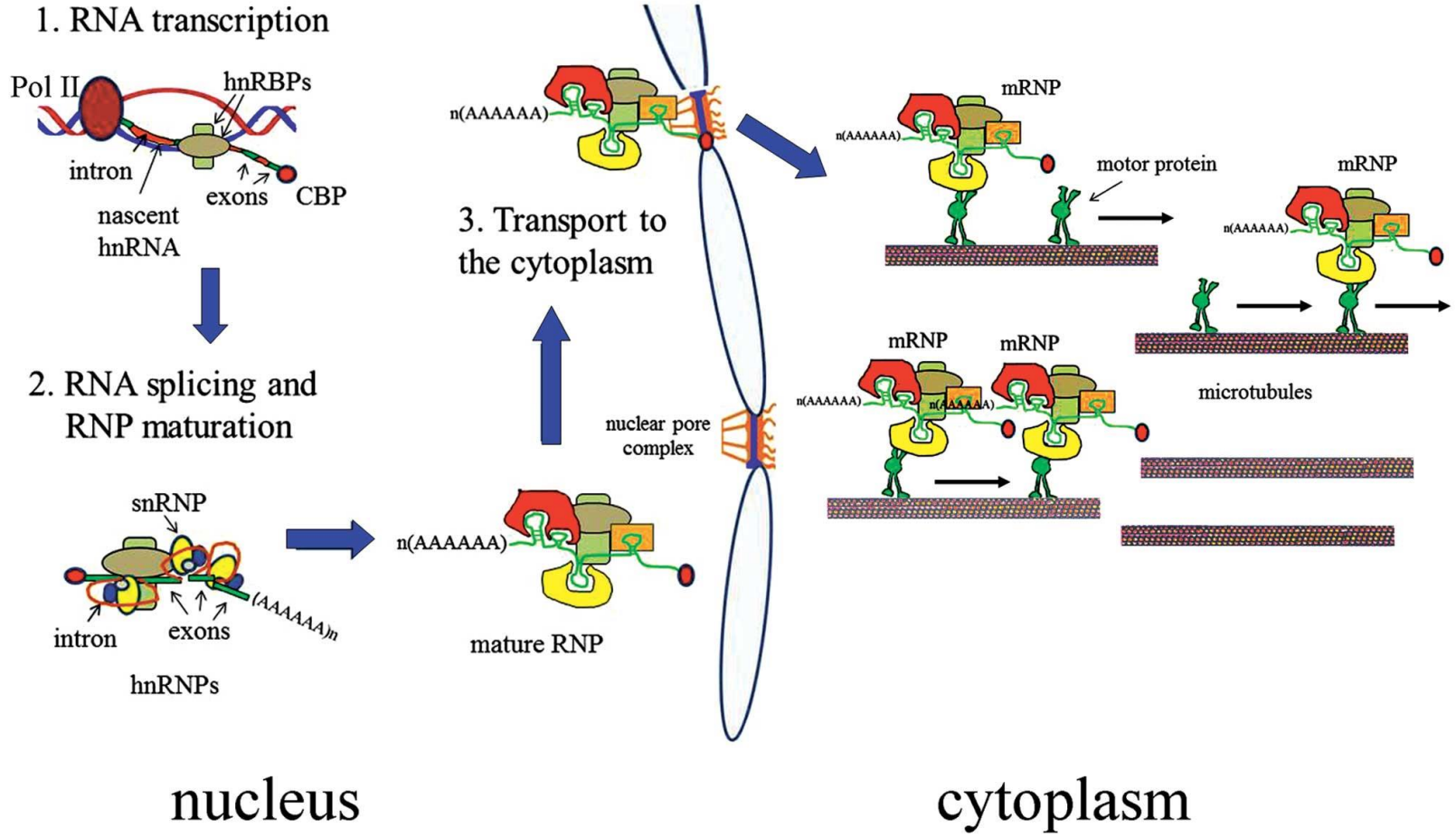
MicroRNAs (miRNAs) are small RNAs that typically are partially complementary to sequences in mRNAs.
Binding of a miRNA to a message can repress translation of that message and accelerate polyA tail removal, thereby hastening mRNA degradation.

Other decay mechanisms

There are other ways by which messages can be degraded, including non-stop decay and silencing by Piwi-interacting RNA (piRNA)

POST-TRANSCRIPTIONAL REGULATION: mRNA AND PROTEIN TRANSPORT

- Nuclear pores control RNA state to let them leave the nucleus.
- The pores let enter only the proteins having a signal sequence adressing them to the nucleus (nuclear localization signal). This protein filtration is essential for transcription regulation.



- DNA MODIFICATIONS
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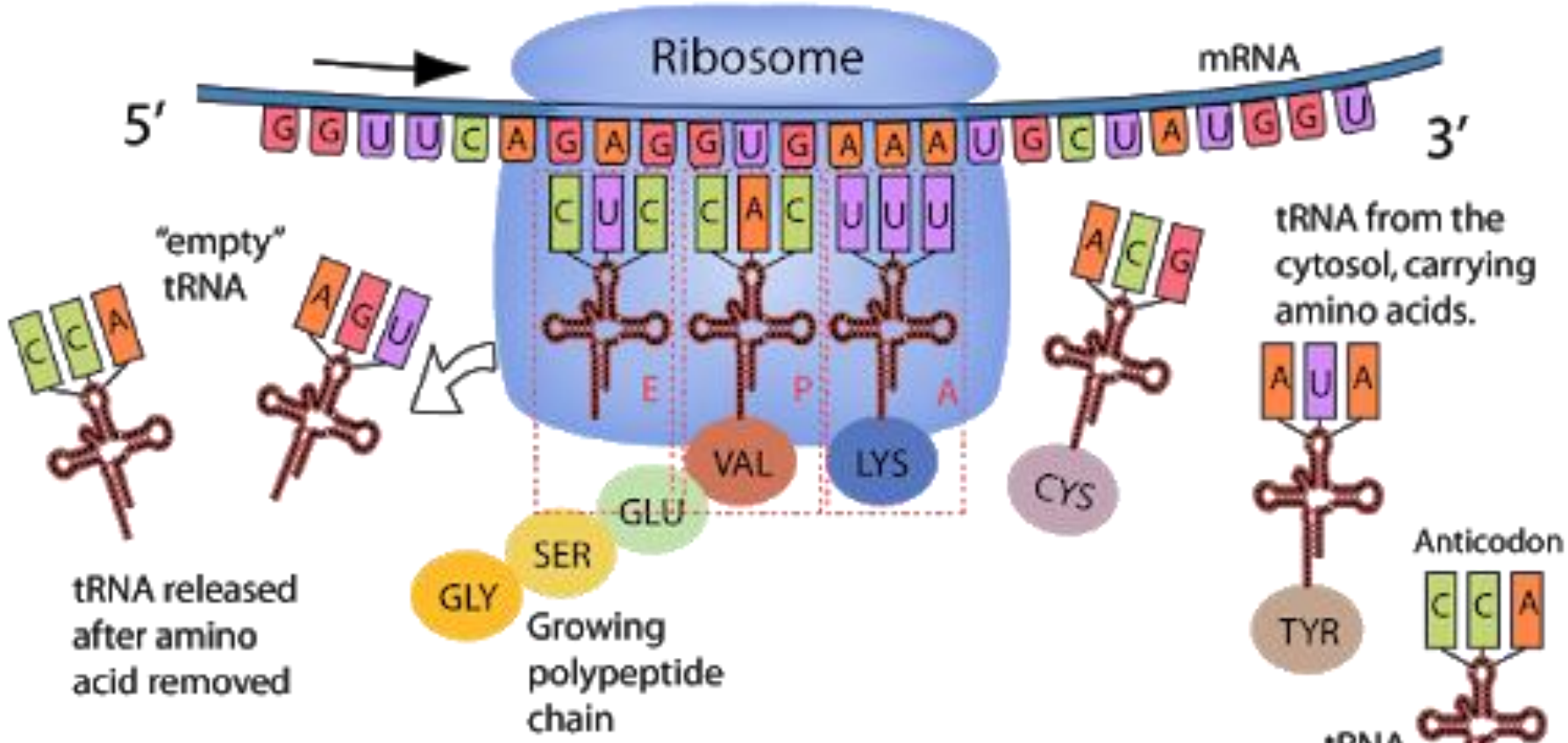
- **TRANSLATIONAL REGULATION**
- POST-TRANSLATIONAL REGULATION

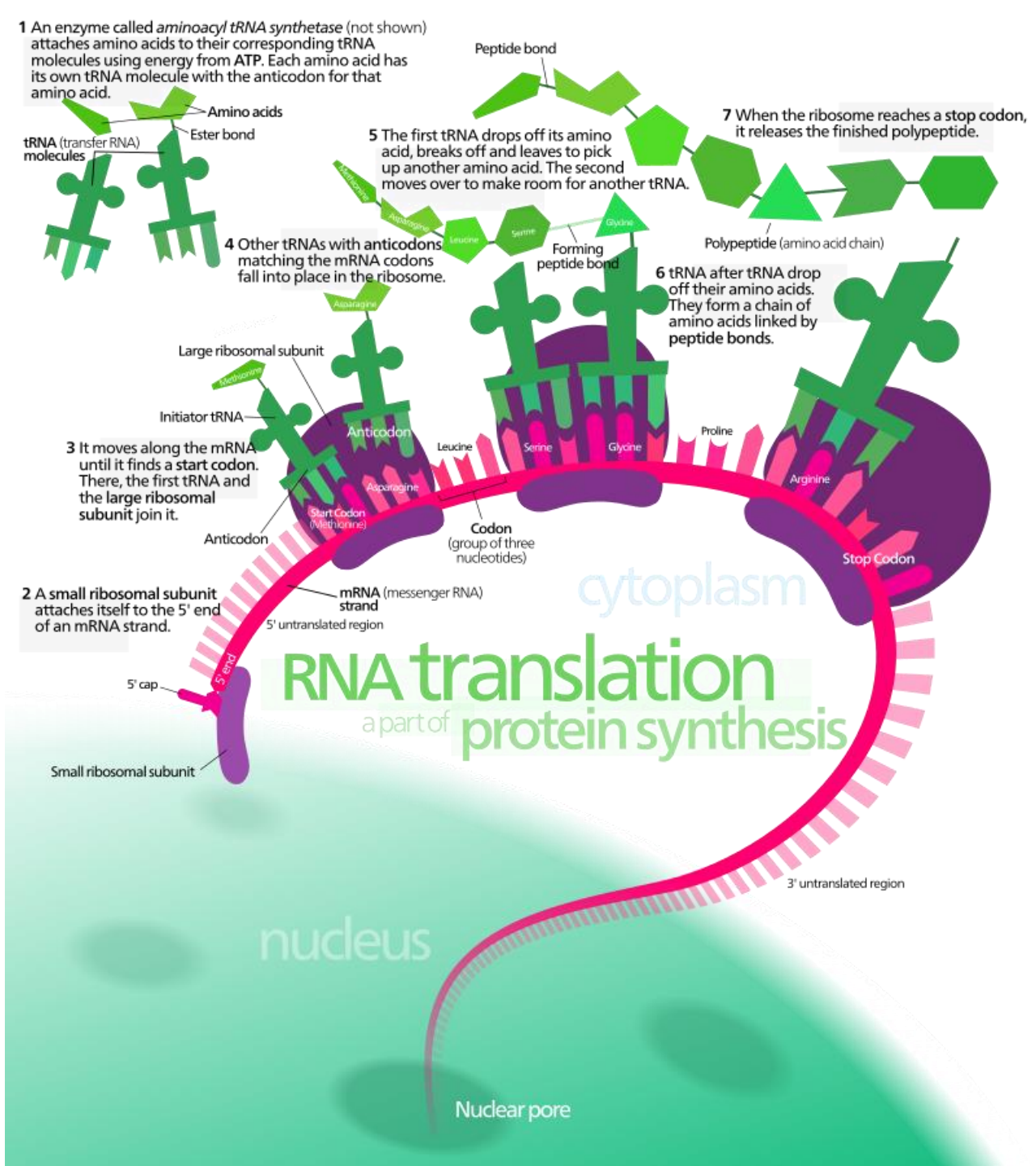
TRANSLATIONAL REGULATION

Translation is the process in endoplasmic reticulum in which ribosomes synthesize protein according to the information contained in the mRNA

There is 3 phases in translation:
1.Initiation: The ribosome assembles around the target mRNA. The first tRNA is attached at the start codon.
2.Elongation: The tRNA transfers an amino acid to the tRNA corresponding to the next codon. The ribosome then moves (*translocates)* to the next mRNA codon to continue the process, creating an amino acid chain.
3.Termination: When a stop codon is reached, the ribosome releases the polypeptide.



The translation process incorporates 20 different amino acids in the precise sequence dictated by the three-base codons built from and alphabet of four bases. The process in the ribosome builds the polypeptide chains tha will become proteins.


1 An enzyme called *aminoacyl tRNA synthetase* (not shown) attaches amino acids to their corresponding tRNA molecules using energy from ATP. Each amino acid has its own tRNA molecule with the anticodon for that amino acid.
Amino acids
Ester bond
tRNA (transfer RNA) molecules
Peptide bond
5 The first tRNA drops off its amino acid, breaks off and leaves to pick up another amino acid. The second moves over to make room for another tRNA.
7 When the ribosome reaches a stop codon, it releases the finished polypeptide.
Polypeptide (amino acid chain)
4 Other tRNAs with anticodons matching the mRNA codons fall into place in the ribosome.
Forming peptide bond
6 tRNA after tRNA drop off their amino acids. They form a chain of amino acids linked by peptide bonds.
Large ribosomal subunit
Initiator tRNA
Anticodon
3 It moves along the mRNA until it finds a start codon. There, the first tRNA and the large ribosomal subunit join it.
Anticodon
Codon (group of three nucleotides)
Start Codon (Methionine)
Methionine
Asparagine
Leucine
Serine
Glycine
Proline
Arginine
Stop Codon
2 A small ribosomal subunit attaches itself to the 5' end of an mRNA strand.
mRNA (messenger RNA) strand
5' untranslated region
5' end
5' cap
Small ribosomal subunit
3' untranslated region
cytoplasm
RNA translation
a part of protein synthesis
nucleus
Nuclear pore

TRANSLATIONAL REGULATION

- Translational regulation refers to the control of the levels of protein synthesized from its mRNA.
- In comparison to transcriptional regulation, it results in much more immediate cellular adjustment through direct regulation of protein concentration.
- The corresponding mechanisms are primarily targeted on the control of ribosome recruitment on the initiation codon, but can also involve modulation of the elongation or termination of protein synthesis.
- In most cases, translational regulation involves specific RNA secondary structures on the mRNA.

TRANSLATIONAL REGULATION: INITIATION

-> The 40S subunit binds to the 5’ cap of mRNA via initiation factors and move on the mRNA until the first AUG codon to initiate translation

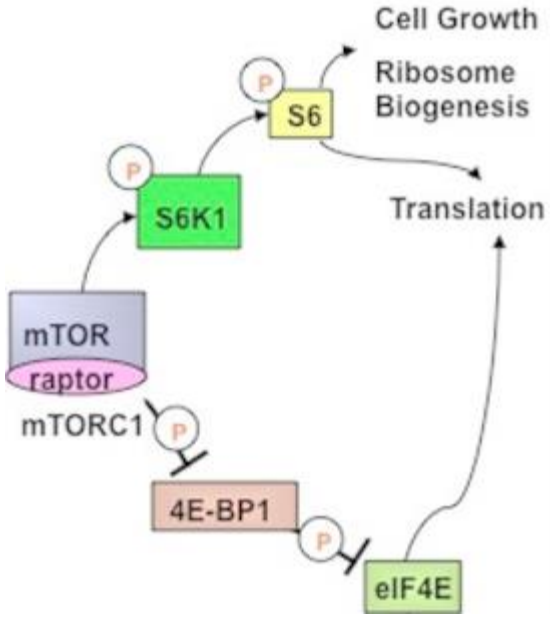
- The initiation codon can be hidden by a antisense RNA to impair ribosome recruitment
- The initiation factor IF-2 (initiation factor 2) can be regulated by phosphorylation (it is inhibited when phosphorylated)
- A secondary structure of mRNA located between the cap and the AUG codon can block the 40S subunit
- Some proteins need a frameshift to be translated

TRANSLATIONAL REGULATION: ELONGATION AND TERMINATION

Eukaryotic elongation factor 2 (eEF2) is a GTP-dependent translocase that moves nascent polypeptide chains from the A-site to the P-site in the ribosome.
Phosphorylation of threonine in position 56 inhibits the binding of eEF2 to the ribosome. Cell stress can activate this process by inducing translational inhibition through this biochemical interaction.

During termination, the release factors eRF1 and eRF3 are responsible of polypeptid chain hydrolysis. In some cases, stop codons can be interpreted as aminoacids. Ex: addition of selenocystein in selenoproteins

TRANSLATIONAL REGULATION: mTOR PATHWAY



mTOR = mammalian target of rapamycin

Serine/threonine kinase

Regulates cell proliferation, cell growth, cell mobility, survival, protein biosynthesis

mTORC1 = complex formed by mTOR, Raptor, PRAS40 (proline-rich Akt substrate of 40kDa), Deptor (DEP domain-containing mTOR-interacting protein) and a protein apparented to b subunit of the G/LST8 complex

This complex controls protein biosynthesis by phosphorylating S6K1 and 4E-BP (eIF4E binding protein) which inhibits eIF4E

The general level of RNA translation is controlled by this factor availability

This complex activity can be stimulated by insulin, growth factors, some aminoacids as leucine and repressed by oxydative stress

- DNA MODIFICATIONS
- TRANSCRIPTIONAL REGULATION
- POST-TRANSCRIPTIONAL REGULATION
- TRANSLATIONAL REGULATION
- **POST-TRANSLATIONAL REGULATION**

POST-TRANSLATIONAL REGULATION

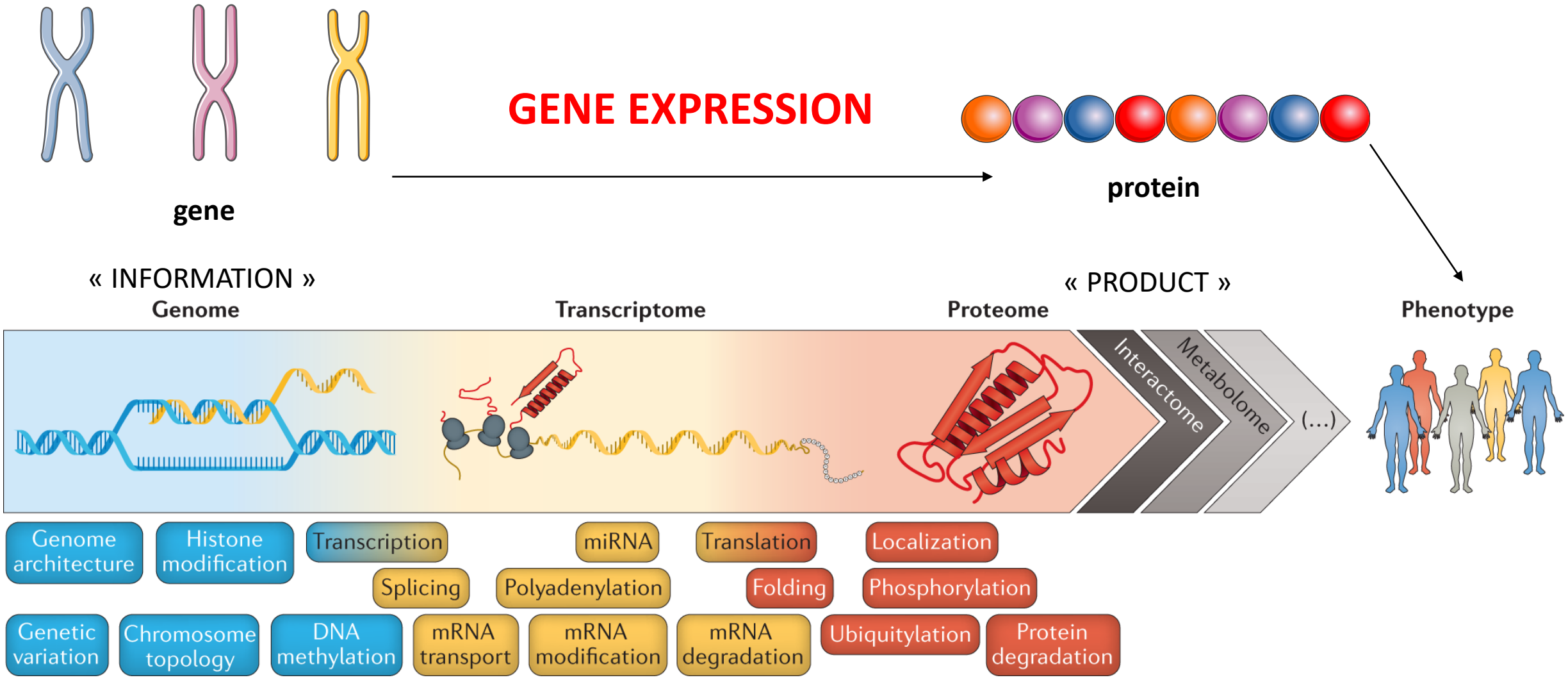
- **Protein activation**

Some proteins are not active when first formed so to be active they undergo modifications:

- Localization (addition of hydrophobic groups)
- Addition of cofactors
- Folding
- Enzymatic cleavage

- **Protein degradation**
  - Ubiquitin proteasome system
  - Autophagy

SUMMARY



Buccitelli&Selbach, Nature Review Genetics, 2020