بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

Molecular Biology L (9-13)

- The stem-loop structure (in E-coli) disrupts the transcription complex.
- Eukaryotic RNA polymerase have 3 types:
  - RNA polymerase I ⟶ transcribes rRNA
  - " " II ⟶ transcribes mRNA, lncRNA & miRNA
  - " " III ⟶ transcribes tRNA & one rRNA.

- While bacterial RNA polymerase is able to initiate transcription without help from other proteins eukaryotic RNA polymerase requires help general transcription factors.
  They assemble on all promoters used by RNA polymerase II

→ Core promoter region is where RNA polymerase and general TFs bind
- TF IIB Recognition element (BRE)
- TATA box (Imp. for RNA polymerase II binding)
- Initiator
- Downstream promotor Element (DPE)

Bidirectional. Separates the 2 DNA strands at the promoter region creating an open promoter complex

⇒ Formation of preinitiation complex :

TFIID ⟶ other subunits ⟶ polymerase ⟶ TFIIH

- TFIIH ⟶ (1) Helicase Activity ⟶ catalyzed by XPB & XPD proteins
  - ⟶ (2) kinase Activity ⟶ Ⓟ the C-terminal of the largest subunit of RNA polymerase II

Required for nucleotide excision & DNA repair

- phosphorylation releases polymerase II from preinitiation complex and leads to initiation of transcription.

*promoter is a DNA element allows for binding of RNA polymerase.

⇒ Termination of transcription

- protein-coding genes have a polyadenylation signal at 3′ end, which is transcribed by RNA endonuclease that cleaves the RNA molecule downstream of the polyadenylation signal.

⇒ Promotor proximal elements. (Response elements) : Upstream of the core promotor region.
They're important for strong expression. Can be shared among different functionally related genes.
Alternative to operons.

→ Examples of response elements :

- Serum response element (SRE) - proliferation
- cAmp respone element (CRE) - " , survival & differentiation.
- Androgen response elements (ARE) - Metabolism & differentiation

- Angiopoietin-like protein 8 (ANGPL8) functions :
  - Metabolism of lipoproteins and triglycerides
  - Inhibition of LpL.

- Inducers of catabolism suppress gene expression of ANGPL8.
- Inducers of anabolism stimulate transcription factors like SREBP-1c & ChREBP

Note : Not all ppEs have a +ve effect

Example : Insulin activates SREBP-1c (TF), which binds to ppEs on the ANGPL8 gene promoting its transcription and enabling lipid synthesis

- Enhancers : Regulatory sequences which are binding sites for TFs that regulate RNA polymerase II
They can regulate transcription regardless of orientation or location due to DNA looping.
They serve 10% or more of total genomic DNA.
Without enhancers, genes are expressed at basal level
- promotor proximal elements regulate specific genes, while enhancers regulate multiple genes.

* TFs bound at enhancers can interact with "mediator" protein or general TFs at promoter. At the preinitiation complex, a Mediator protein facilitates the interaction between proteins bound to enhancers and those bound to promotor region.

* Enhancers are restricted to interact with promoters in the same domain

- The boundaries of DNA loops are stabilized by Cohesin & CTCF proteins.
- Mediator protein interacts both with general TFs & with RNA polymerase, and links the general TFs to the gene-specific TFs which interact withe enhancers.

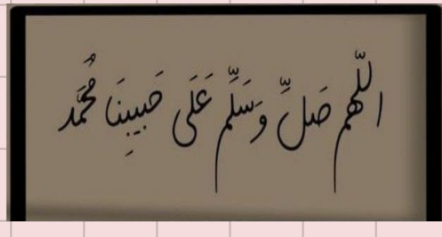
- TF binding sites can be identified by chromatin immunoprecipitation

- Capping: Addition of a 7-methylguanosine molecule to the 5′ end of new RNA molecule

Importance of capping:
- Differentiates mRNA from other RNA molecules
- Stabilizes mRNA
- Signals the 5′ end
- Recruits proteins necessary for splicing & polyadenylation.
- Helps in exporting RNA to cytoplasm
- Helps in translation.

Importance of poly-A sequence?
- mRNA transport to cytosol
- Helps in translation.
- Stabilizes mRNA

* poly-A polymerase does NOT require a templet.
The poly-A sequence is NOT encoded in the genome



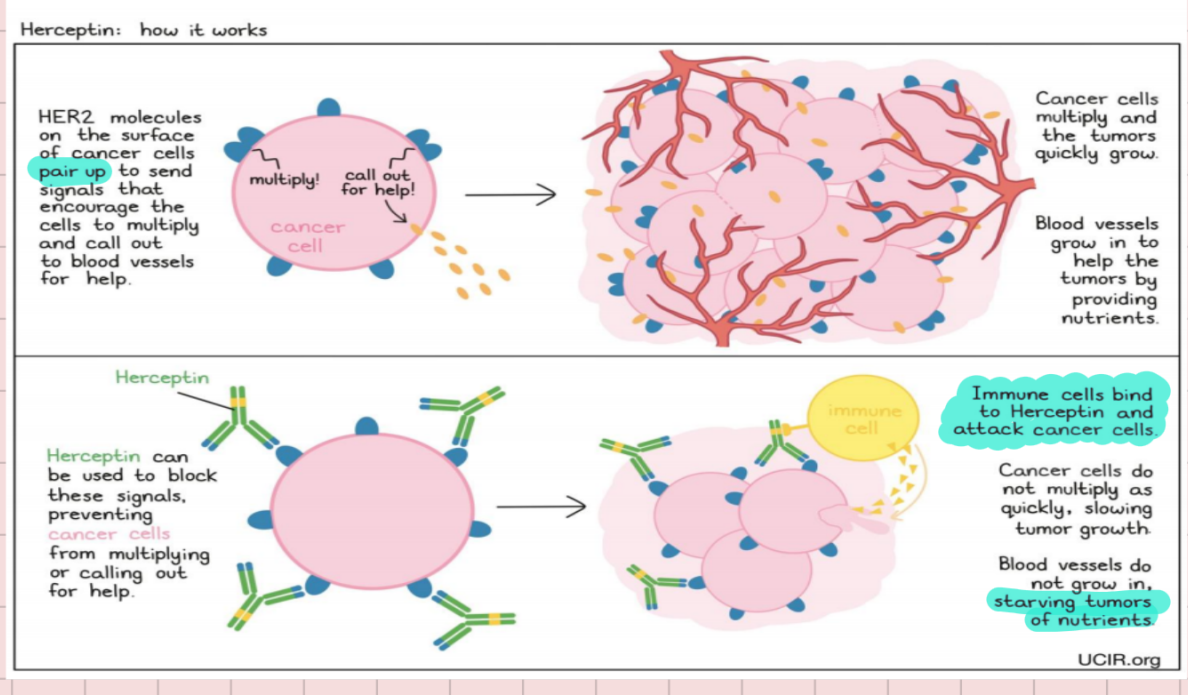
⇒ Degradation of mRNA:
(1) Shortening of poly-A tail, and then by one of 2 mechanisms:
ⓐ (2) 3′ to 5′ exonuclease.
ⓑ (2) decapping and then 5′ to 3′ exonuclease.

- Protein isoforms: similar function with different regulation, structure & binding affinity.

- B lymphocytes produce Igs. Each cell can produce only 1 type of an Ig.
- "Site specific recombination" of genes that encode unique antigen-binding variable regions.
  Antigen binds to Ig on the surface of B-lymphocyte ⟶ One of each gene is combined with one of the others by site-specific recombination.
- "Somatic hypermutation": Multiple mutations are introduced during DNA replication within the rearranged Ig variable regions

So, diversion of Igs comes from:
a. Combinatorial diversity (Gene rearrangement)
b. Imprecise joining
c. Somatic hypermutation
- T cell receptor on the surface of T-lymphocyte is produced by "site-specific rec." as well
  "CAR-T" cells in cancer therapy.

- Cancer cells use gene amplification to develop resistance from methotrexate, whereby the target gene, dihydrofolate reductase" is amplified.
  ↳ DNA synthesis
- How gene amplification is detected?
  we start with immunohistochemistry, if unequivocal ⟶ staining with FISH.



* Enzymes have diff. heterogenous substrates, and diff. reactions are catalyzed in diff. tissues.

- Exons 2,3,4 & 5 encode the catalytic domain that enteracts with UDP-glucoronic acid.
- Exon 1 determines substrate specifity. It contains 9 tandemly arrayed first exons, each one has its own promoter.
  So each sub exon can be transcribed independently of others, which leads to 9 different binding sites that can react with 9 different substrates.
- Exons 2-5 are shared among all UGT1A transcripts. Exon 1 is specific to each transcript, each one with its own promoter, which allows for independent regulation and alternative splicing.

→ Transcription can be terminated at diff. poly-A sites generating short & long mature mRNAs
The long mRNA is regulated differently than the short mRNA.

* Enhancers, silencers & promotors are transcription factors.
* Template strand = antisense strand
* Termination of transcription is accompanied by the enzyme endonuclease.

⇒ Operon: A cluster of genes transcribed from one promoter producing a single polycistronic mRNA that is used to make several proteins (different in structure and function, but participate in the same pathway

- Lac operon have 3 proteins:
  1- β-galactosidase
  2- permease (lac transport to inside the cell)
  3- Transacetylase (acetylation of toxic thiogalactosidase)

⇒ Operator: Binding site of the (lac suppressor) protein. It is a part form the promoter
- Lac repressor prevents RNA polymerase binding to the promoter ⟶ Blocking transcription
  ↳ synthesized by (I) gene.

- Allolactose binding to the repressor increases expression of lac operon which increase lac metabolism.

- Allolactose binding to the repressor prevents it from binding to the operator and activating transcription. This is called "positive regulation"

* Some promoters are leaky

- Trans regulatory elements are produced from trans-acting elements (genes), e.g. I gene.

Feature	Cis-Regulatory Elements	Trans-Regulatory Elements
Definition	DNA sequences on the same molecule as the gene they regulate	Proteins or RNAs encoded by distant genes
Location	Always on the same DNA molecule	Can be on the same or a different chromosome
Mobility	Immobile (fixed within the DNA)	Mobile (can diffuse or be transported)
Examples	Promoters, enhancers, silencers	Transcription factors, regulatory RNAs
Mode of Action	Provide binding sites for regulatory proteins	Bind to cis elements to regulate genes

4. How Cis and Trans Elements Work Together

- **Cis-acting elements** provide the platform for regulation, while **trans-acting factors** recognize and bind to these elements to modulate gene activity.
- For instance:
  - A **transcription factor** (trans) binds to an **enhancer** (cis), recruiting RNA polymerase to initiate transcription.

- CAP binds to regulatory sequences upstream of the promoter. It then interacts with RNA polymerase facilitating its binding to the promoter

CAP-cAmp complex binds to CAP-binding site upstream the promoter, enhancing RNA polymerase binding.

* Lactose induces lac operon while glucose suppresses it

+ve regulation — by inhibiting the repressor

-ve regulation — by inhibiting cAmp production.

* Repressor binds downstream of the promoter while CAP binds upstream

⇒ Regulation of transcription in eukaryotes

- Cis-acting elements e.g. promoters, promoter proximal elements, enhancers & silencers
- Trans-acting elements e.g. transcriptional regulatory proteins (activators, repressors)

- Transcription factors cause epigenetic/epigenomic changes in DNA and chromatin.

→ Epigenetic changes: Alterations in gene expression without a change in DNA sequence via external or internal factors

🇵🇸 "وأُخرى تُحِبُّونَها نَصرٌ مِنَ اللَّهِ وفَتحٌ قَرِيبٌ وبَشِّرِ المُؤمِنينَ"

15/Jan./25 ♡

⇒ Nucleosome

- Nucleosome core particle: DNA wrapped around an octamer of histone proteins.

* Histone 1 can bind to DNA outside the nucleosome core.

* There is a free linker DNA between every 2 nucleosome core particles.

- Cells exchange DNA structures between euchromatin & heterochromatin depending on their needs. This is epigenetic control.

- Methylation causes nucleosomes to pack tightly ⟶ prevent gene expression
- Acetylation results in loose packaging of nucleosomes ⟶ enhance gene expression.

⇒ Compactness change of chromatin can result by plenty of mechanisms, including:-

(1) Chromatin structure and position change.

▶ Chromatin remodeling factors

They facilitate the binding of TFs.

They can be associated with transcriptional activators or inhibitors.

(2) Chemical modification of histones

- The core histones have 2-domains:

1- A histone fold

for interaction with other histones + DNA wrapping around the nucleosome core particle.

2- An amino terminal tail.

extends outside of nucleosome. Rich in lysine.

a. Acetylation of lysine (k)

b. Methylation / Phosphorylation of histones

* The effect, whether transcriptional activation or repression, depends on the modification site.

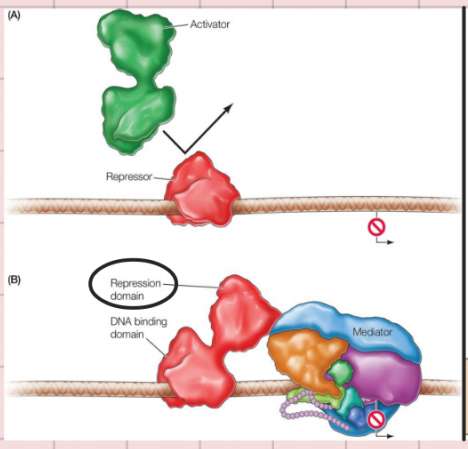
→ Positive transcription factors have at least 2 indendent domains:

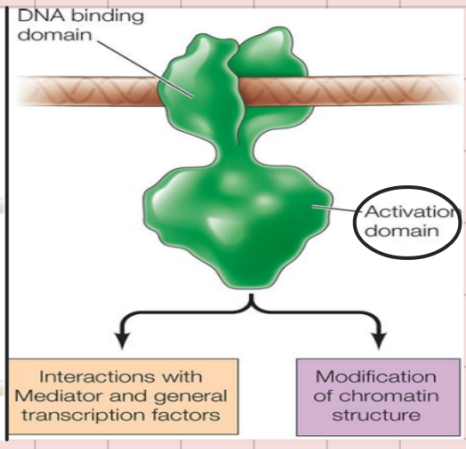
1- DNA-binding domain

- for interaction with mediator proteins & general TFs such as TFIID (to recruit RNA polymerase & facilitate the assembly of a transcription complex on the promoter.
- for modifying chromatin.

TFIID also interacts with histone acetyltransferase.

2- Activation domain.





* Transcriptional activators and repressors are associated with coactivaters and corepressors which have HAT & HDAT respectively.

(3) Chemical modification of cytosine.

Cytosine residues are methylated at the 5′ carbon specifically at CG sequences (CpG islands) near promoters.

This reduces transcription by blocking activator binding to DNA & inducing heterochromatin formation.

Also plays a key role in "genetic imprinting" → in 75 genes.

* Methylation is inherited following DNA replication.

(4) Binding of non-coding RNAs to DNAs

* More than 5000 long (>200) noncoding RNAs (cis or trans)

They can complex with general or specialized TFs, Mediator or RNA processing proteins.

LncRNAs can be homologous to certain DNA sequences and form complexes with chromatin and DNA modifiers to activate or repress gene expression via chromatin modification.

⇒ "Xist" gene is transcribed into lncRNA, which in turn coats the X chromosome in cis and triggers its inactivation by deacetylation & cytosine methylation.

→ The inactivated X chromosome is called "Barr body" and is crumbled.

- Some enhancers can be transcribed into eRNA that can regulate transcription of adjacent genes.

* Identical twins have the exact same genetic information BUT their epigenomics become increasingly different over time. So, "epigenetics" is heritable.