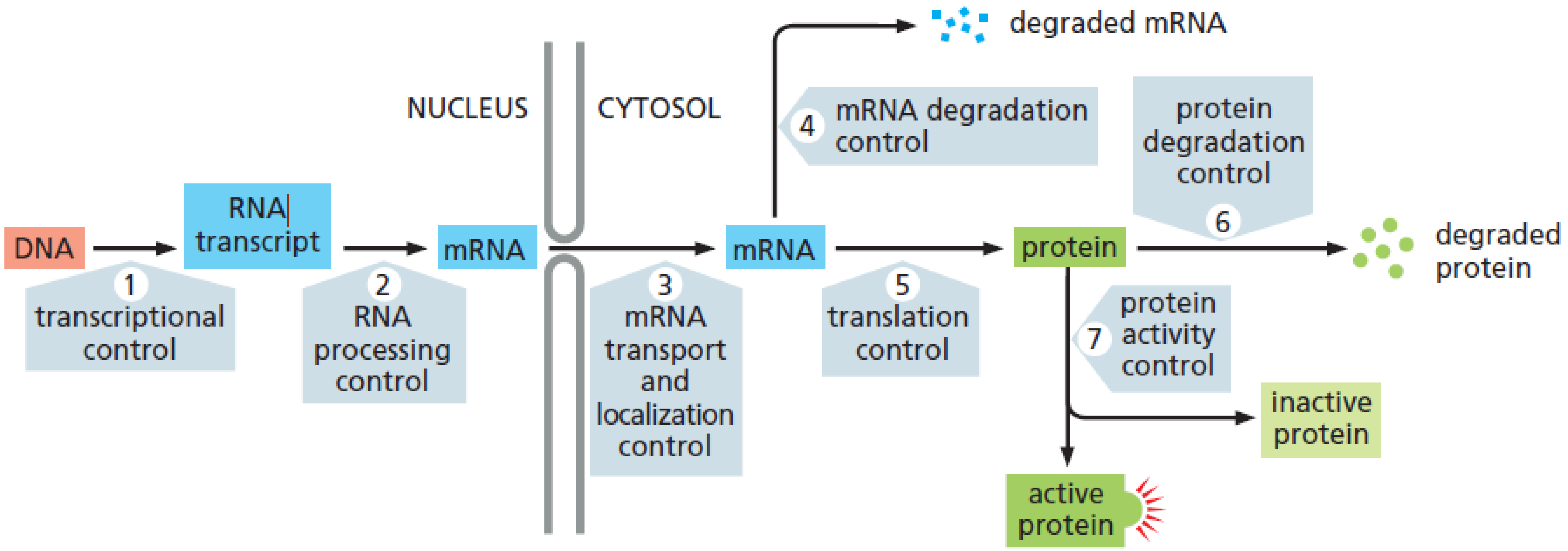
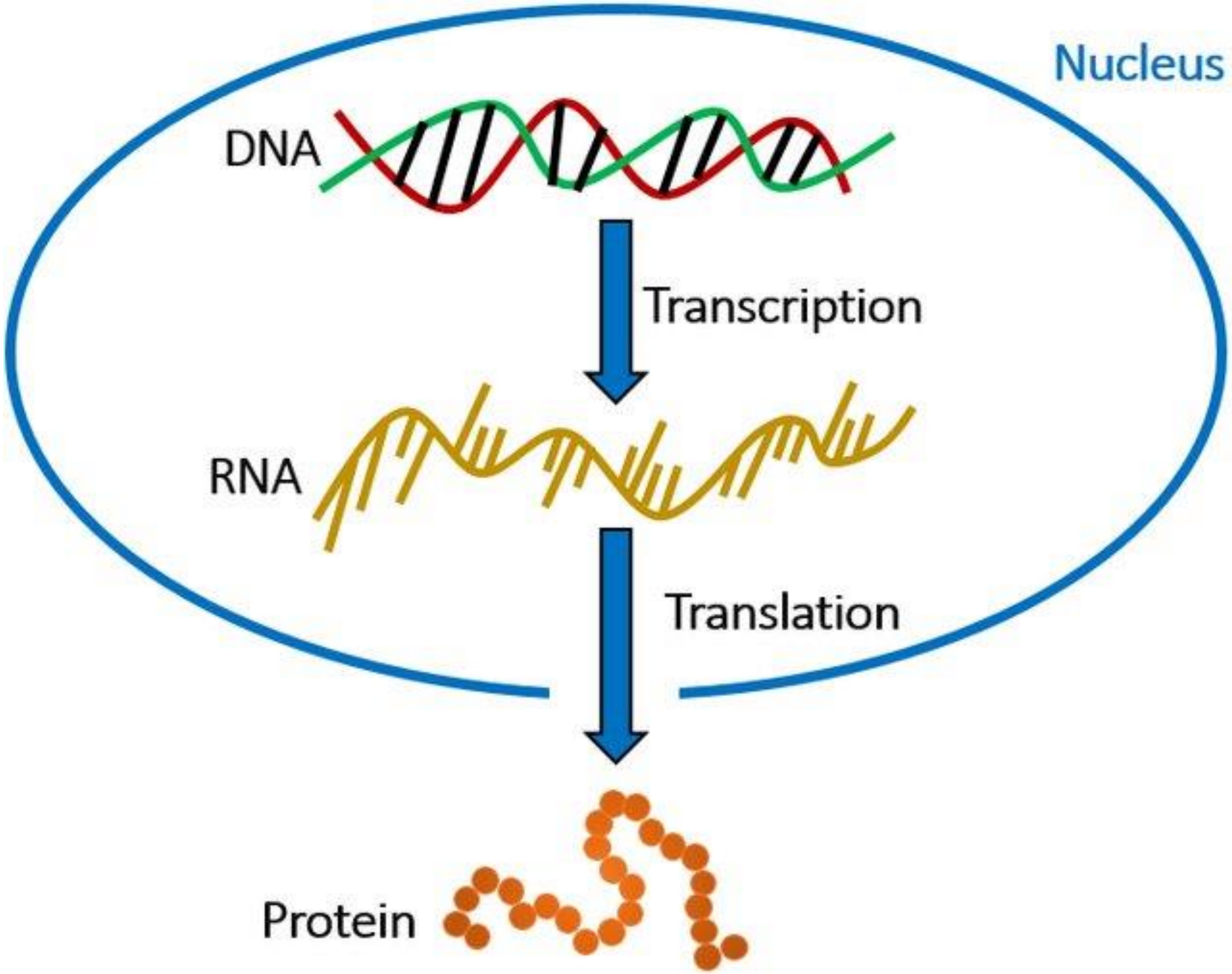
Regulation of Gene Expression I



จารุพงษ์ แสงบุญมี, พ.บ., ปร.ด.

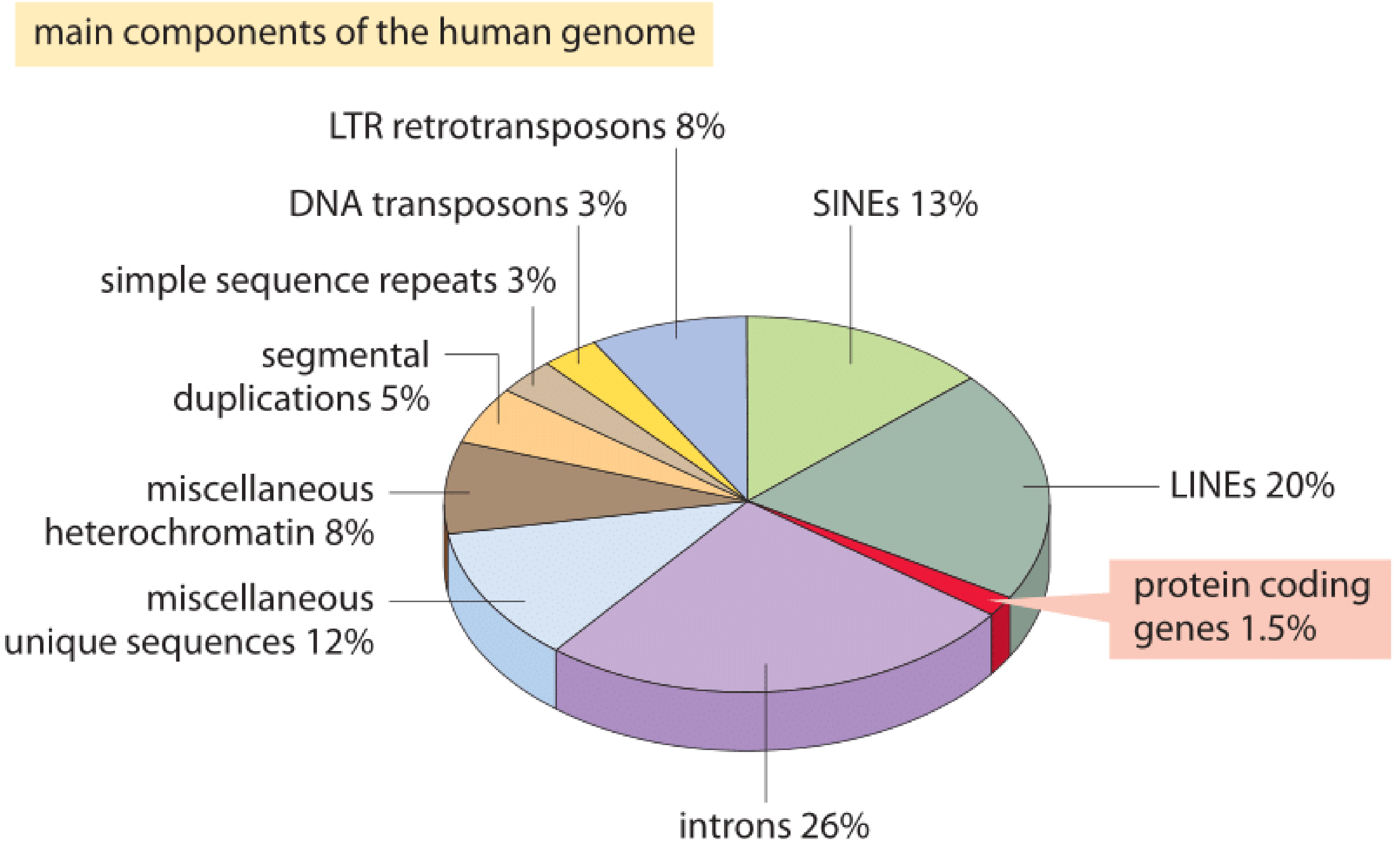
สาขาวิชาชีวเคมี คณะแพทยศาสตร์

Central dogma: flow of genetic information



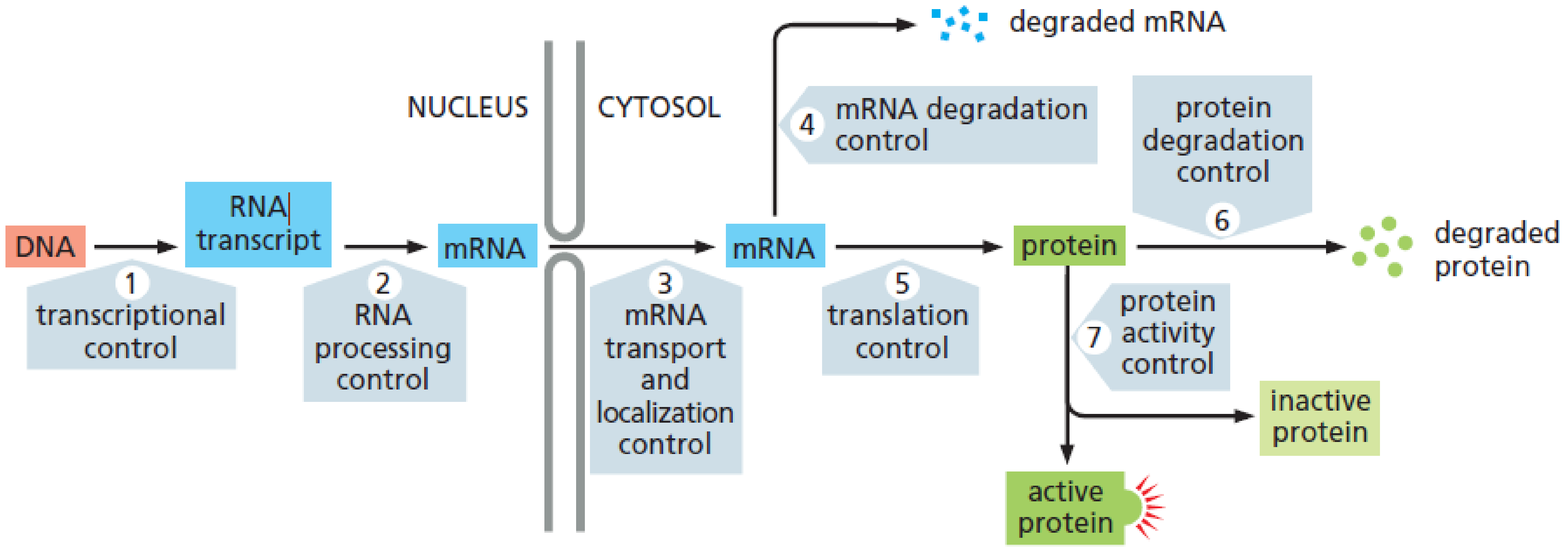
- Tahvildari R., 2017

Protein coding genes are minor proportion.



http://book.bionumbers.org/how-many-genes-are-in-a-genome/

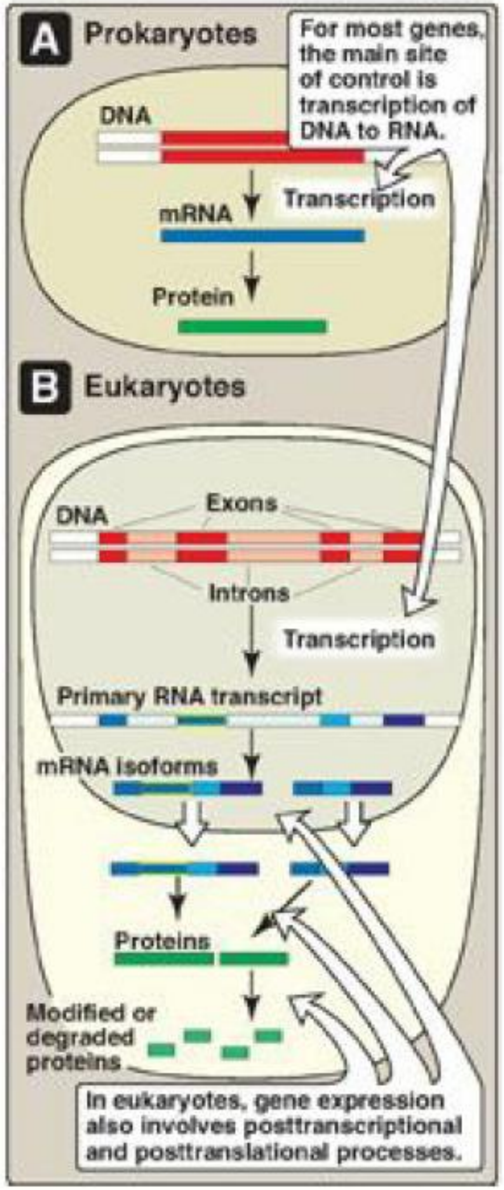
Gene expression can be regulated at multiple step.



The main step of the control is transcription.

Albert et al. (Editors). Essential Cell Biology. 5th Ed. 2019.

Overview of gene expression



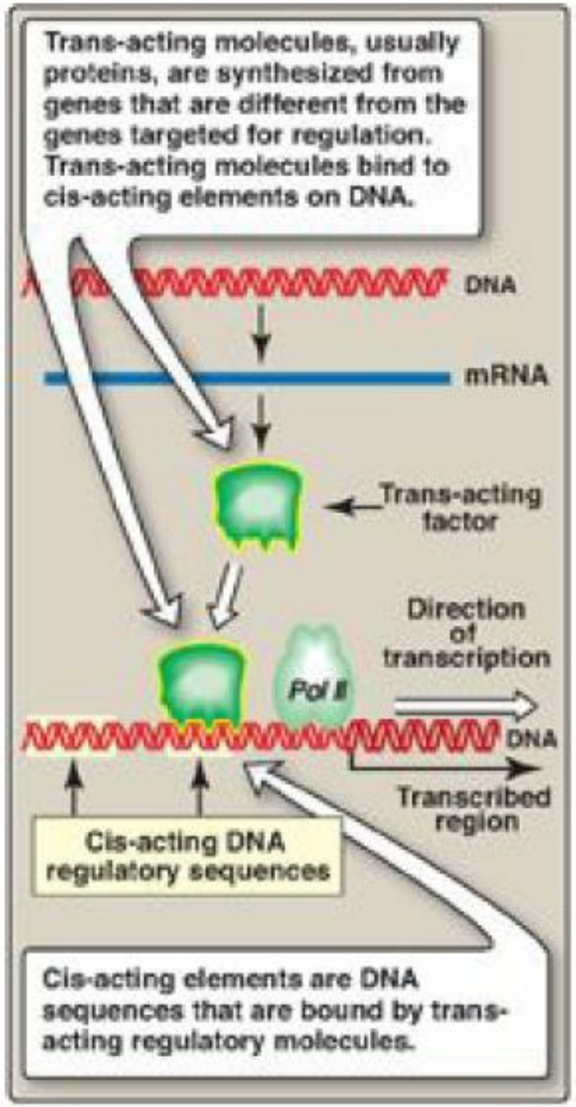
- Gene expression refers to the multistep process that ultimately results in the production of a functional gene product, either ribonucleic acid (RNA) or protein.
- Transcription is the primary site of regulation in both prokaryotes and eukaryotes.
- In eukaryotes gene expression also involves extensive posttranscriptional and posttranslational processes.

Harvey RA. Lippincott’s Illustrated Reviews Biochemistry, 6th ed. 2014.

Overview of gene expression

- Not all genes are regulated. For example, “housekeeping” genes are continually expressed.
- Regulated genes are expressed only under certain conditions.

Regulatory sequences and molecules



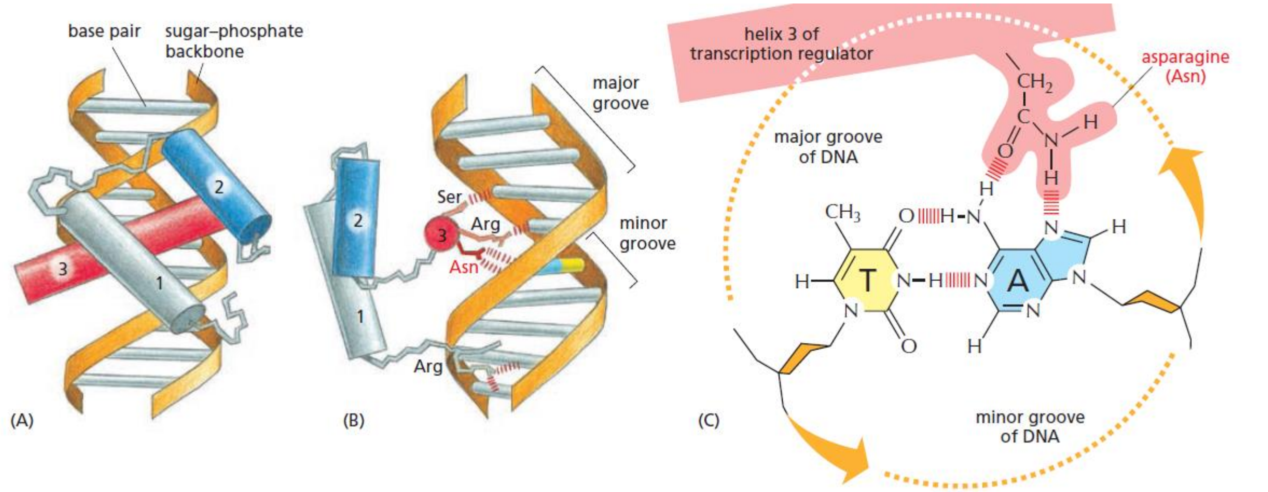
Trans-acting molecules, usually proteins, are synthesized from genes that are different from the genes targeted for regulation. Trans-acting molecules bind to cis-acting elements on DNA.

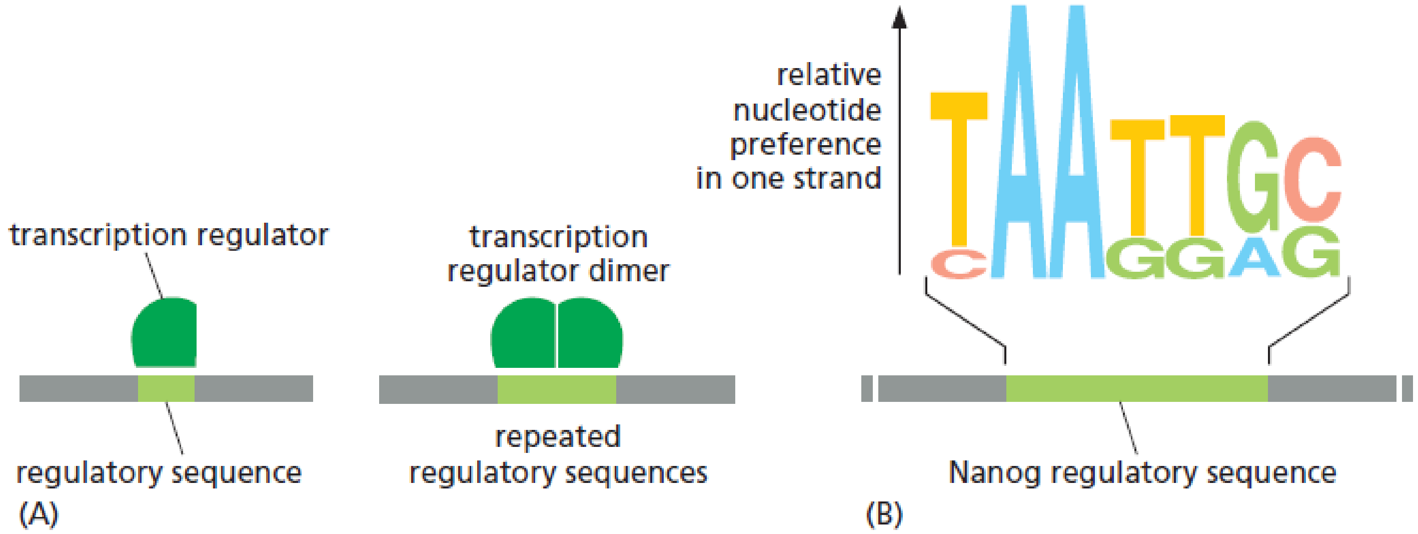
Cis-acting elements are DNA sequences that are bound by trans-acting regulatory molecules.

- The initial step in all gene expression controlled by regulatory sequences of DNA.
- The interaction between DNA segments and regulatory molecules can engage or repress the transcription.
- DNA sequences flanking a gene to regulate the transcription are called cis-acting.
- Trans-acting factor is the regulatory molecule which can transit (diffuse) through the cell from its site of synthesis to its DNA-binding site

Harvey RA. Lippincott’s Illustrated Reviews Biochemistry, 6th ed. 2014.

- Binding of transcription regulators (transcription factor) to a regulatory DNA sequence acts as the switch to control transcription

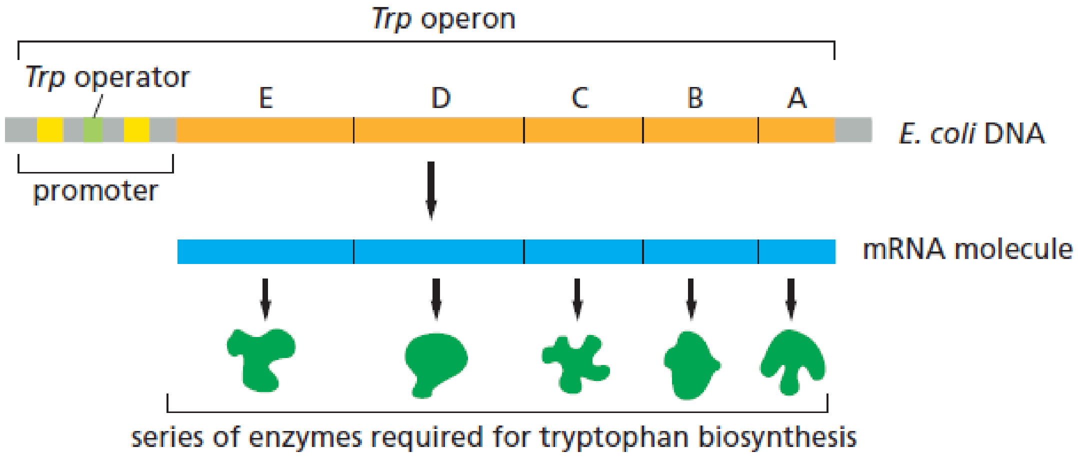




Albert et al. (Editors). Essential Cell Biology. 5th Ed. 2019.

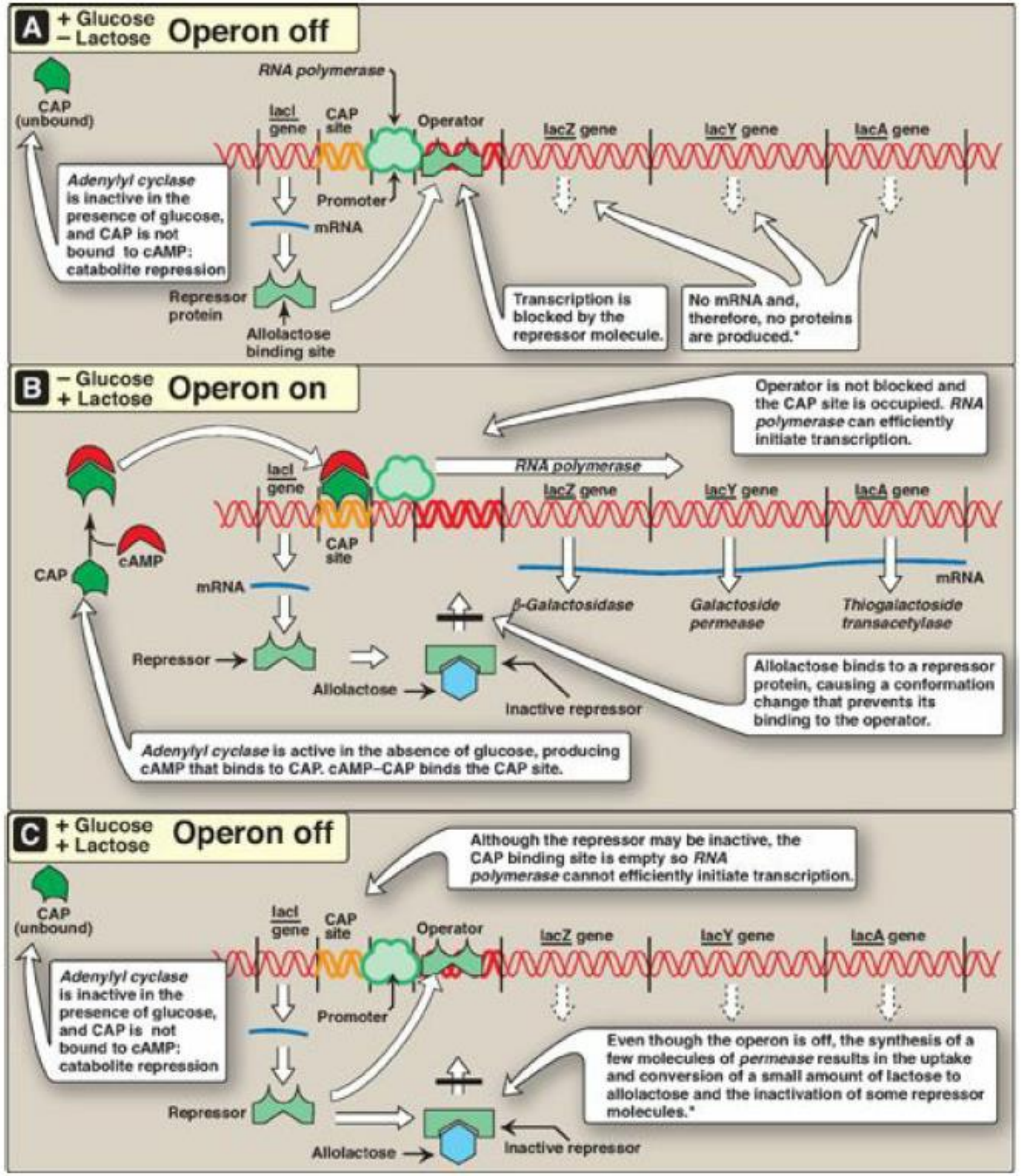
Regulation of prokaryotic gene expression

- Regulation occurs at the level of transcription, mediated by the binding of transacting proteins to cis-acting regulatory elements.
- Transcriptional control in prokaryotes can involve the initiation or premature termination of transcription.
- The structural genes that code for proteins involved in a particular metabolic pathway are often found sequentially grouped along with the cis-acting regulatory elements call "operon".



Albert et al. (Editors). Essential Cell Biology. 5th Ed. 2019.

The lac operon



Adenylyl cyclase is inactive in the presence of glucose, and CAP is not bound to cAMP: catabolite repression

Transcription is blocked by the repressor molecule.

No mRNA and, therefore, no proteins are produced.*

Operator is not blocked and the CAP site is occupied. *RNA polymerase* can efficiently initiate transcription.

Allolactose binds to a repressor protein, causing a conformation change that prevents its binding to the operator.

Adenylyl cyclase is active in the absence of glucose, producing cAMP that binds to CAP. cAMP–CAP binds the CAP site.

Although the repressor may be inactive, the CAP binding site is empty so *RNA polymerase* cannot efficiently initiate transcription.

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 synthesis 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.*

Harvey RA. Lippincott's Illustrated Reviews Biochemistry, 6th ed. 2014.

Lac I: repressor protein (a trans-acting factor) binding to the O site with high affinity

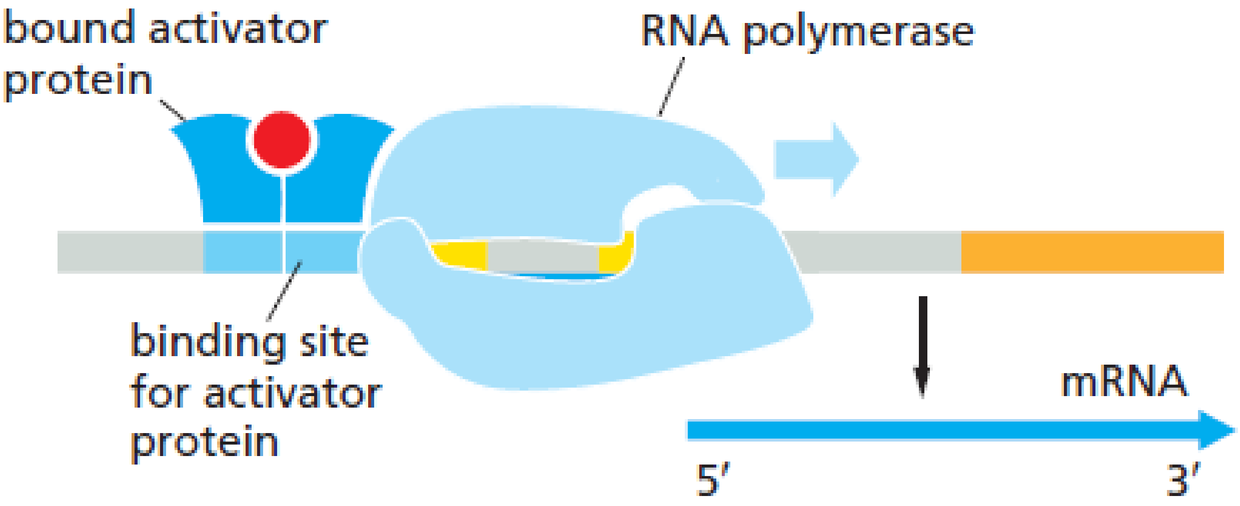
Lac Z: β-galactosidase

Lac Y: permease

Lac A: thiogalactoside transacetylase

The lac Z, lac Y, and lac A genes are expressed only when the O site is empty, and the CAP site is bound by a complex of cAMP and the catabolite activator protein (CAP) or cAMP regulatory protein (CRP).

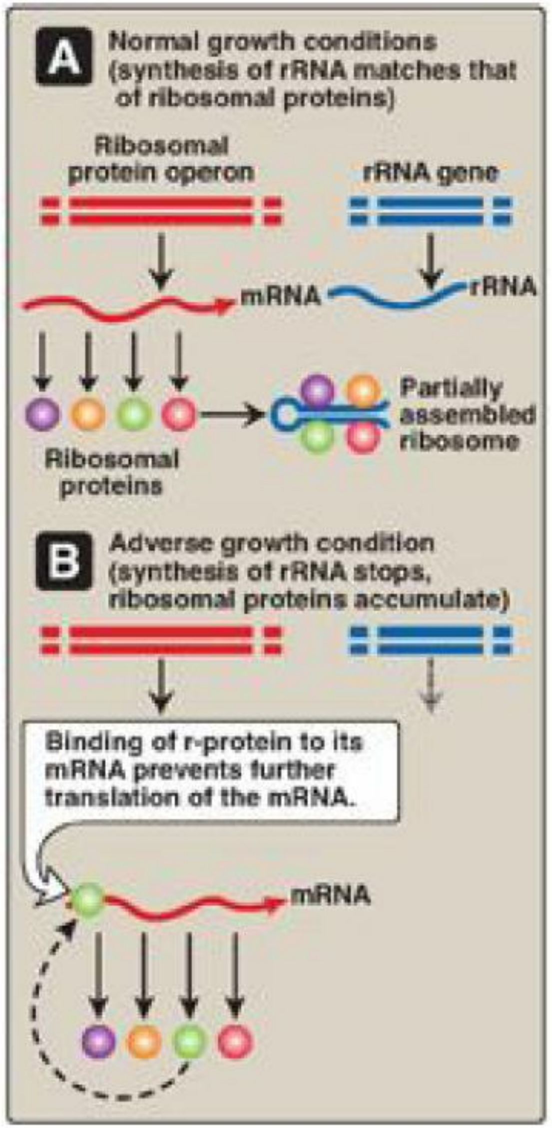
Activator = a positive regulator



An activator protein binds to a regulatory sequence on the DNA and then interacts with the RNA polymerase to help it initiate transcription. Without the activator, the promoter fails to initiate transcription efficiently.

Albert et al. (Editors). Essential Cell Biology. 5th Ed. 2019.

Coordination of transcription and translation in prokaryotes



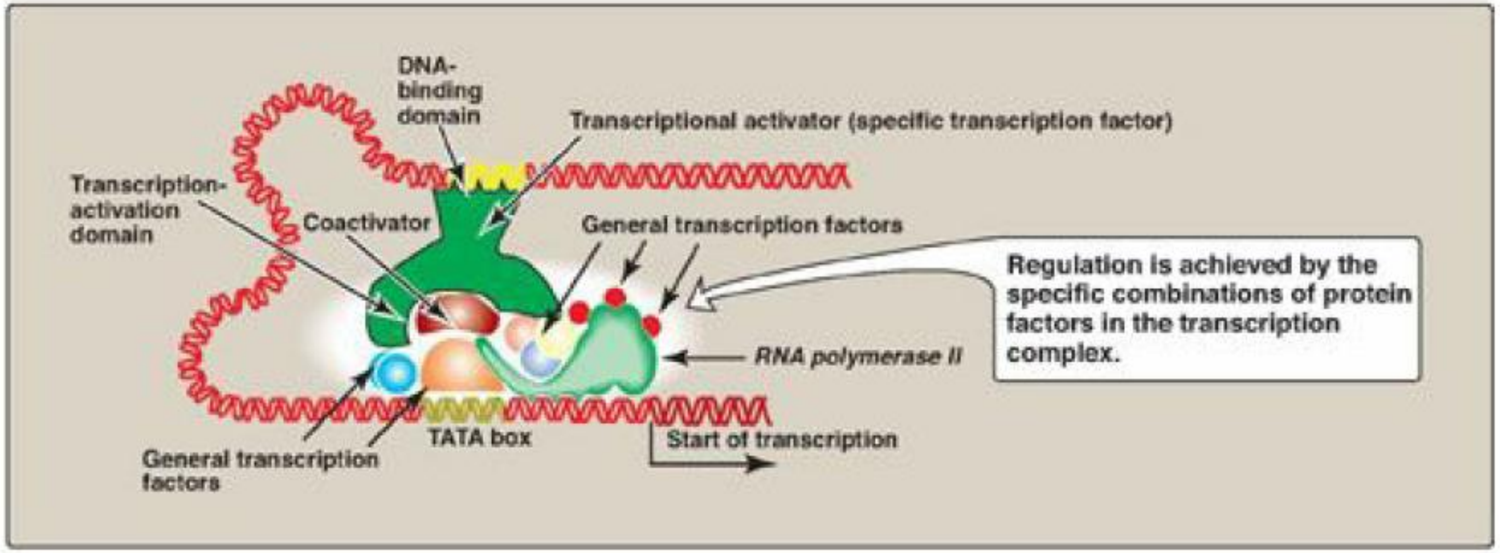
- Operons for ribosomal proteins (r-proteins) can be inhibited by an excess of their own protein products.
- The r-protein binding to the Shine-Dalgarno (SD) sequence acting as a physical impediment to the binding of the small ribosomal subunit to the SD sequence.
- One r-protein thus inhibits synthesis of all the r-proteins of the operon.

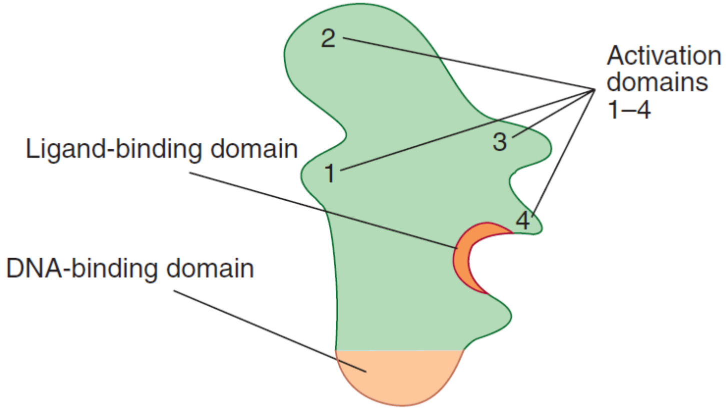
Harvey RA. Lippincott’s Illustrated Reviews Biochemistry, 6th ed. 2014.

Regulation of Eukaryote Gene Expression

- The primary site of regulation is at the level of transcription.
- The theme of trans-acting molecules binding to cis-acting elements is also seen.
- Operons are generally not found in eukaryotes.
- Gene expression is regulated at multiple levels other than transcription [mRNA splicing, control of mRNA stability, and control of translational efficiency].

Trans-acting molecules



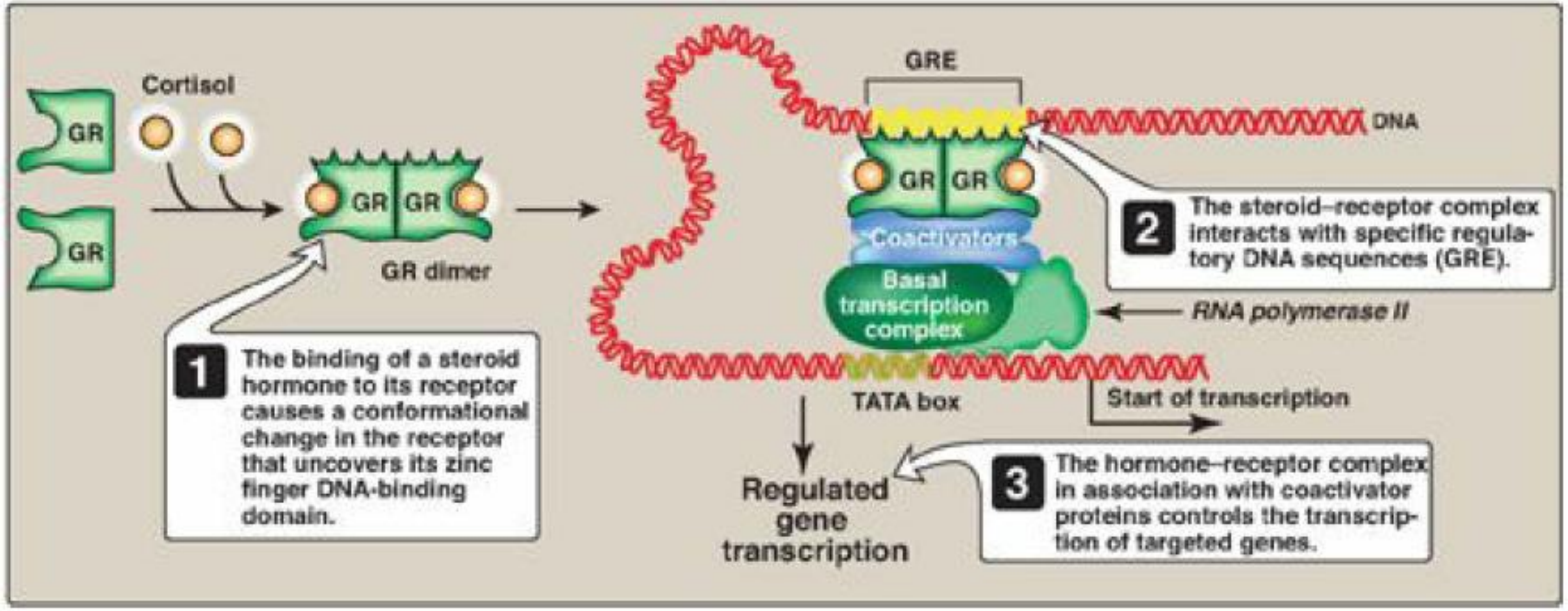


- Specific transcription factors are trans-acting DNA-binding proteins functions as an activators.
- They have at least two binding domains: the DNA-binding and the transcription-activation domain.
- The transcription-activation domain recruits other proteins, such as the GTF and coactivators.
- These facilitate formation of the transcription initiation complex (RNA polymerase II plus the GTFs) at the promoter, and activate transcription.

Harvey RA. Lippincott's Illustrated Reviews Biochemistry, 6th ed. 2014.

Rodwell et al. Harper's Illustrated Biochemistry, 30th ed, 2015

Cis-acting regulatory elements



1 The binding of a steroid hormone to its receptor causes a conformational change in the receptor that uncovers its zinc finger DNA-binding domain.

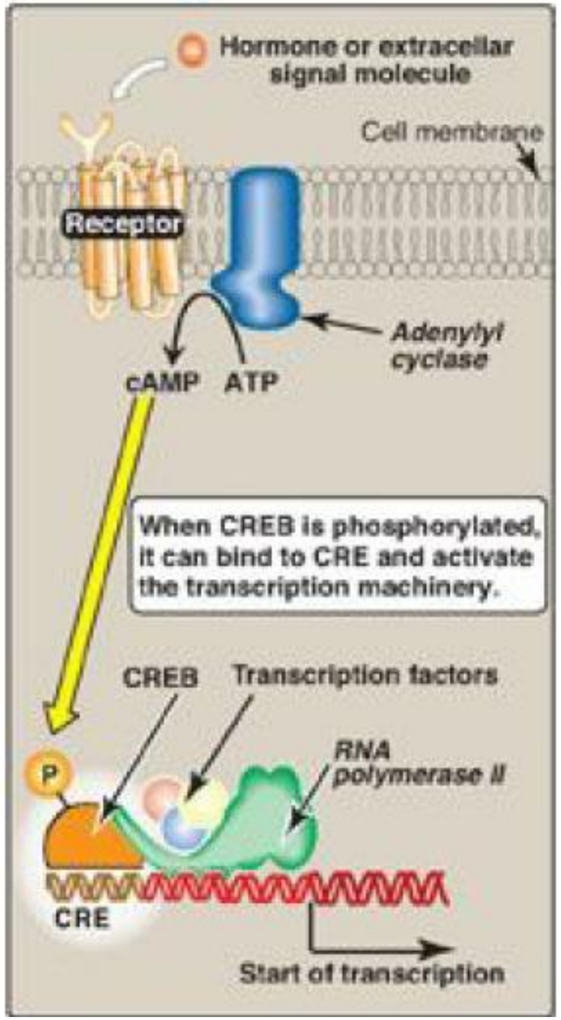
2 The steroid–receptor complex interacts with specific regulatory DNA sequences (GRE).

3 The hormone–receptor complex in association with coactivator proteins controls the transcription of targeted genes.

- The binding of specific protein to a regulatory consensus element on each of the genes can affect their expression.
- For example, hormone-response elements (HREs) are cis-acting DNA sequences that bind trans-acting protein factors and regulate gene expression in response to hormonal signals.

Harvey RA. Lippincott’s Illustrated Reviews Biochemistry, 6th ed. 2014.

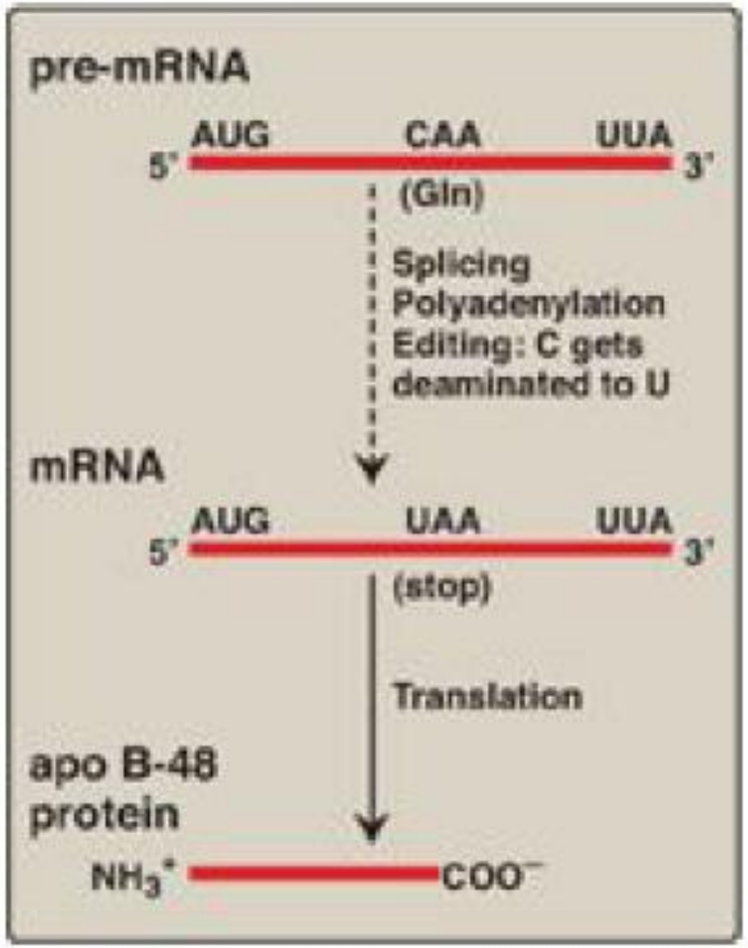
Regulatory signals mediated by cell-surface receptors



Harvey RA. Lippincott's Illustrated Reviews Biochemistry, 6th ed. 2014.

Regulation by processing of messenger RNA

- Messenger RNA editing

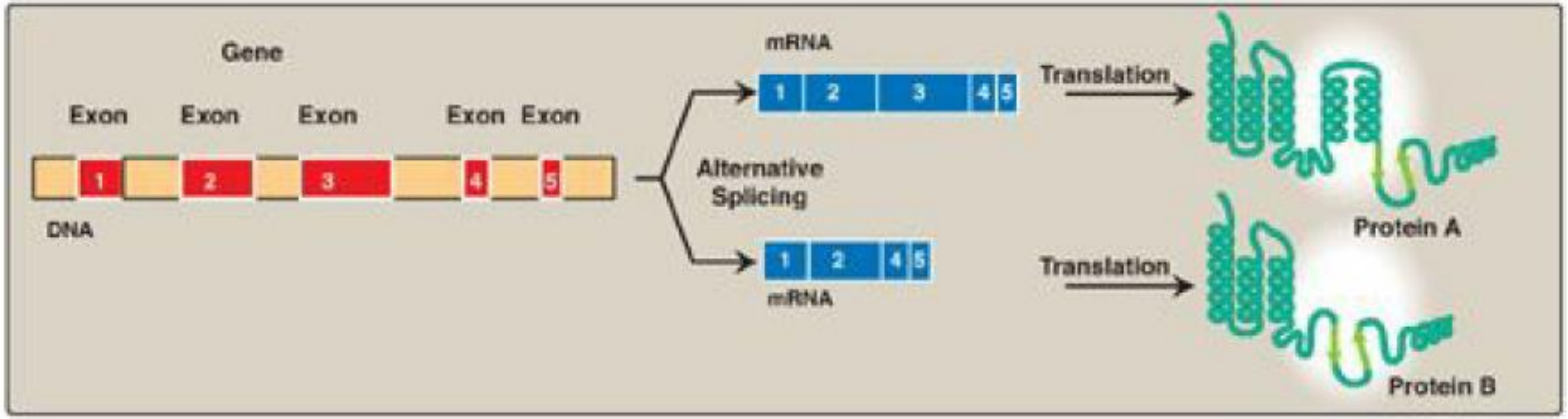


- Apolipoprotein (apo) B is a component of chylomicrons and very low density lipoproteins (VLDL).
- In the intestine only, the C residue in the CAA codon for glutamine is deaminated to U, changing to a stop codon resulted in a shorter protein (apo B-48).
- Apo B48 represents 48% of apo B-100, full-length, incorporated into VLDL synthesized in liver.

Harvey RA. Lippincott's Illustrated Reviews Biochemistry, 6th ed. 2014.

Regulation by processing of messenger RNA

- RNA splicing

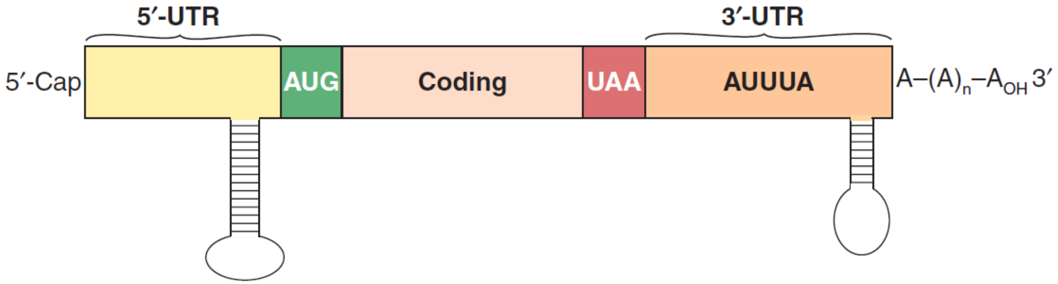


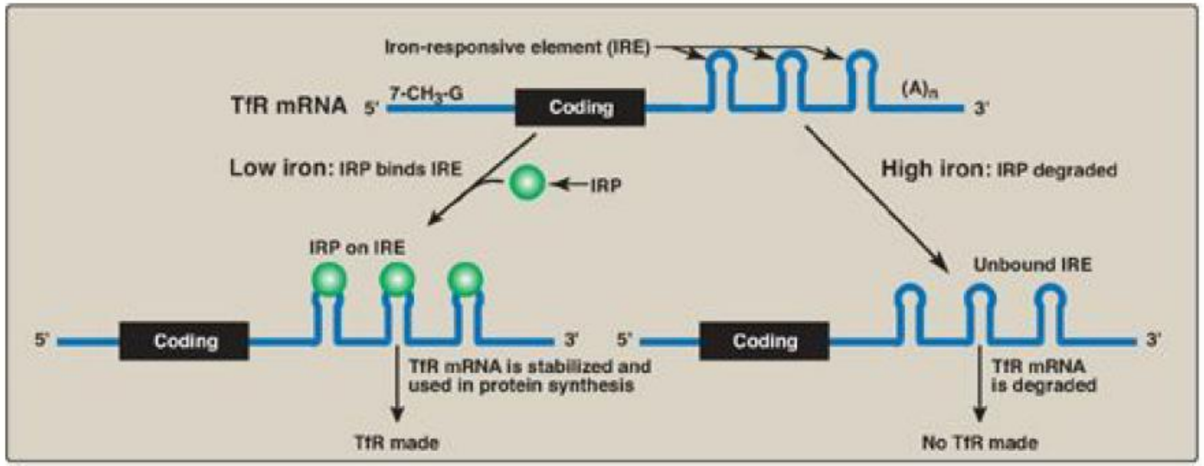
“Over 60% percent of the approximately 25,000 genes in the human genome undergo differential splicing. The use of alternative polyadenylation and transcription start sites is also seen in many genes. This explains, at least in part, how 25,000 genes can give rise to hundreds of thousands of proteins.”

Harvey RA. Lippincott’s Illustrated Reviews Biochemistry, 6th ed. 2014.

Regulation by processing of messenger RNA

- Messenger RNA stability





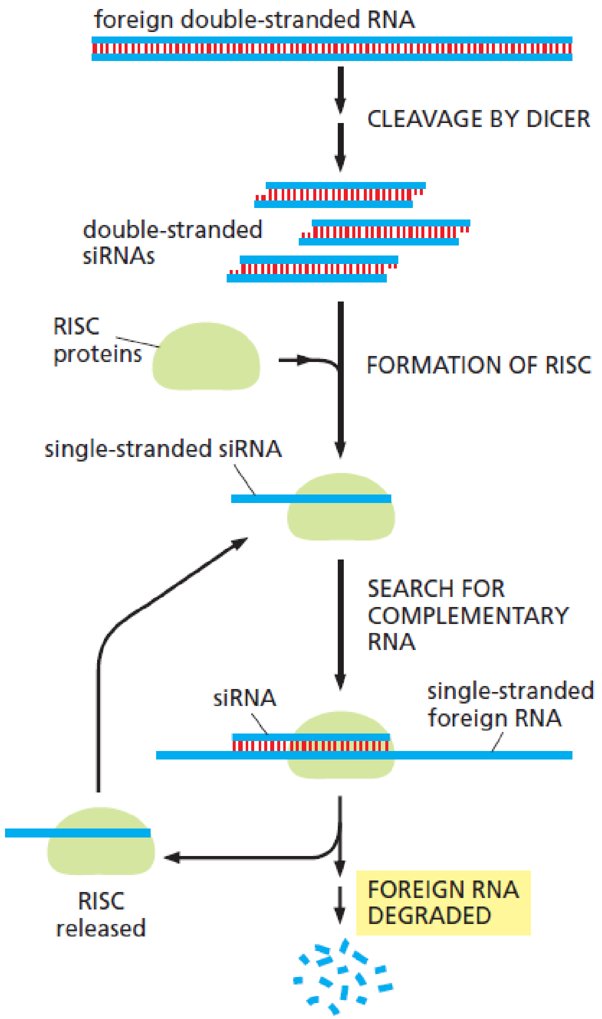
- The mRNA for the transferrin receptor (TfR) has several cis-acting iron-responsive elements (IREs) at its 3-end.
- IREs have a short stem–loop structure that can be bound by trans-acting iron regulatory proteins [IRPs].
- When the iron concentration in the cell is low, the IRPs bind to the 3-IREs and stabilize the mRNA for TfR, allowing TfR synthesis.
- When intracellular iron levels are high, the IRPs are degraded.

Rodwell et al. Harper's Illustrated Biochemistry, 30th ed, 2015

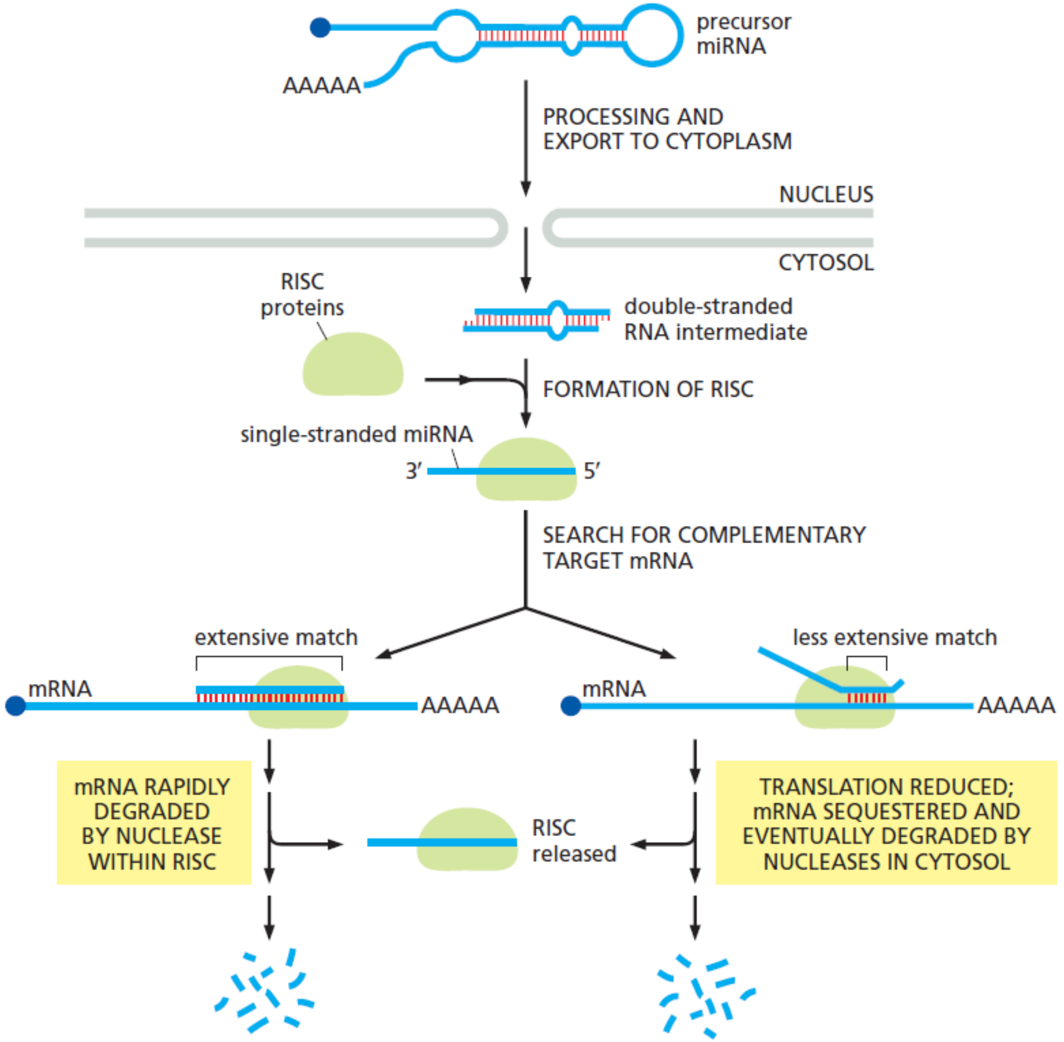
Harvey RA. Lippincott's Illustrated Reviews Biochemistry, 6th ed. 2014.

Regulation by processing of messenger RNA

- RNA interference (RNAi)

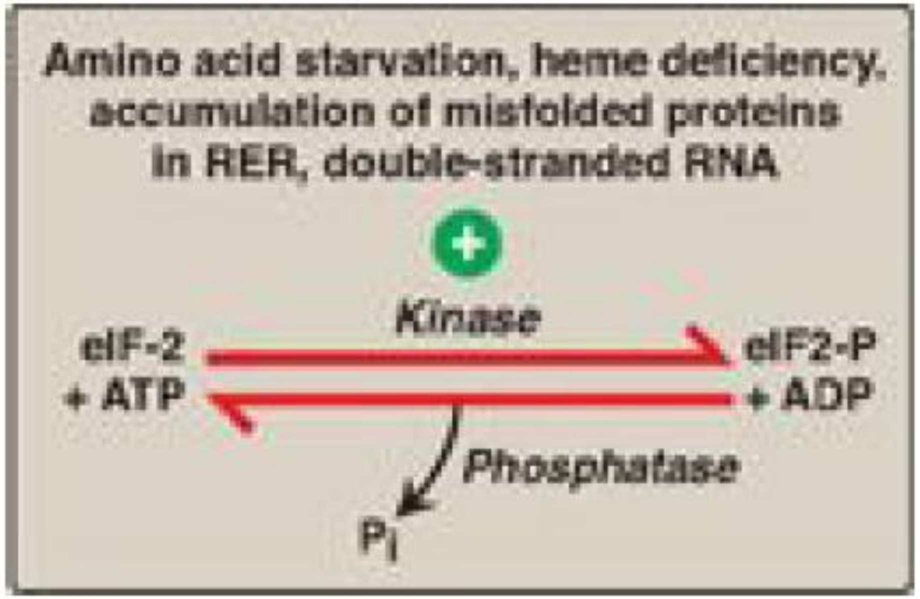


- microRNA (miRNA)



Albert et al. (Editors). Essential Cell Biology. 5th Ed. 2019.

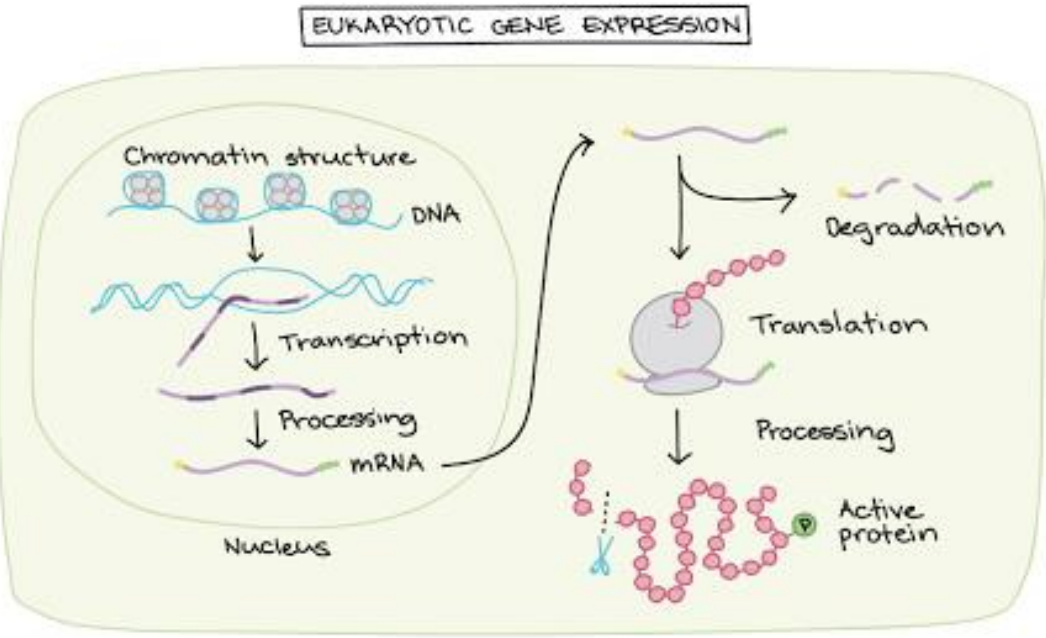
Translational control of messenger RNA



- Regulation of gene expression can also occur at the level of translation.
- One mechanism is through phosphorylation of the eukaryotic translation initiation factor, eIF-2 to inhibit the translation at the initiation step.

Harvey RA. Lippincott's Illustrated Reviews Biochemistry, 6th ed. 2014.

Regulation of Gene Expression II



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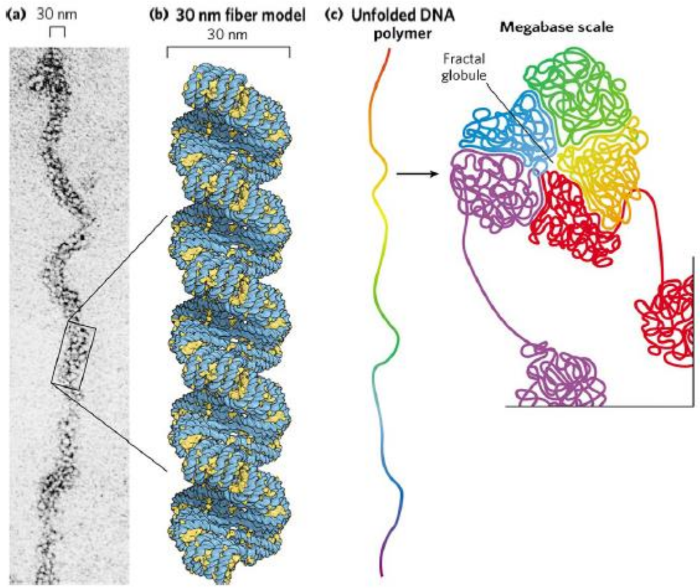
https://www.khanacademy.org/science/biology/gene-regulation/gene-regulation-in-eukaryotes/a/overview-of-eukaryotic-gene-regulation

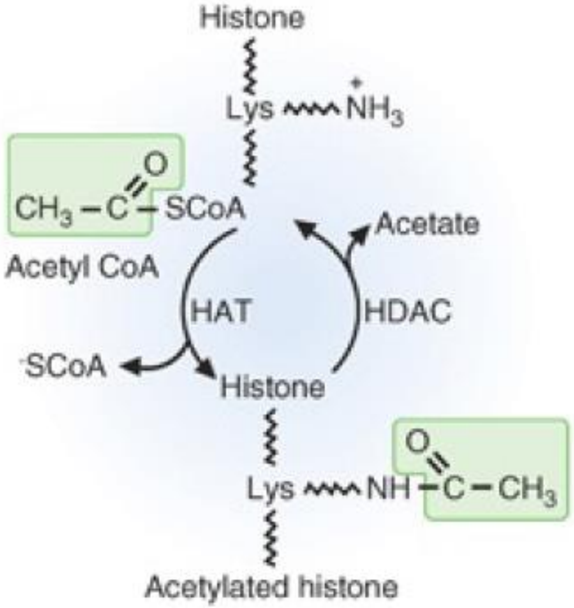
Regulation through modifications to DNA

- Gene expression in eukaryotes is also influenced by the availability of DNA to the transcriptional apparatus, the amount of DNA, and the arrangement of DNA.
- The term *epigenetics* is used to refer to changes in gene expression without altering the sequence of the DNA.

1. Chromatin Remodeling

- Displacement of the nucleosome from specific DNA sequences to allow transcription of the genes
- ATP–driven chromatin remodeling; uses energy from ATP hydrolysis to unwind sections of DNA from the nucleosome core
- Covalent modification of the histone tails through acetylation

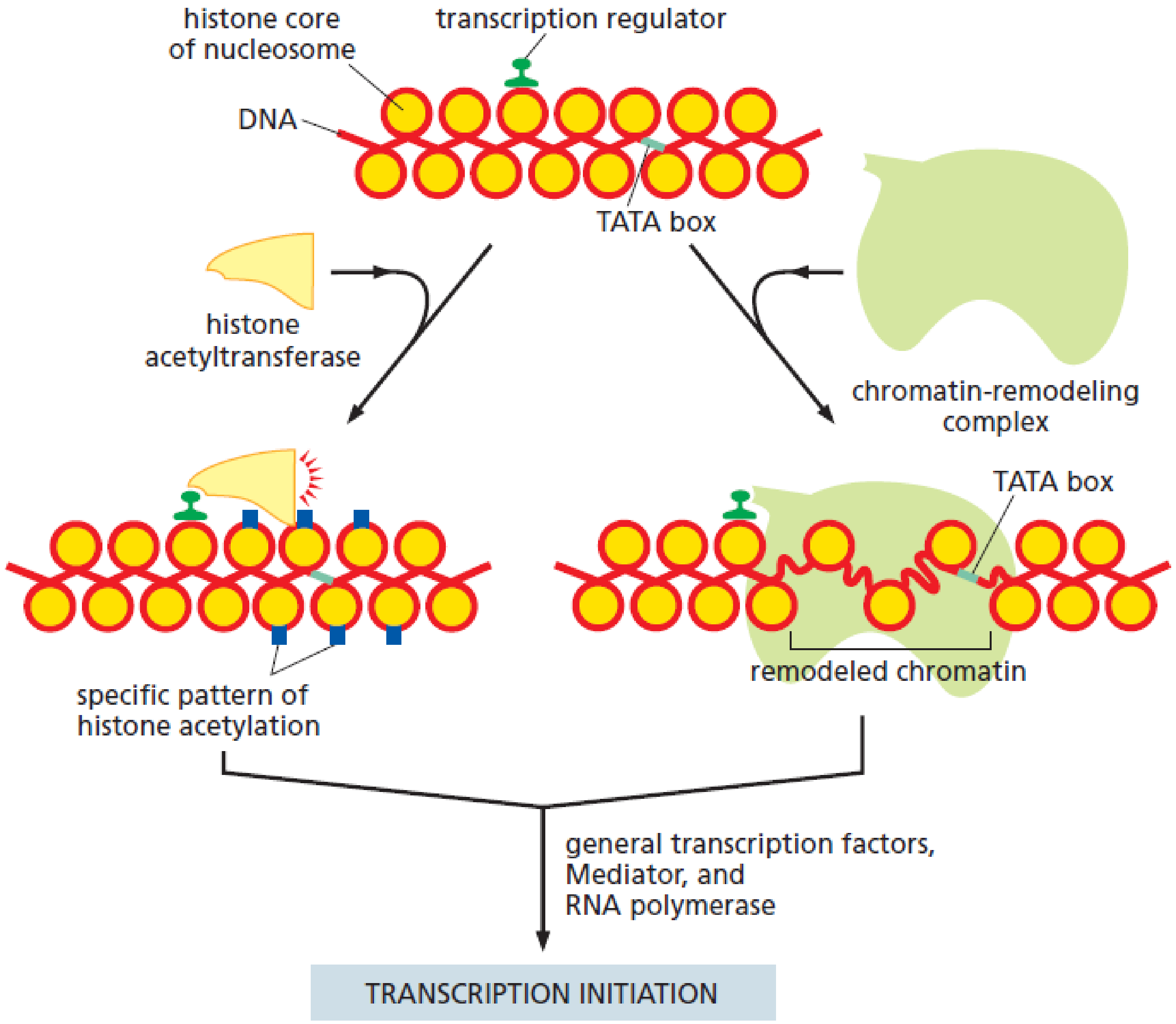


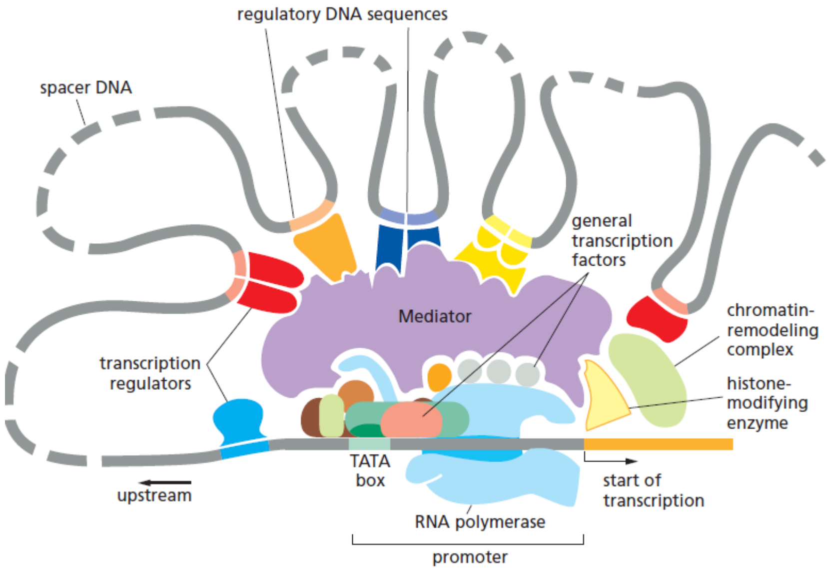


Nelson DL and Cox MM. Lehninger Principle of Biochemistry. 7 ed,2018.

Lieberman and Peet. Marks' Basic Medical Biochemmistry. 5th ed, 2018.

Eukaryotic Transcription Regulators Help Initiate Transcription by Recruiting Chromatin-Modifying Proteins



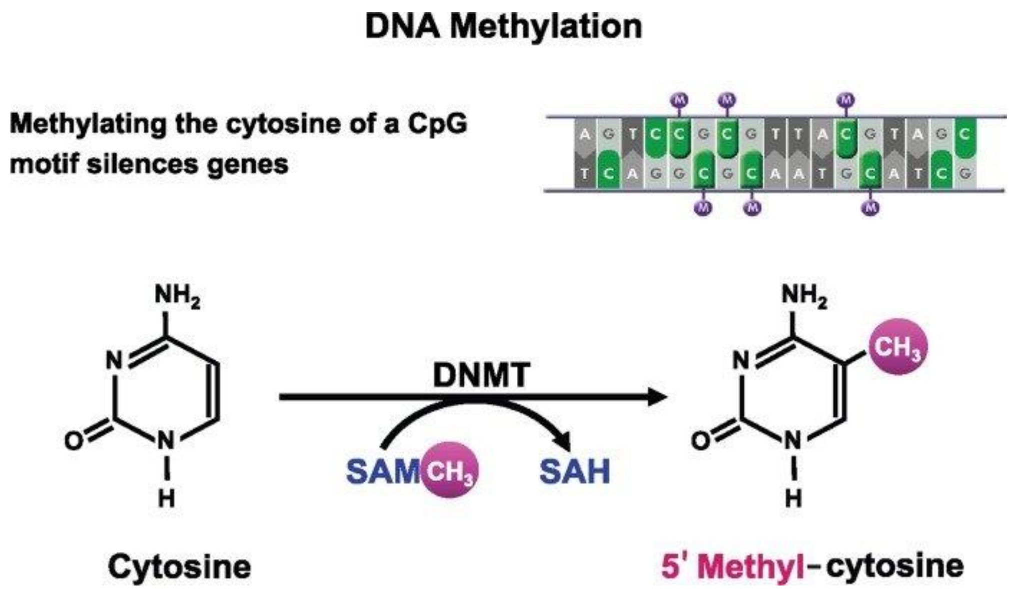


Transcription regulators work together as a “committee” to control the expression of a eukaryotic gene.

Albert et al. (Editors). Essential Cell Biology. 5th Ed. 2019.

2. Methylation of DNA

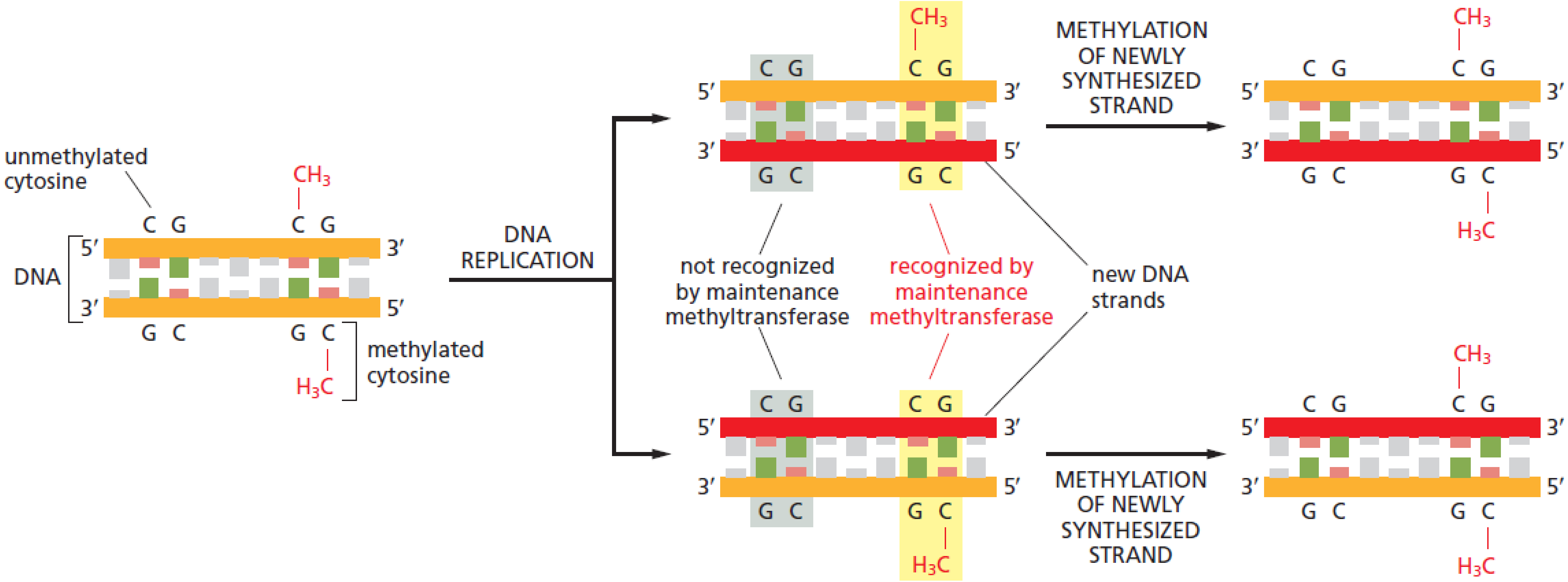
- Cytosine residues in DNA can be methylated to produce 5-methylcytosine.
- The methylated cytosines are located in CG-rich sequences (called *CG* or *CpG islands*), which are often near or in the promoter region of a gene.
- Genes that are methylated are less readily transcribed.
- Methylation is a mechanism for regulating gene expression during differentiation, particularly in fetal development.



DNMT = DNA methyltransferase
SAM = S-adenosylmethionine
SAH = S-adenosylhomocysteine

https://www.researchgate.net/publication/259209496_Alcohol_Metabolism_and_Epigenetics_Changes/figures?lo=1

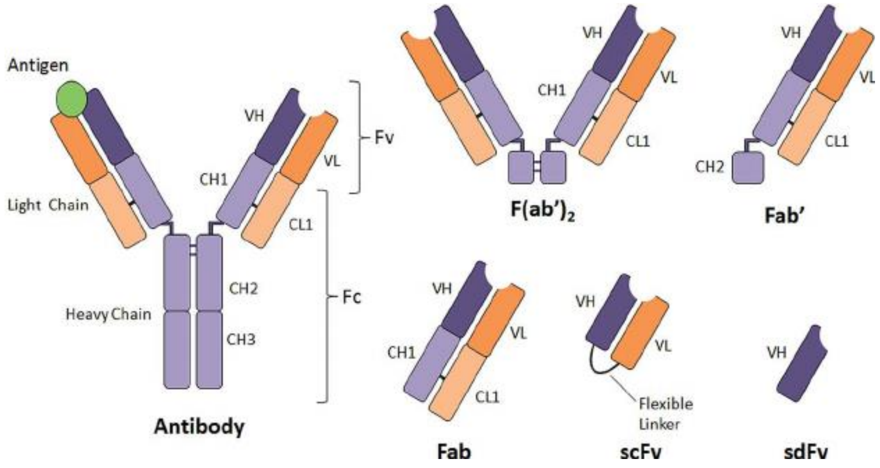
DNA methylation patterns can be faithfully inherited when a cell divides.

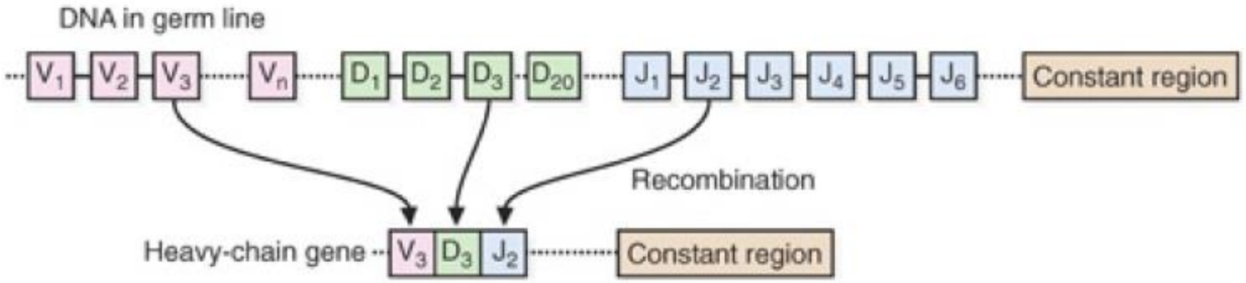


Albert et al. (Editors). Essential Cell Biology. 5th Ed. 2019.

3. Gene Rearrangement

- Segments of DNA can move from one location to another in the genome so that different proteins are produced



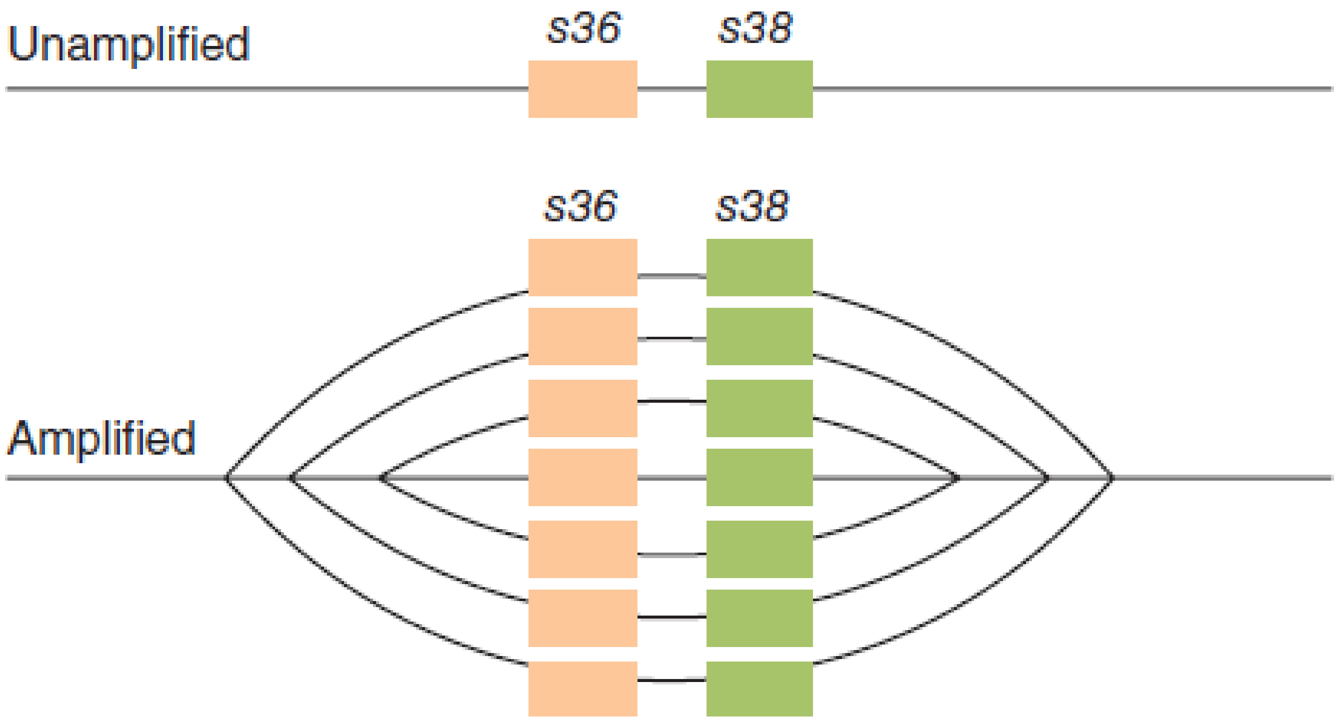


Stefanelli V. and BarkerTH. Journal of Materials Chemistry, 2015.

Lieberman and Peet. Marks' Basic Medical Biochemmistry. 5th ed, 2018.

4. Gene Amplification

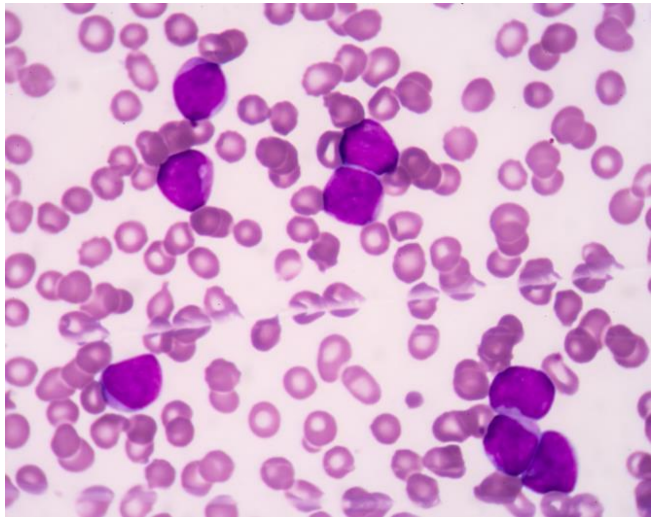
- Gene amplification is not the usual physiologic means of regulating gene expression in normal cells.
- It occurs in response to certain stimuli if the cell can obtain a growth advantage.

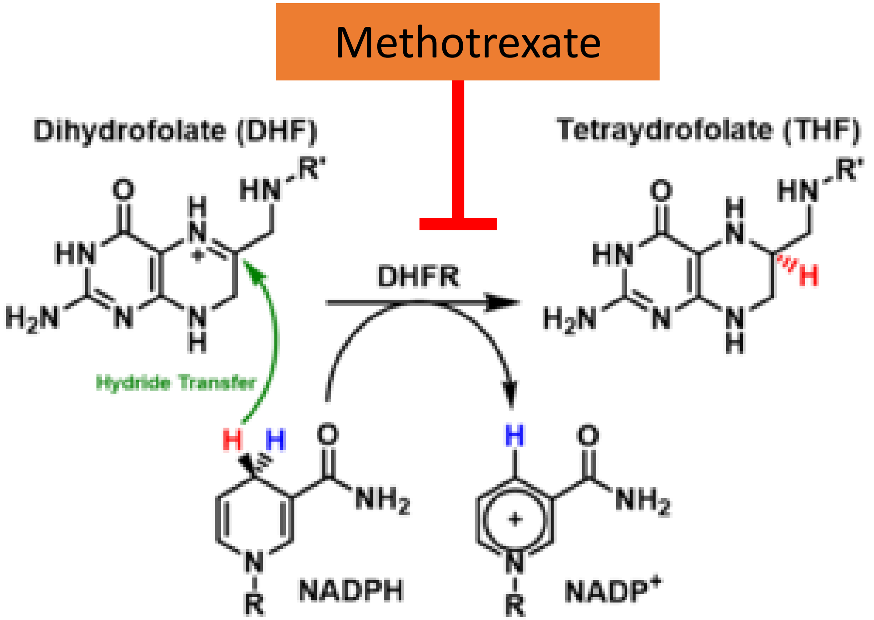


Rodwell et al. Harper's Illustrated Biochemistry, 30th ed, 2015

Gene amplification cause methotrexate resistance in cancer cells

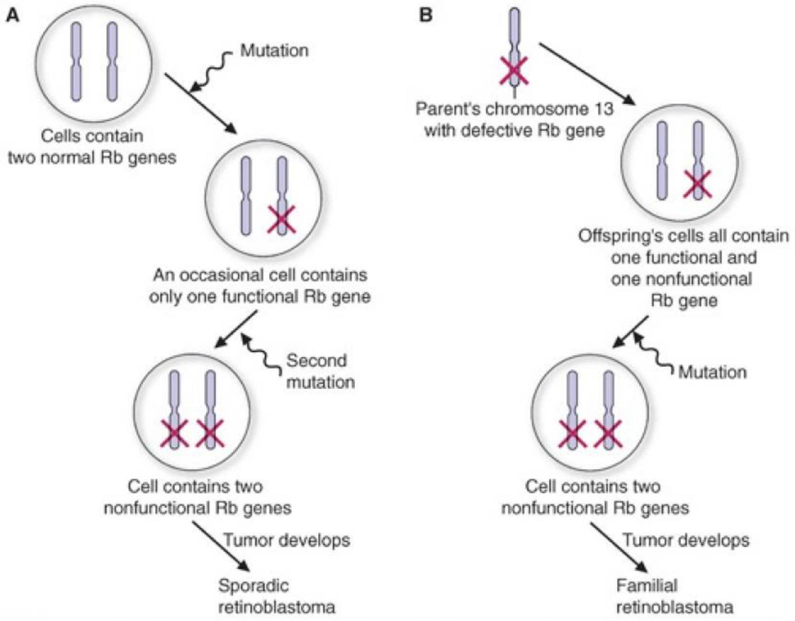
- Historically, non–Hodgkin lymphoma was treated with methotrexate (this drug is still used in certain cases of lymphoma).
- It inhibits cell proliferation by inhibiting dihydrofolate reductase.
- Methotrexate resistance was frequently observed in patients. Sometimes, cancer cells treated with methotrexate amplify the gene for dihydrofolate reductase, producing hundreds of copies.
- Methotrexate is no longer used for the treatment of non–Hodgkin lymphoma





5. Gene Deletions

- The deletion of genetic material is likewise not a normal means of controlling transcription.
- Gene deletions can occur through errors in DNA replication and cell division and are usually noticed only if a disease results.
- For example, various types of cancers result from the loss of a good copy of a tumor-suppressor gene, leaving the cell with a mutated copy of the gene.

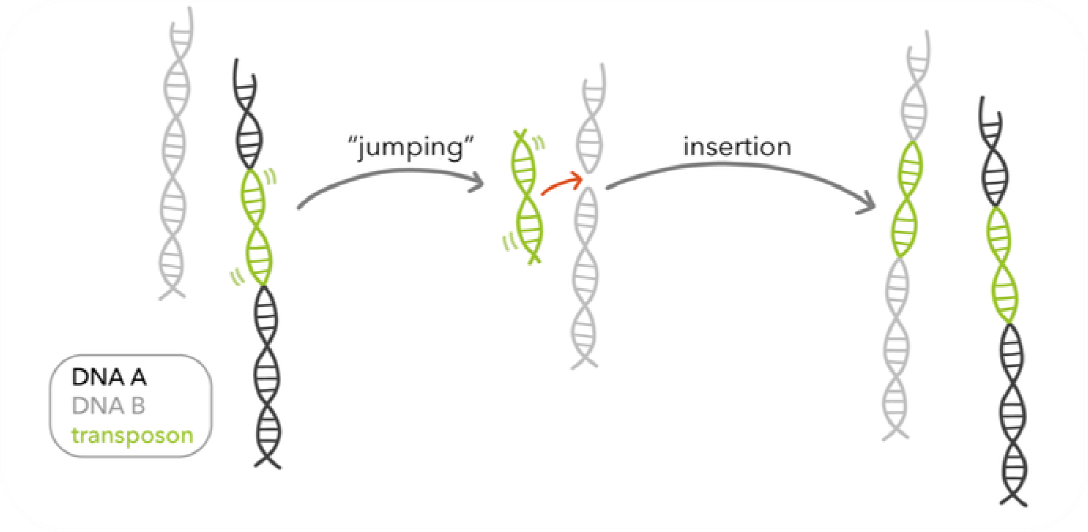


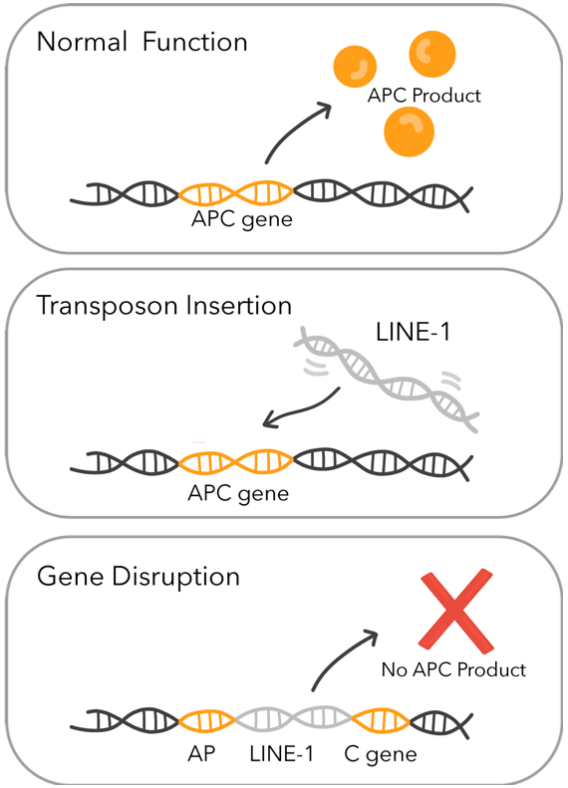
E 18.9 Mutations in the retinoblastoma (*Rb*) gene. **A.** Sporadic retinoblastoma. **B.** Familial retinoblastoma.

Lieberman and Peet. Marks' Basic Medical Biochemmistry. 5th ed, 2018.

6. Mobile DNA elements

- Transposons (Tns) are mobile segments of DNA that move in random manner from one site to another on the same or a different chromosome.
- Transposition has contributed to structural variation in the genome but also has the potential to alter gene expression and even to cause disease.





http://sitn.hms.harvard.edu/flash/2018/transposons-your-dna-thats-on-the-go/

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