بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 16

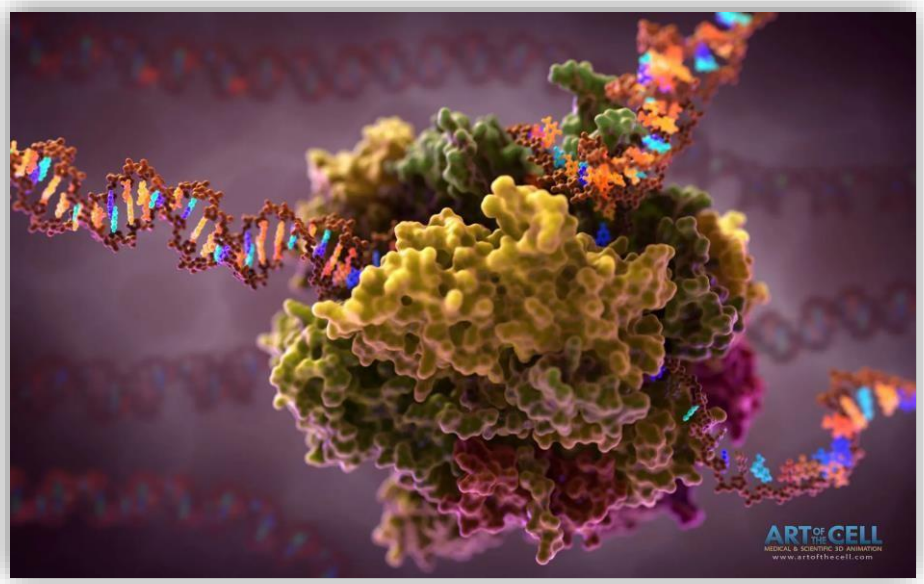
Transcriptional Phenomena in Human

Written by : DST
NST

Reviewed by : NST members

Quiz on the previous lecture

Click on the picture



(اللَّهمّ أخرِجنا من ظُلُمات الجَهْل و أكرِمنا بنور الفَهْم و افتح علينا بمَعرِفة العِلْم ، و حَسّن أخلاقنا بالحلم و سهّل لنا أبواب فضلك و انشر علينا من خزائن رحمتك يا أرحم الرَّاحمين)

Recap of the relatable information from the transcription lectures to maximize your understanding of this lecture.

(Skip these 2 slides if you feel confident in your recall.)

Transcription is the process of how the cell makes RNA from DNA.

RNA polymerase is the enzyme that builds RNA using one DNA strand as a template.

- RNA is made in the **5' to 3'** direction in three steps:

Initiation: RNA polymerase starts at a promoter region on DNA.

Elongation: RNA grows as nucleotides are added.

Termination: Transcription stops when it reaches a signal.

- **Transcription in Eucaryotic genes:**

Promoters: Where RNA polymerase binds to start transcription.

Enhancers: Regions of DNA that increase transcription. They can work even if far from the gene or flipped.

- **RNA in Eukaryotes:**

5' Cap: Added to protect RNA and help it function.

Splicing: Introns (non-coding parts) are removed, and exons (coding parts) are joined. Alternative splicing creates different proteins from the same gene.

Poly-A Tail: A chain of adenines is added to the RNA's end for stability and export.

❖ **Regulation of transcription**

Promoter-Proximal Elements: Regions near the promoter that help control gene expression.

Transcription Factors: Proteins that turn specific genes on/off depending on the cell type.

➢ **The relation of the recap with our lecture :**

Gene Rearrangement: Splicing and enhancers explain how immunoglobulin genes can rearrange to make many types of antibodies.

Gene Amplification: Extra copies of genes help cancer cells resist drugs.

Alternative Polyadenylation: Using different poly-A sites creates RNA molecules of varying lengths and functions.

Transcriptional phenomena in humans

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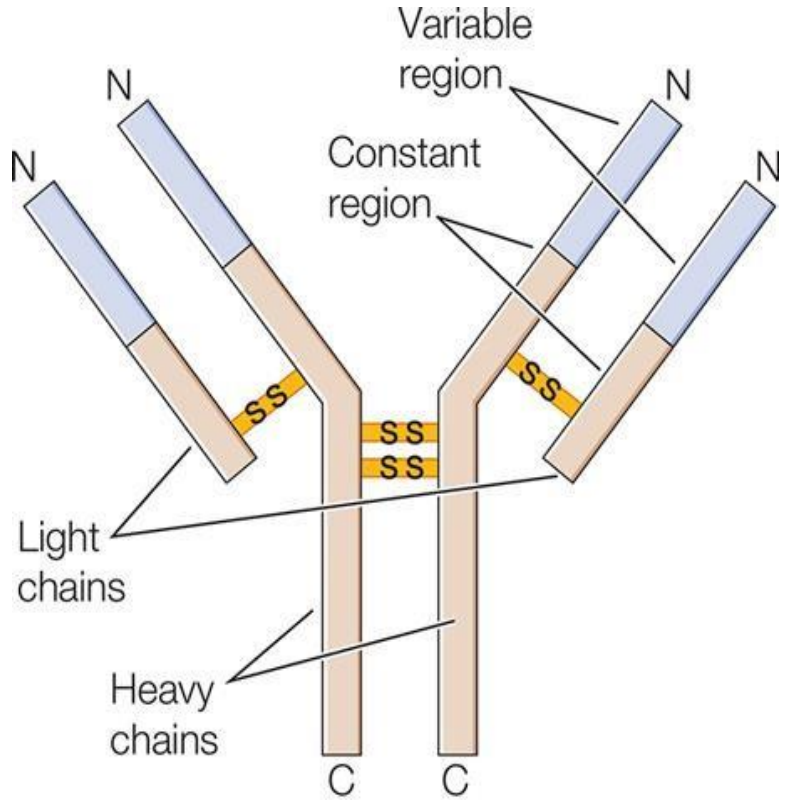
School of Medicine

Second year, First semester, 2024-2025

Gene rearrangement

Immunoglobulins

- The human body can possess a population of approximately 10^{12} B lymphocytes that can produce and release immunoglobulins (antibodies), but each cell can produce one type of an immunoglobulin.
- Each antibody has a unique antigen-binding variable region (it's the site responsible for binding with antigens) that is encoded by unique genes formed by site-specific recombination during B-lymphocyte development. Variable regions must be heterogeneous to recognize and bind الكمّ الهائل of antigens.



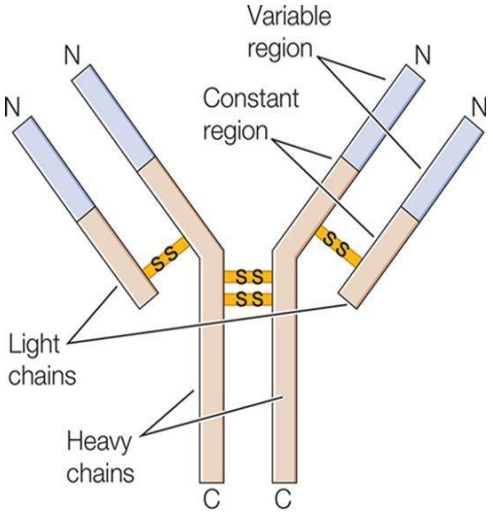
Immunoglobulins- Explanation

- They are **diverse** proteins that interact with **foreign antigens** (proteins or carbohydrates) as a mechanism to **eliminate** these from the body.

➢ Composed of **4** polypeptide chains, held together by **disulfide bonds**;

- 2 identical light chains.
- 2 identical heavy chains.

✓ Light chains are composed of a constant region and a variable region, while heavy chains are composed of a variable region and a constant region of **three domains**. Differences in the constant region of the heavy chain lead to the formation of **five** immunoglobulin classes: IgG, IgA, IgM, IgE, and IgD.

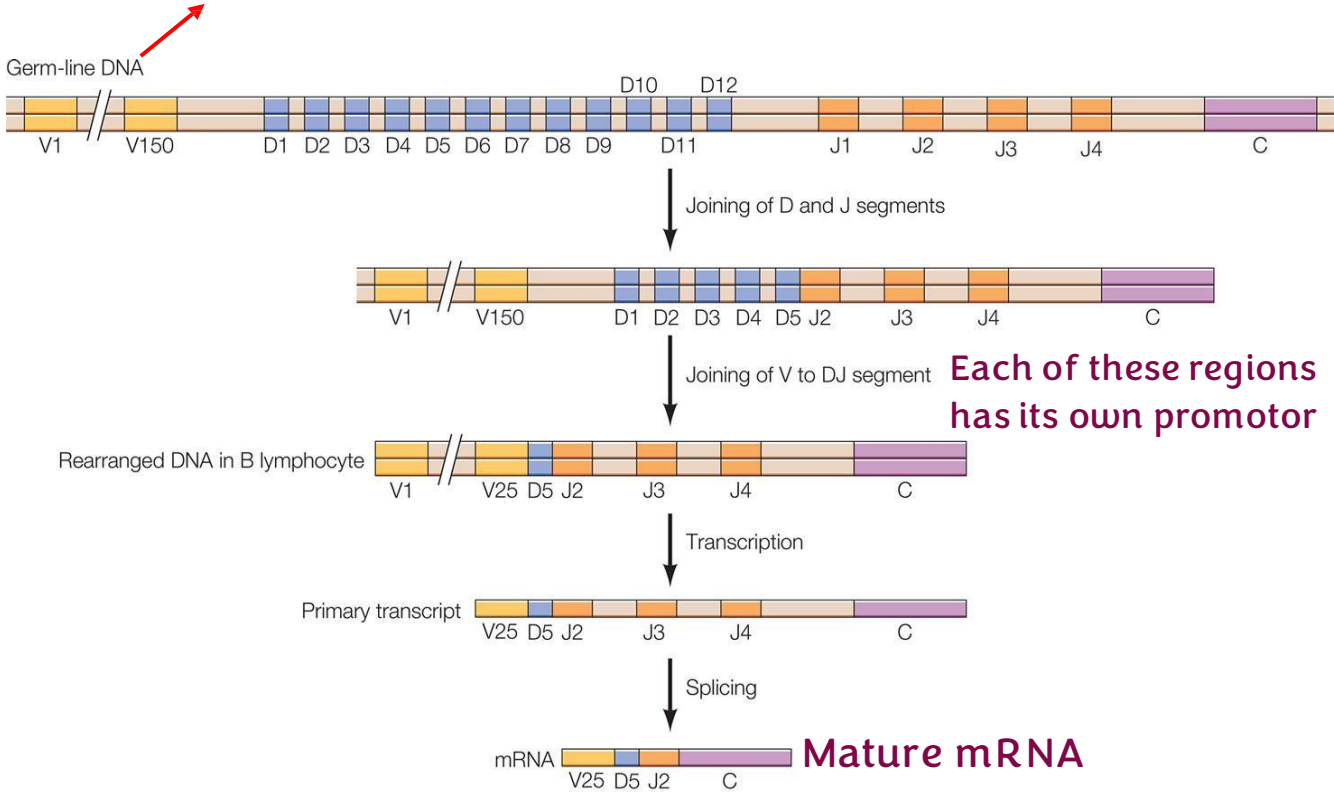


Similar for both, the light and the heavy chains.

The mechanism

- Each heavy gene consists of 150 variable regions (V), 12 diversity exons (D), 4 joining (J) exons, and one constant exon (C).
- During lymphocyte development, one of each is combined with one of the others by site-specific recombination.
- The total number of heavy chains that can be generated is about 7200 (150 × 12 × 4).
- 600 light chains are produced by the same mechanism resulting in a possible 4×10^6 different combinations.
- The joining of the different segments often involves the loss or gain of one to several nucleotides resulting in 10^{11}. different immunoglobulins.

This is the gene of **naïve B cell** (a B cell that hasn't been exposed to an antigen yet; **not** activated/ stimulated)



Each of these regions has its own promotor

Mature mRNA

Once we have the DNA arranged, it can't change anymore.

Somatic hypermutation is an additional mechanism where multiple mutations are introduced during DNA replication within the rearranged immunoglobulin variable regions.

Further Elaboration

- B Cell Activation; occurs when an **antigen** binds to the immunoglobulin on the B cell surface, stimulating recombination and diversity.

Mechanism;

1. D exon (D5) rearranges and aligns with a J exon (J2).
   - So the intervening DNA between these exons (J1 to D6) is deleted
2. Then a V exon (V25) recombines with the already rearranged D exon
   - The DNA between V25 and D6 is deleted, bringing them close together.
3. The primary transcript includes the selected V, D, and J exons, followed by the constant (C) region.
4. The RNA transcript undergoes splicing, removing the sequence between the J exon (J2) and the C exon.

Further elaboration- Diversity

1. Combinatorial Diversity:

- Heavy Chain:
  - 150 V × 12 D × 4 J = 7,200 combinations.
- Light Chain:
  - 600 combinations are possible through a similar mechanism.

✓Total diversity from heavy and light chains = **4 million** combinations !

2. Imprecise Joining:

- During recombination, nucleotides may be added or deleted, creating additional diversity.

3. Somatic Hypermutation:

- Random mutations occur during B cell proliferation, further enhancing diversity.

➢ Combined Mechanisms,

- The diversity from recombination, imprecise joining, and hypermutation ensures the immune system can produce a vast array of antibodies to recognize diverse antigens.

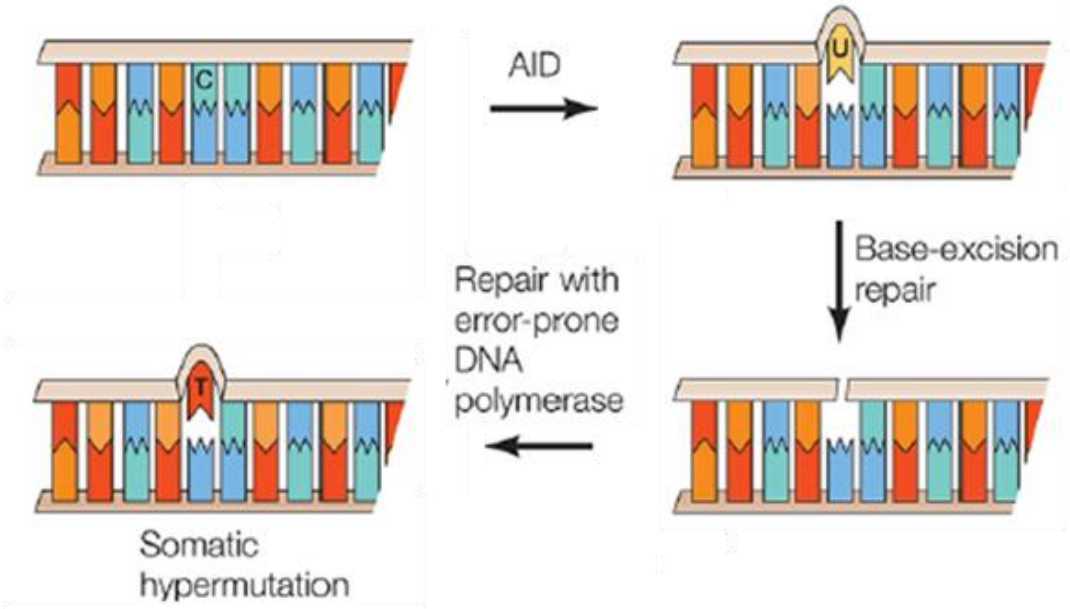
Somatic hypermutation

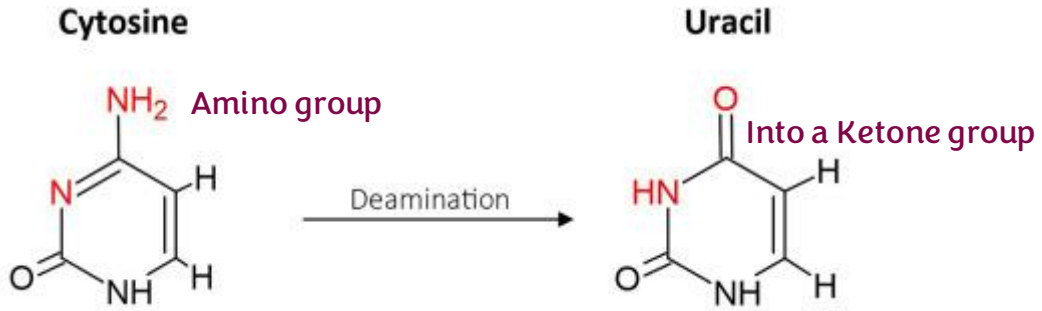
طفرة

In somatic cells <B lymphocytes>

A lot

- In the variable region, the activation-induced deaminase enzyme (AID) converts C's to U's in the DNA.
- The U's are removed (after the cells recognize that عندنا U base in DNA) By a repair mechanism we'll talk about it later on, leaving a single-strand gap that is filled in by a specialized error-prone DNA polymerase and creating more diversity.





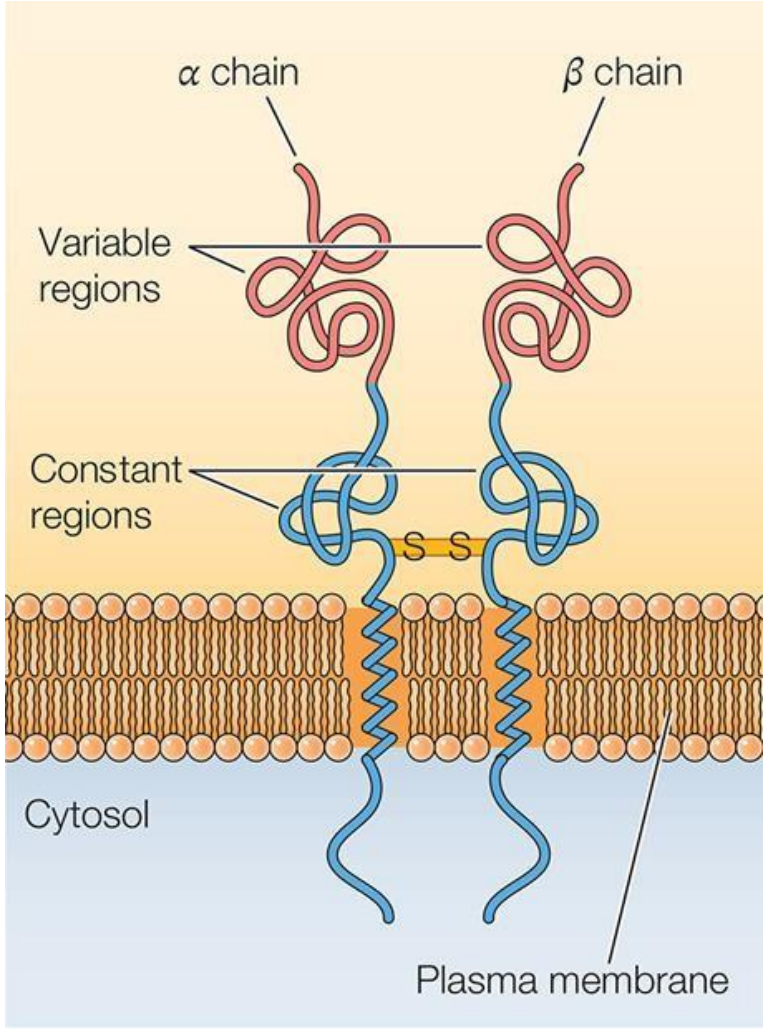
T cells and CAR T cells

Like before

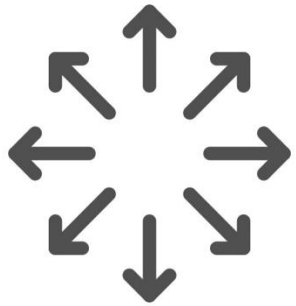
- The T cell receptor on the surface of T lymphocytes is produced by site-specific recombination.
- A new type of cancer treatment (CAR-T cell therapy) utilizes a patient's T cells are genetically engineered to express an artificial T-cell receptor that recognizes antigens on the surface of tumor cells.

❖ Mechanism of CAR-T cell production:
T cells are removed from the patient → then they are engineered in the laboratory → injected back to the patient → then they recognize and bind the cancer cells and helps in the removal of it.

➢ These cells are engineered in way that T cell receptors recognize cancer cells.



https://www.cancer.gov/about-cancer/treatment/research/car-t-cells

Geneamplification 

مُطمئن
@u5yin

ورد في القرآن

يَا لَيْتَنِي قَدَّمْتُ لِحَيَاتِي

(لِحَيَاتِي) وليس في (حياتي)

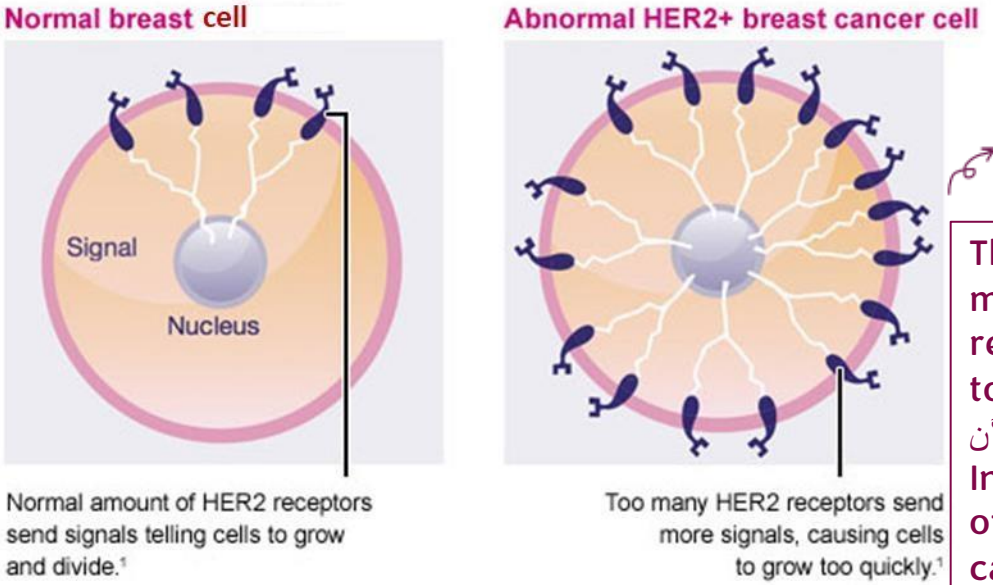
حياتنا لم تبدأ!

Gene amplification

It happens naturally as a mechanism for fighting drugs for example.

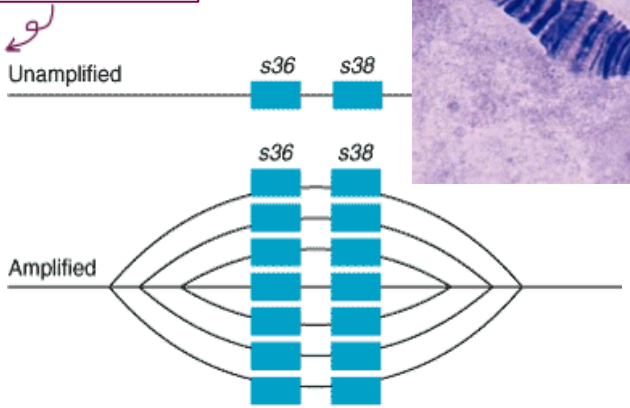
- It is an increase in copy number of a restricted chromosome region increasing the quantity of DNA in these regions and, hence, increasing RNA and protein levels.
- Cancer cells use it to develop resistance from methotrexate (cancer chemotherapeutic drug)

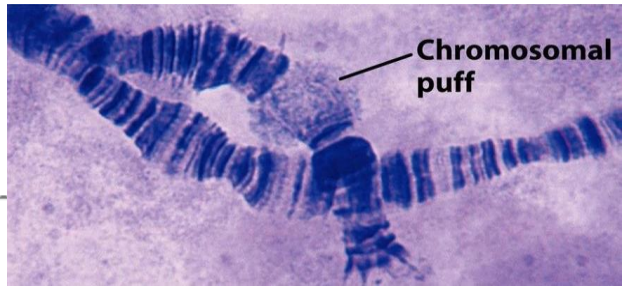
  whereby the target gene, dihydrofolate reductase (an enzyme that plays a key role in DNA synthesis), is amplified.
- Breast tumor cells amplify the human epidermal growth factor receptor 2 (HER2) making them highly proliferative and more aggressive in growth and progression.



Breast Cancer Cells can amplify the gene that encodes the HER2

This amplification mechanism makes the receptor more sensitive to the ligand غير المعروفة حتّى الآن
Increasing proliferation of tumer cells leading to cancer cells





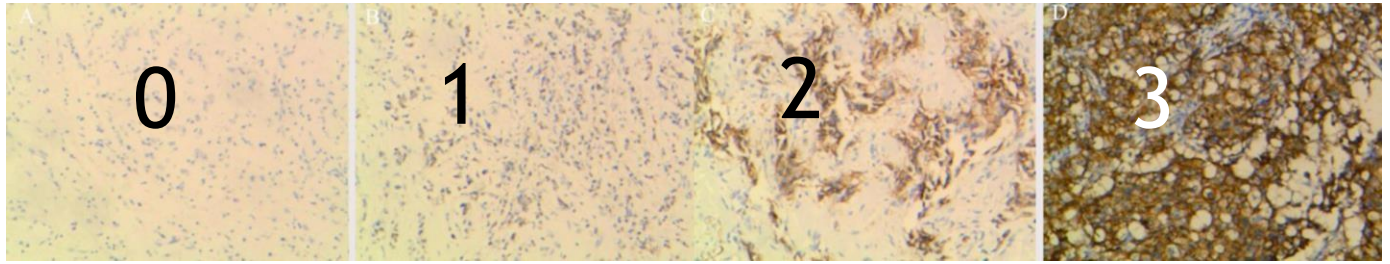
How is it detected?

We start with → immunohistochemistry → (If unequivocal) FISH

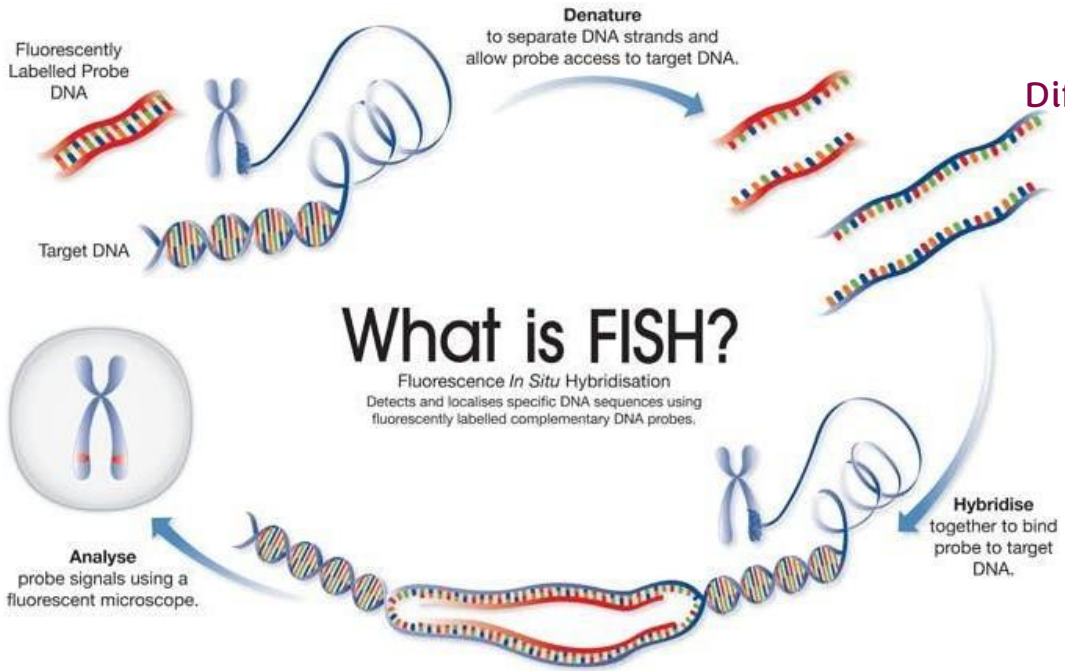
It is basically using antibodies on tissue sections

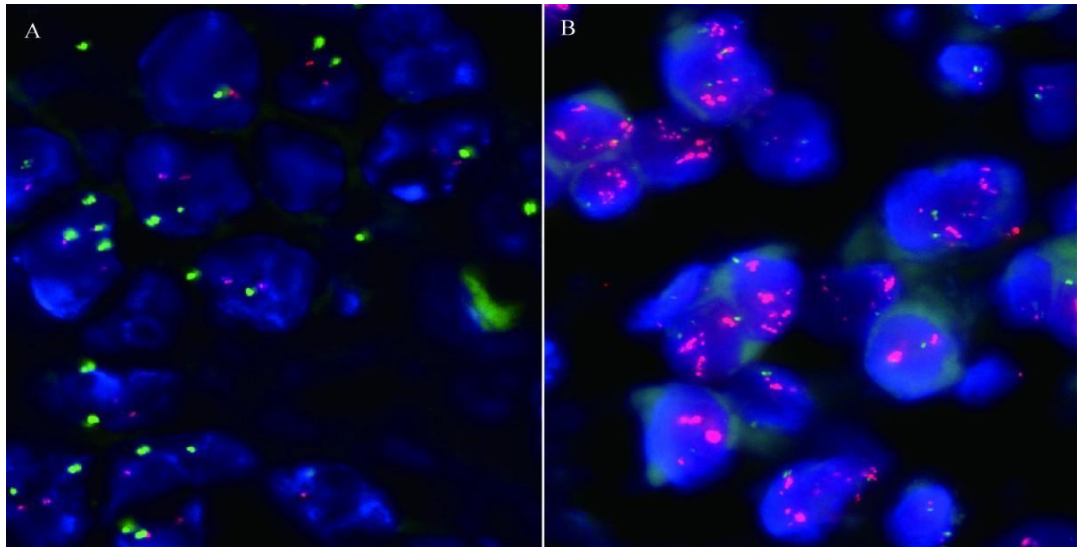
- If immunohistochemistry shows unequivocal (hard to determine the result) staining, then FISH is done.

Recall the FISH mechanism from previous lectures and try to connect the dots. (FISH = fluorescence in situ hybridization)



Different scoring for determining the gene amplification using immunohistochemistry



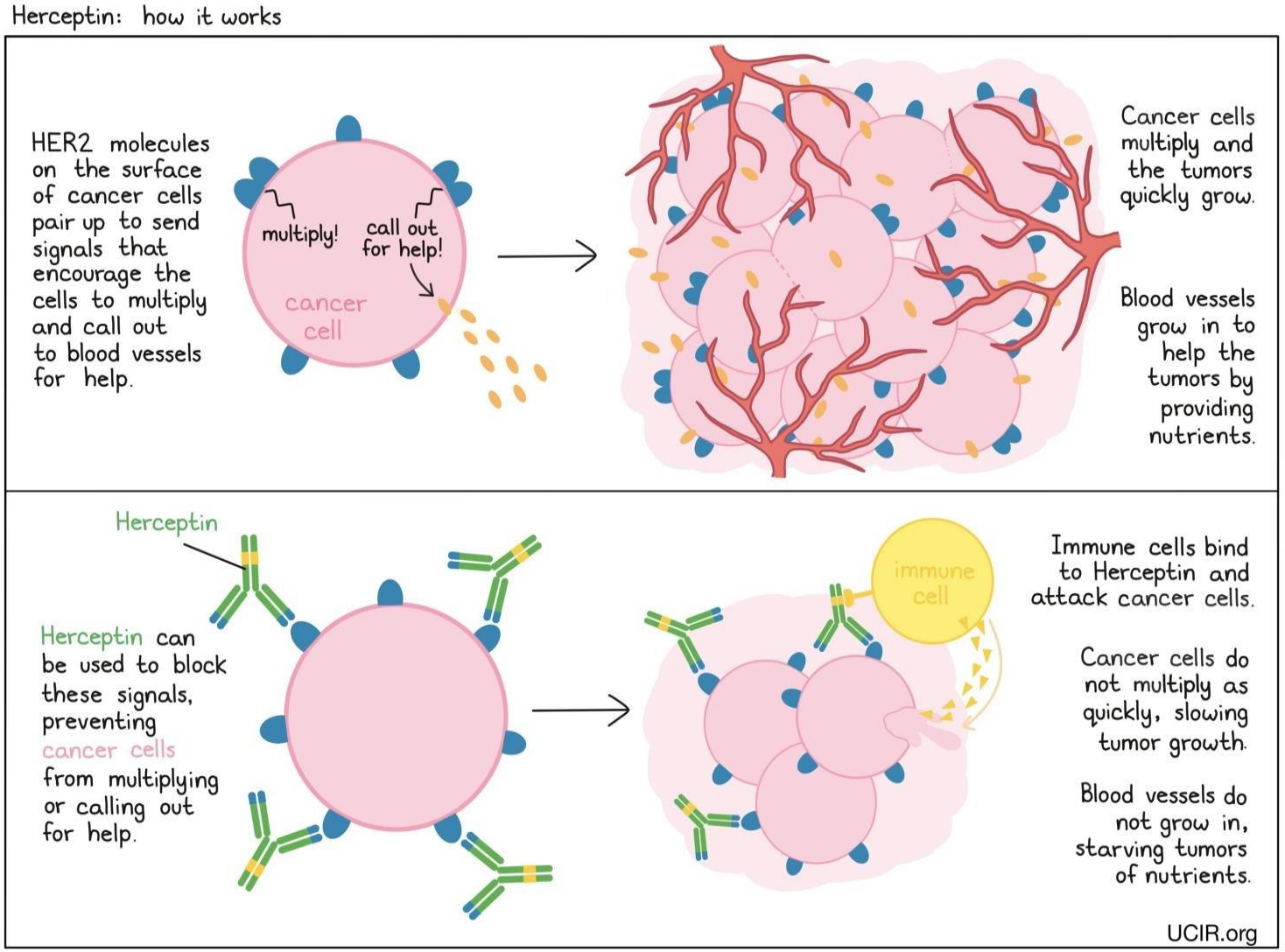


The high amount of red/pink dots indicate the presence of gene amplification (A- normal state / B- gene amplification)

How are HER2-enriched cancers treated?

Herceptin (trastuzumab) Mab= monoclonal antibodies

Take a look at the picture and endorse it.



Remember;
Monoclonal Ig: An antibody from a single B cell that specifically binds a single antigen epitope with consistent affinity.

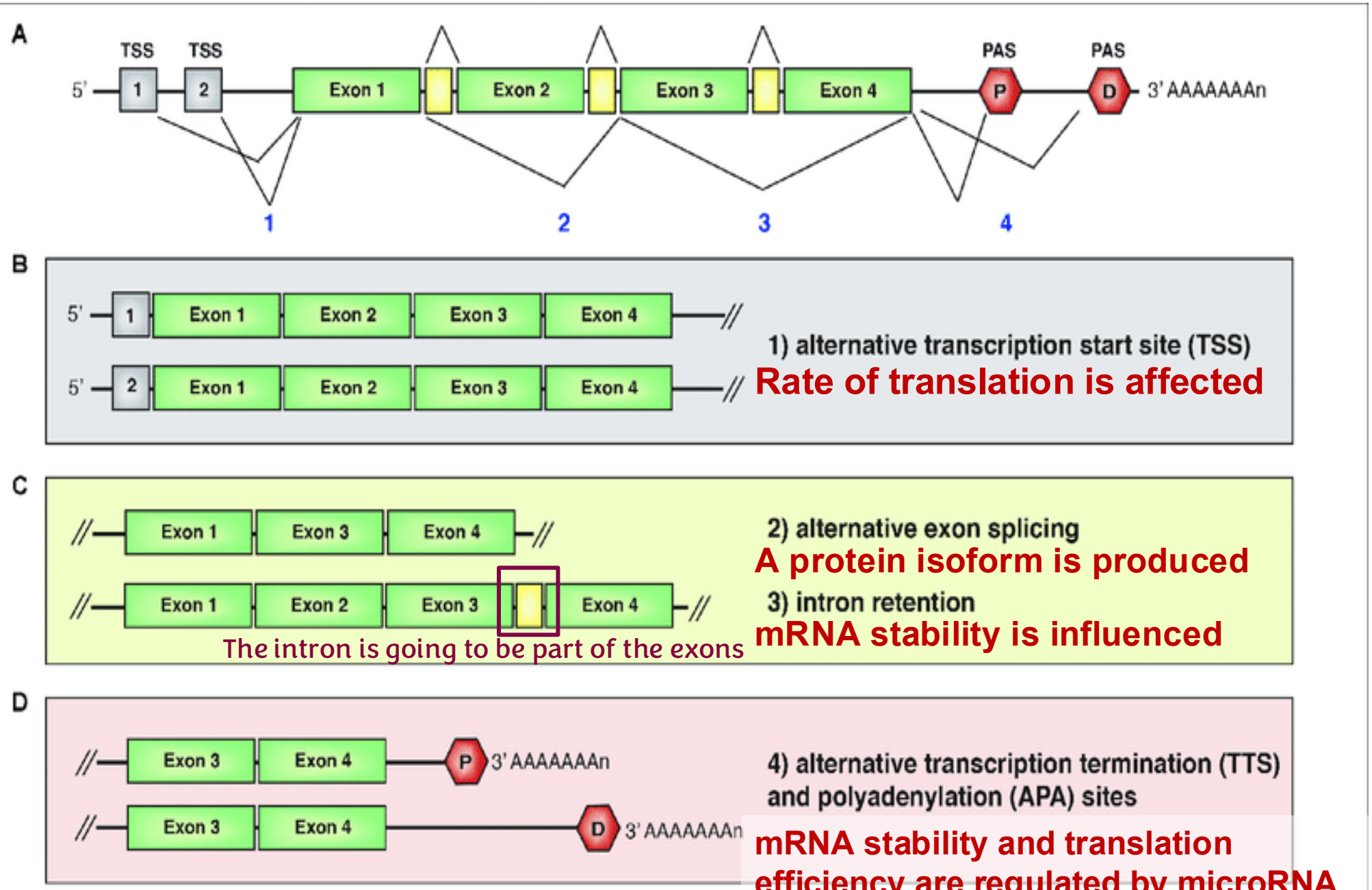
Briefly:
The Herceptin targets HER2 on cancer cells preventing its action, which leads to the death of the cancer cell.

الحمد لله

The alternatives:

Alternative transcription start site, alternative splicing, and alternative polyadenylation sites

- This what increases the diversity of proteins.

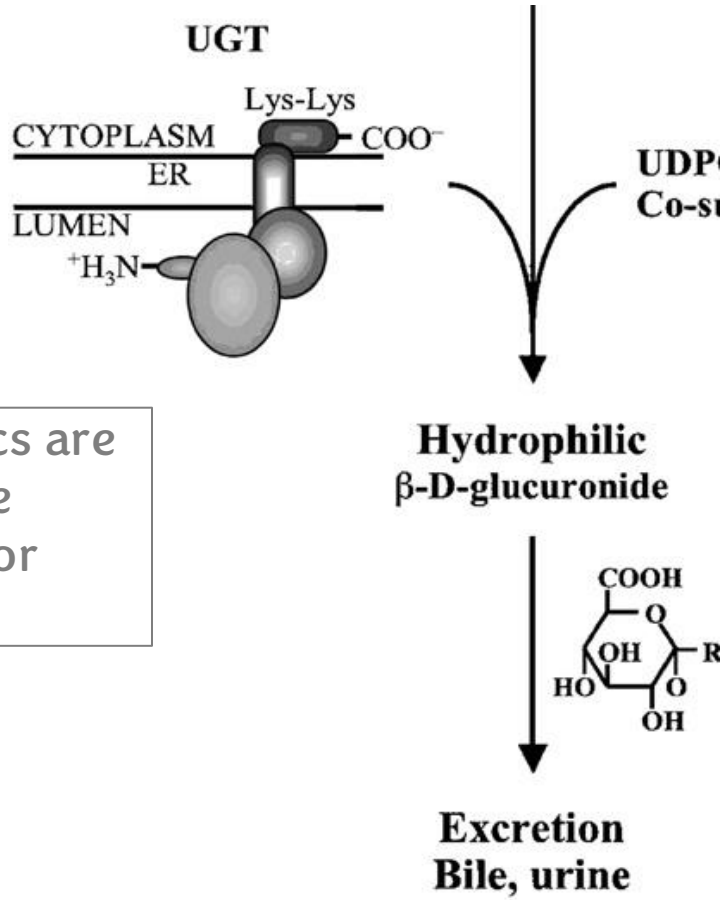


- It affects the binding capability with microRNA

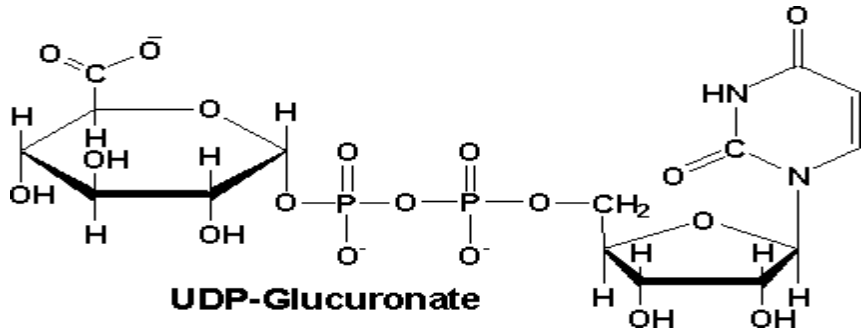
One gene, multiple promotors, multiple exons

An example of alternative splicing:

UDP-glucuronosyltransferase (UGT)



Lipophilic substrate



remember;, Xenobiotics are foreign things that are harmful to humans, for example Drugs.

The uridine diphosphate glucuronosyltransferase (UGT) enzymes transfer glucuronic acid onto xenobiotics and other endogenous compounds making them water soluble (by adding a sugar molecule -gulcuronosly moiety-)**and allowing for their biliary or renal elimination.**

وَجَّهتُ وَجهي نحو بابِكَ راجياً و الحالُ لا يَخْفى و أنتَ عَليمُ

The enzyme(s) has many heterogenous substrates

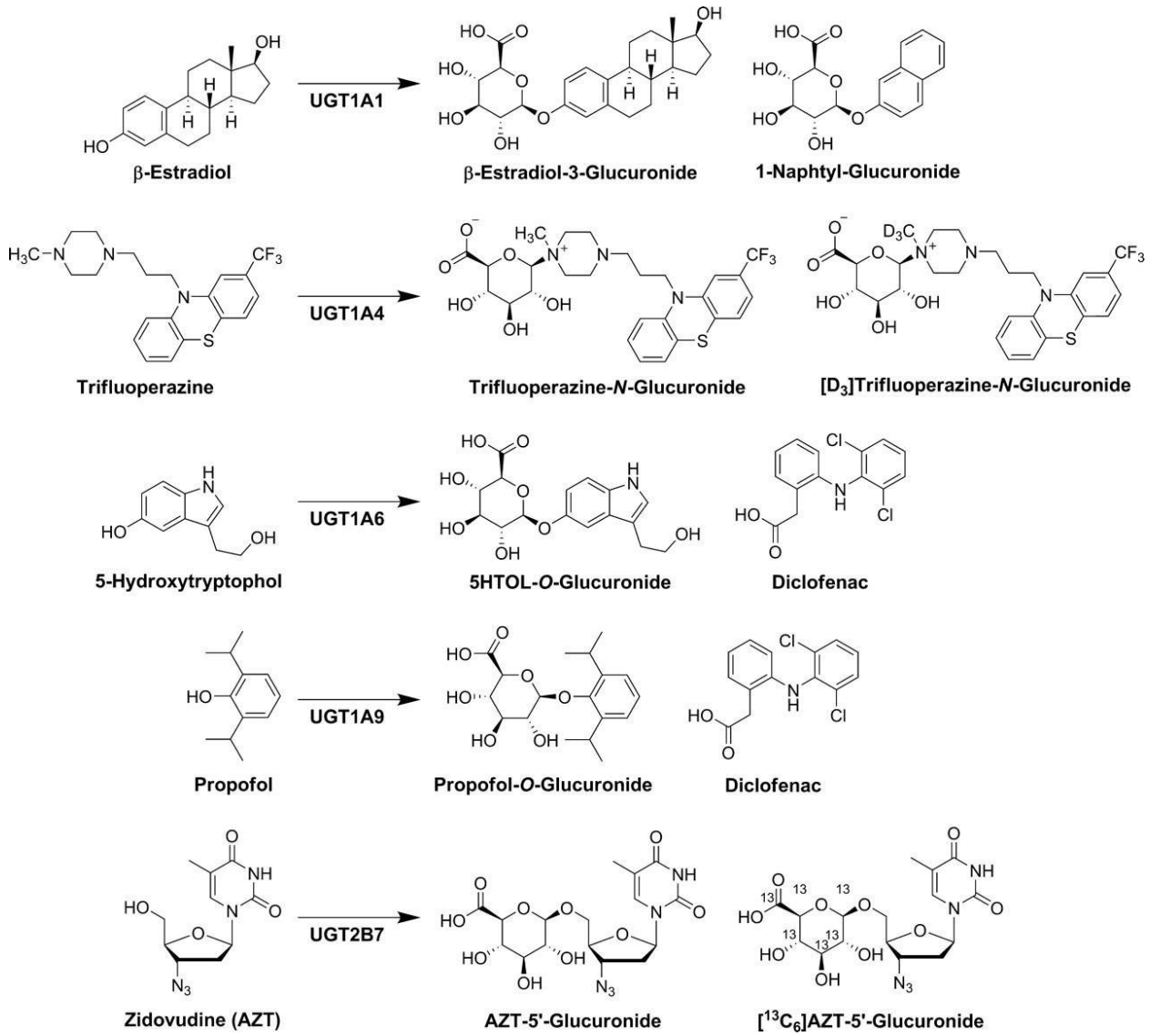
Lipophilic substrate

Therapeutic drugs	Biliary acids
Carcinogens	Steroïds
Environmental toxicants	Retinoic acids
Dietary constituents	Fatty acids
Bilirubin	

It is a family of enzymes that is responsible for the glucuronidation of hundreds of compounds, including hormones, flavonoids, and environmental mutagens.

How can such an enzyme recognize all of these substrates and react with it ?

Seek next slides for the answer



and different reactions are catalyzed in different tissues

Not to memorize, just observe the mind-blowing variation, ﴿وَفِي أَنفُسِكُمْ أَفَلَا تُبْصِرُونَ﴾

Substrates	Place of reaction
Etoposide	Biliary tissue, colon, intestine, liver, stomach
Genistein	Biliary tissue, colon, liver, stomach
Tamoxifen	Biliary tissue, colon, intestine, liver
PCBs	Biliary tissue, brain, colon, kidney, larynx, liver, lung, stomach
Heterocyclic amines	Esophagus, intestine, kidney, larynx
Benzo[a]phrene	Colon, esophagus, intestine, kidney, larynx
Nicotine	Breast, colon, esophagus, liver, kidney, ovary, prostate, skin, testis
Raloxifene	Biliary tissue, colon, esophagus, intestine, orolaryngeal tissue, stomach

Get this concept, first…

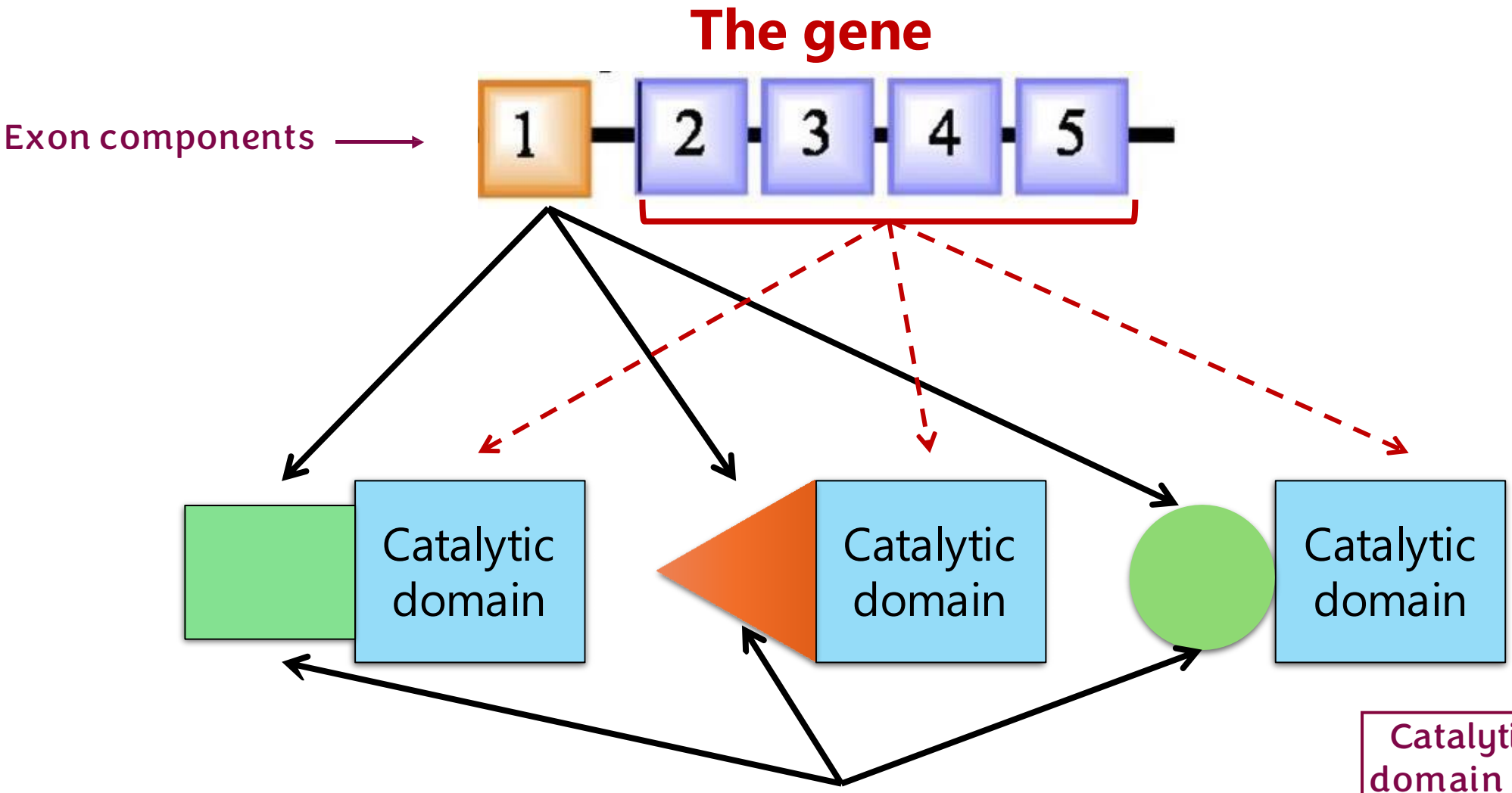
One drill, many flutes

One head, many hats

Drill= Enzyme Flutes= substrate.
You can use the same drill with many flutes.

- So, same enzyme has multiple substrates (same function but different substrate)

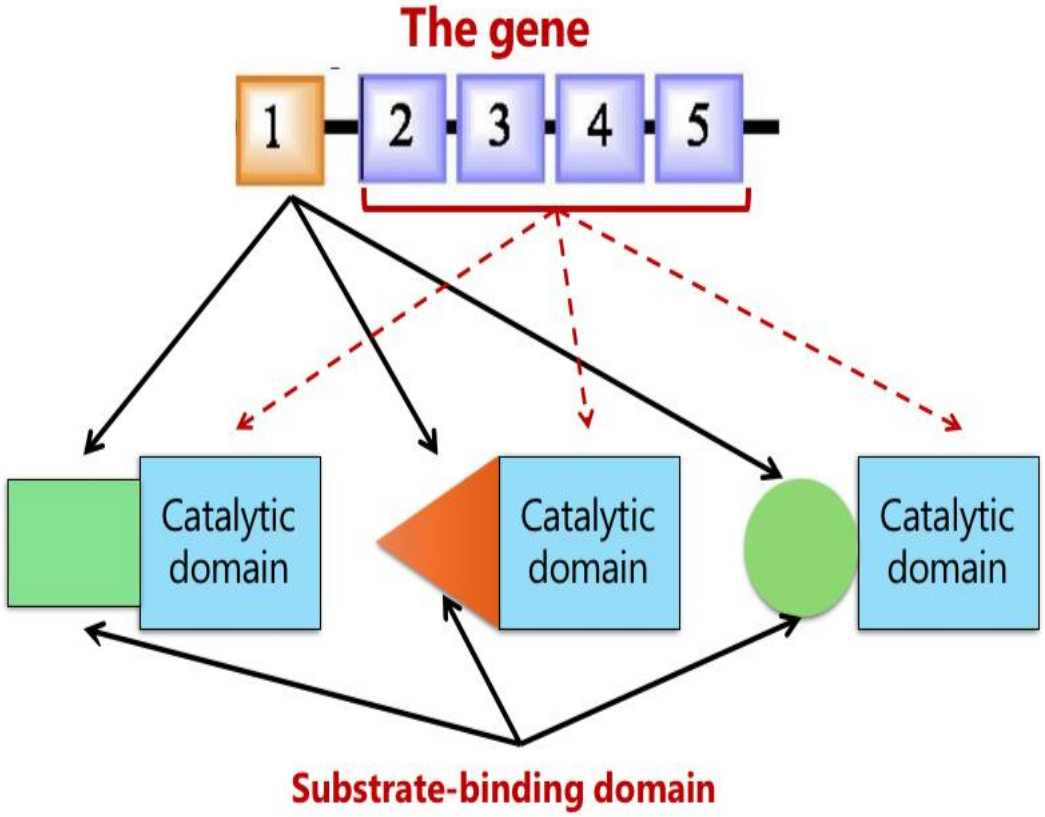
Then this…



Catalytic domain: the domain responsible for transferring the glucuronic acid.

✓ For better understanding...

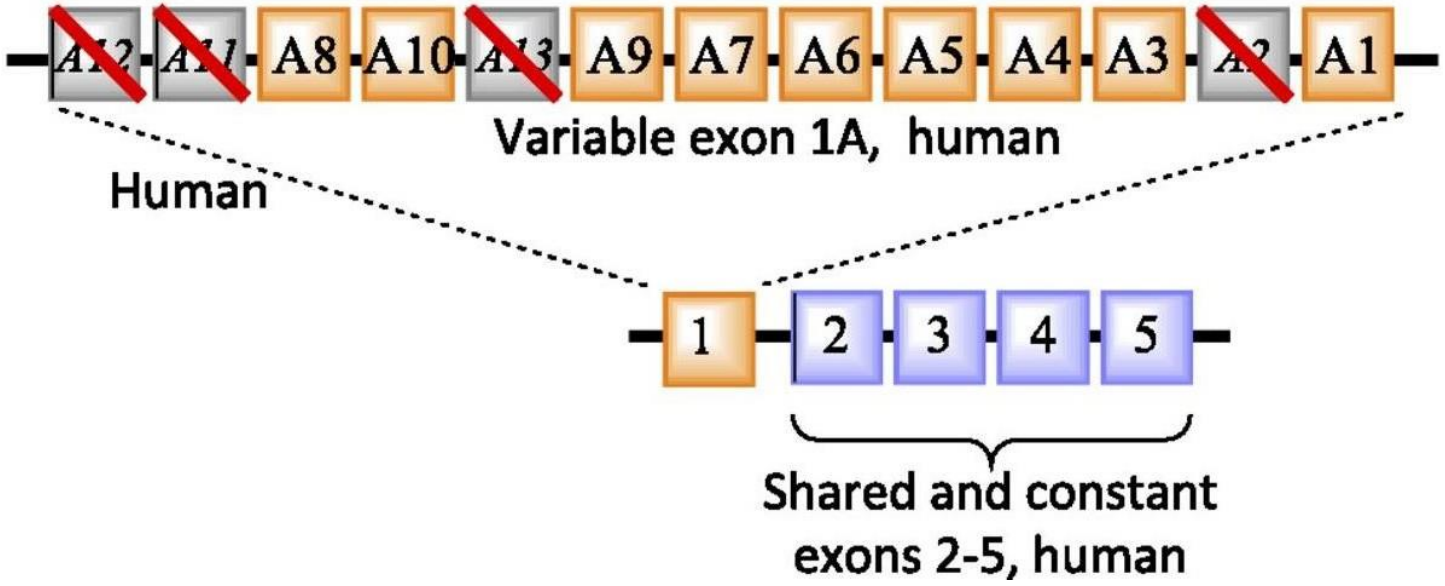
- This gene consists of **five exons**.These exons encode the **catalytic domain** that **carries out the addition of a sugar molecule to the substrate.**
- The **catalytic domain is identical in all enzyme variants**, so the type of reaction remains the same.In contrast, **exon 1** determines substrate specificity because **it forms the substrate-binding domain**.Therefore, the enzymes share the **same enzymatic function but act on different substrates due to variations in the substrate-binding domain.**



قال تعالى : (وَاصْبِرْ فَإِنَّ اللَّهَ لَا يُضِيعُ أَجْرَ الْمُحْسِنِينَ)

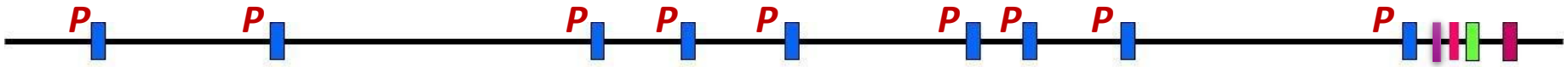
How does UGT1A do this?

- Exons 2, 3, 4, and 5 encode the catalytic domain that interacts with UDP-glucuronic acid, and exon 1 determines substrate specificity, but…
- Exon 1 contains NINE tandemly arrayed first exons and each one has its own promoter.
- The 9 exons determine substrate specificity and one of them is spliced to exon 2 generating 9 possible UGT1A transcripts.

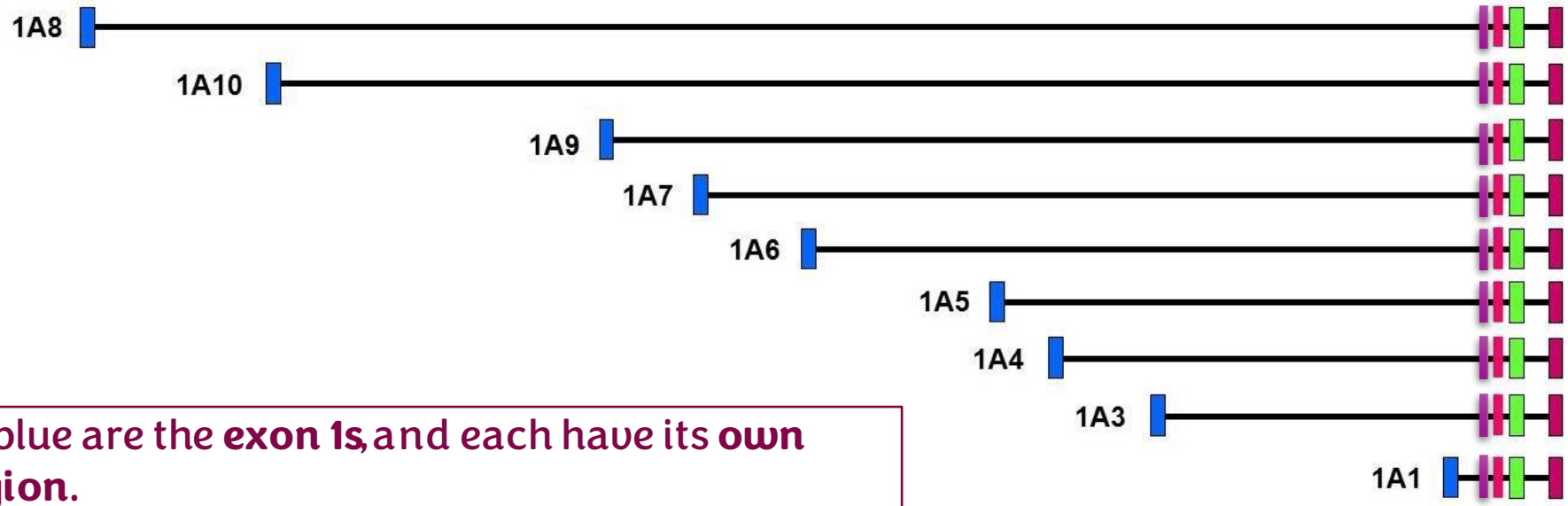


So, each sub exon can be transcribed independently of others > which will lead to 9 different binding sites that can react with 9 different substrates.

Splice variants for UGT1A



The possible transcripts



Structures in blue are the **exon 1s**, and each have its **own promotor region**.
So, **9** different **transcripts** and **different** substrate **binding** domain but the ***same catalytic*** domain.

Explaining the substrate specificity and tissue distribution

- The variation of proteins produced from the same gene is the reason behind the diversity of humans

Gene	Where expressed	Substrates
UGT1A1	Biliary tissue, colon, intestine, liver, stomach	Etoposide
UTG1A3	Biliary tissue, colon, liver, stomach	Genistein
UGT1A4	Biliary tissue, colon, intestine, liver	Tamoxifen
UGT1A6	Biliary tissue, brain, colon, kidney, larynx, liver, lung, stomach	PCBs
UGT1A7	Esophagus, intestine, kidney, larynx	heterocyclic amines
UGT1A8	Colon, esophagus, intestine, kidney, larynx	Benzo[a]phrene
UGT1A9	Breast, colon, esophagus, liver, kidney, ovary, prostate, skin, testis	Nicotine
UGT1A10	Biliary tissue, colon, esophagus, intestine, orolaryngeal tissue, stomach	Raloxifene

Regulation of mRNA stability

Physiology of iron

- Iron is an essential metal for the human body.
  - Oxygen transport
  - Enzyme function
- But, too much iron can be toxic.
  - Organ failure
  - Bacterial infection(iron attracts bacterial growth , so high levels of it increase bacterial infection)
- The level of iron is intricately maintained.

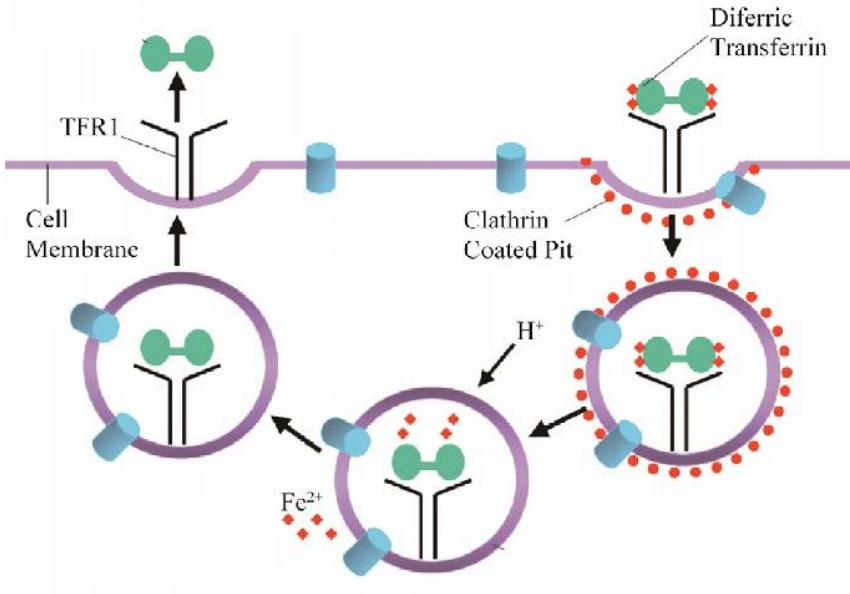
The players

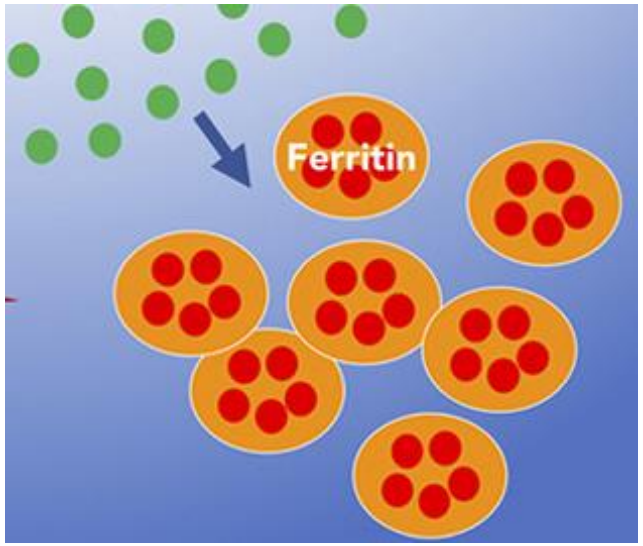
- Liver ferritin protein stores iron when abundant (in the liver).

✓It's function is to bind to iron , so it is a storage place of iron in the body .

- Transferrin receptor mediates iron entry via receptor-mediated endocytosis into peripheral cells when needed.
- When iron is high, expression of ferritin should be up-regulated (for iron storage) and expression of transferrin receptor should be down-regulated (Iron should not be transported into the cell because adequate levels are already present, and excess iron can be toxic), and vice versa.

✓The regulation of ferritin is the opposite of the regulation of transferrin receptor (inverse regulatory relationship).



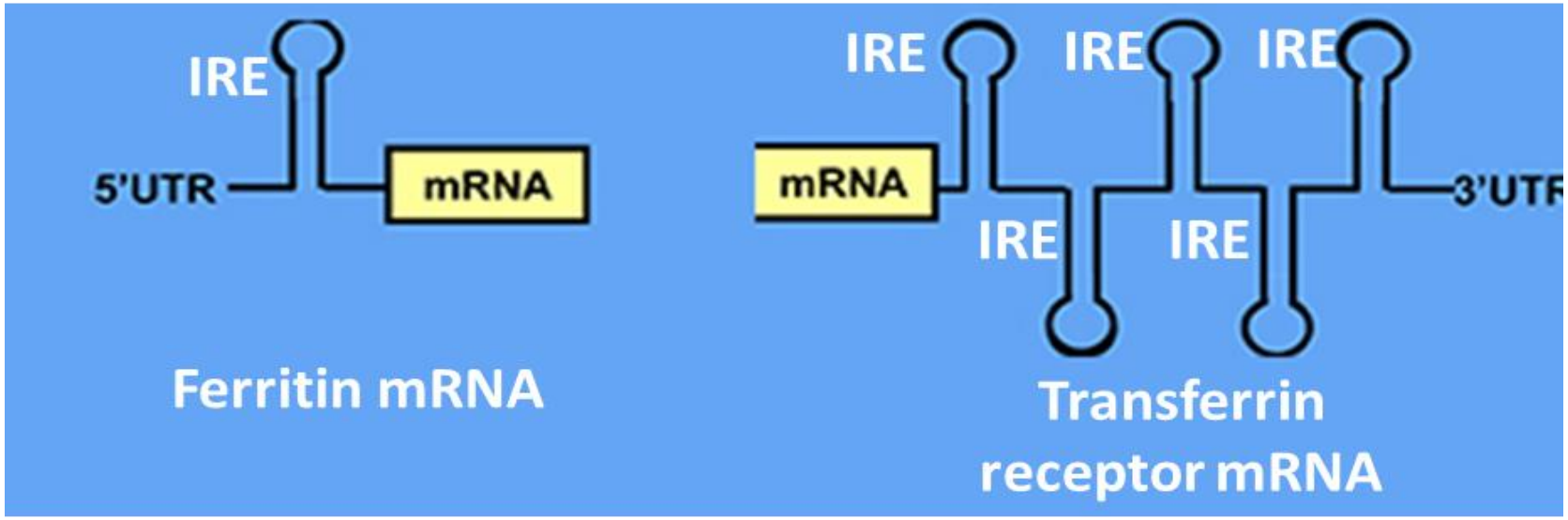


Iron-response elements

- In human iron-regulatory genes, there are genetic regions (of mRNAs, as well) called **iron response elements (IREs),** these regions regulate transferrin receptor and ferritin levels.
- These regions also exist within the mRNAs of ferritin and transferrin receptor but at different sides.

✓**In ferritin mRNA**, the **IRE** is located at the **5' end**.

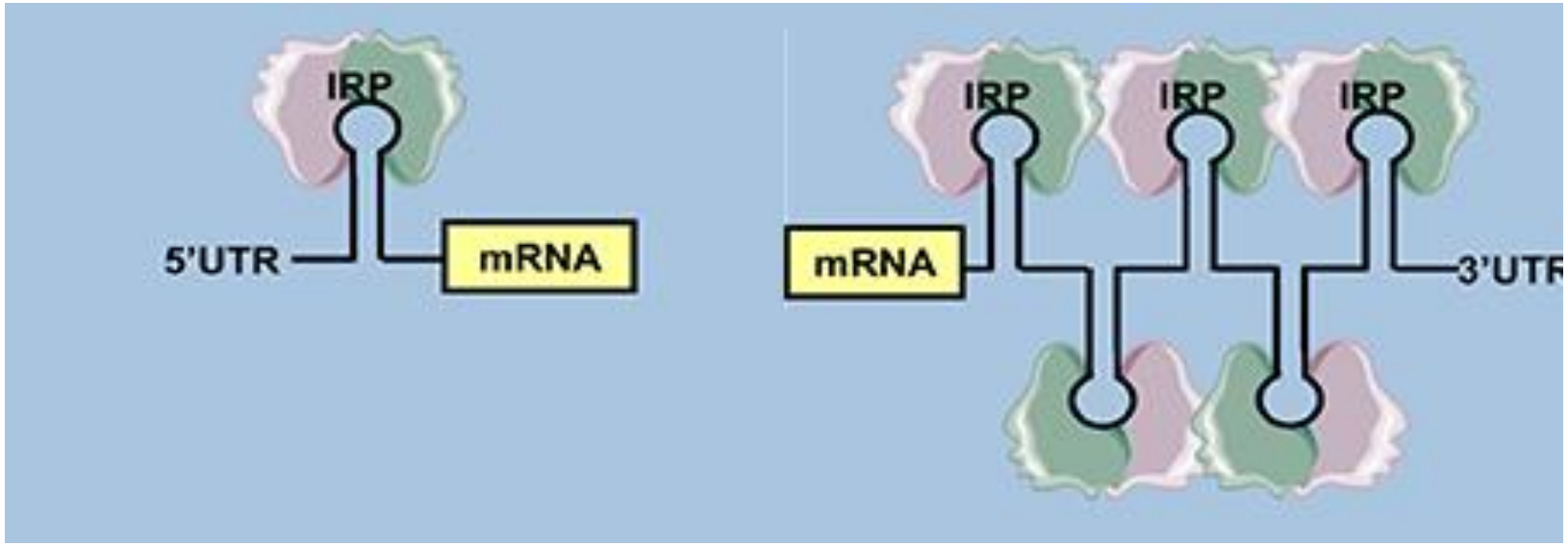
✓**In transferrin receptor mRNA**, the **IRE** is located at the **3' end**.



Iron regulatory protein

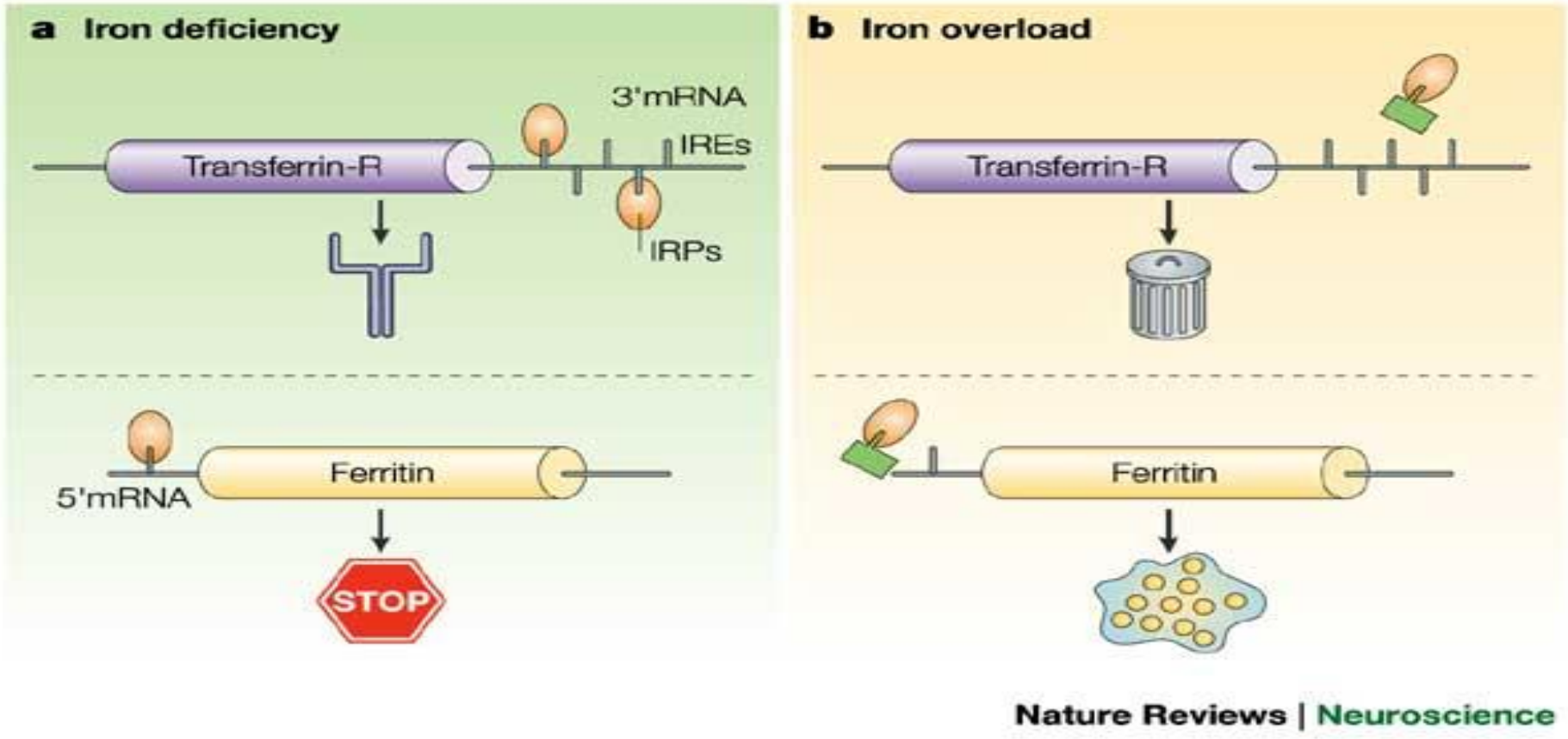
✓ Iron response elements are the binding sites for iron regulatory proteins, which can sense and respond to cellular iron levels.

- When iron is low, the iron regulatory protein (IRP) – IRP are active when free of iron –binds to IREs influencing protein expression.
  - Remember, this binding happens when iron is low.
- When iron is high, iron binds to IRP preventing its binding to the IRE.



Effect on expression

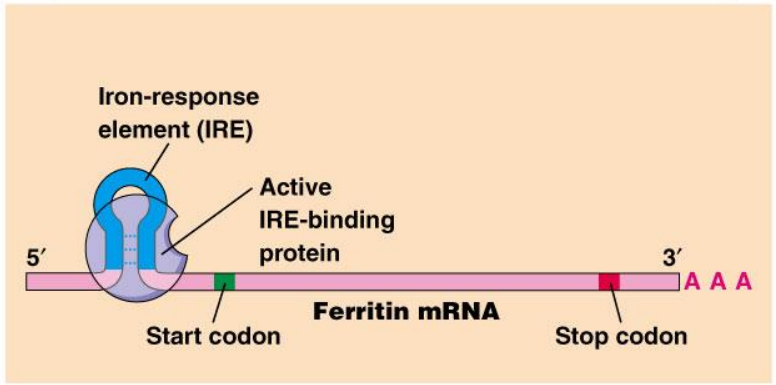
- **When iron is abundant (high) in the cells**, it binds to IRP, disabling the binding of IRP to the mRNAs of transferrin receptor and ferritin.
  - Transferrin receptor: mRNA is **destabilized** and is degraded, **lowering protein level, and, hence, iron uptake.**
  - Ferritin: Translation is activated and **storage increases**.
- **When iron is low,** the IRP is iron-free and can bind to the mRNAs of transferrin receptor and ferritin.
  - Transferrin receptor: mRNA is **stabilized**, more protein is made, and, hence, **iron uptake into the cells increases**.
  - Ferritin: Translation (protein synthesis) is blocked, and **less protein is available for storage.**



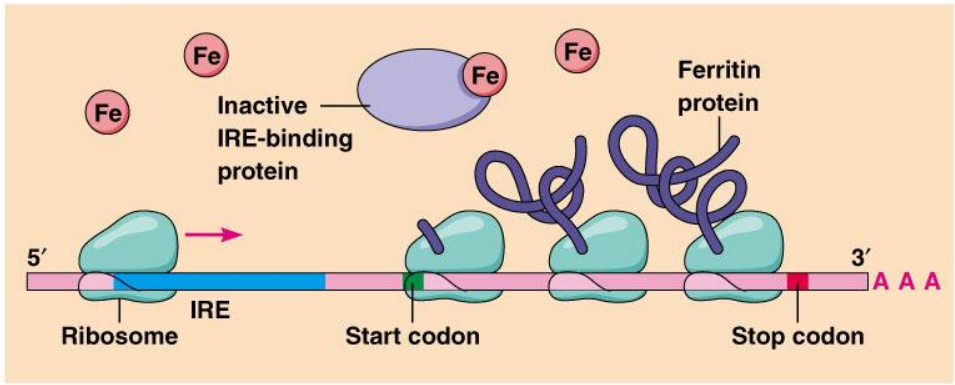
Quick recap:

- ✓ When iron is high: IRP binds iron → cannot bind mRNA
- Transferrin receptor: mRNA is degraded → protein decreases → iron uptake decreases.
- Ferritin: Translation is activated → storage increases.
- ✓ When iron is low: IRP is iron-free → binds mRNA
- Transferrin receptor: mRNA is stabilized → protein increases → iron uptake increases.
- Ferritin: Translation is blocked → storage decreases.

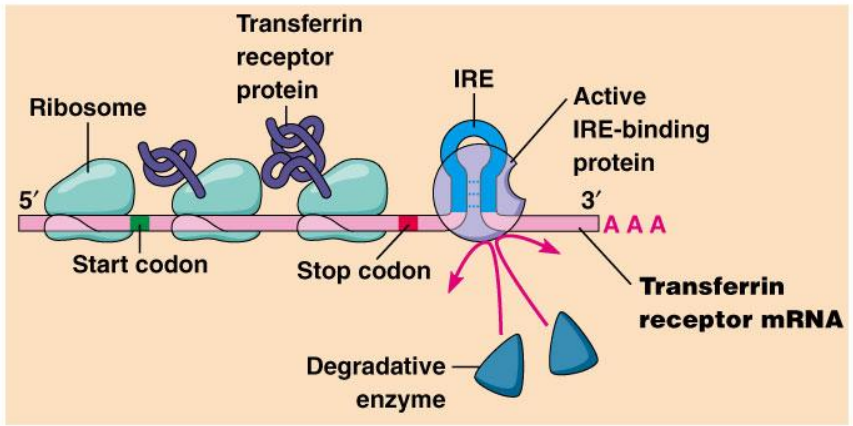
(a) Low iron concentration. IRE-binding protein binds to IRE, so translation of ferritin mRNA is inhibited.



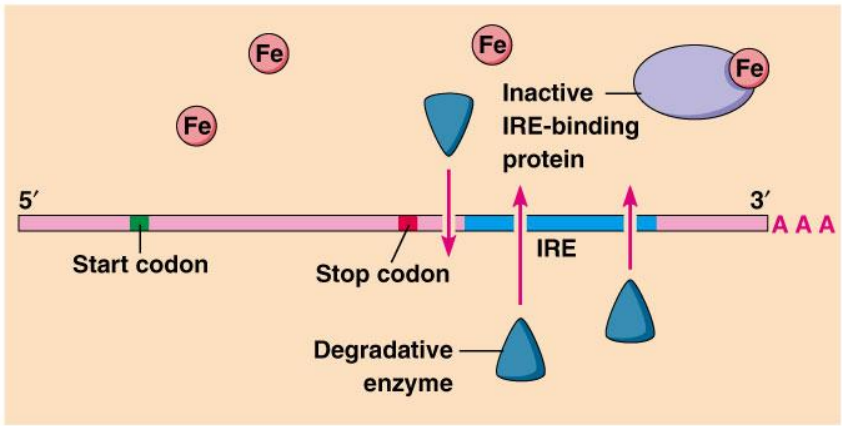
(b) High iron concentration. IRE-binding protein cannot bind to IRE, so translation of ferritin mRNA proceeds.



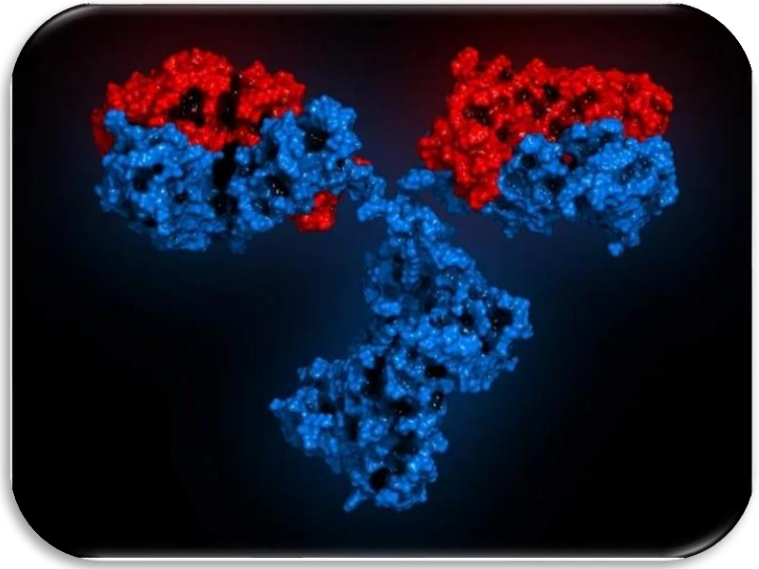
(a) Low iron concentration. IRE-binding protein binds to the IRE of transferrin receptor mRNA, thereby protecting the mRNA from degradation. Synthesis of transferrin receptor therefore proceeds.



(b) High iron concentration. IRE-binding protein cannot bind to IRE, so mRNA is degraded and synthesis of transferrin receptor is thereby inhibited.



Quiz on this lecture



رسالة من الفريق العلمي:

وقد عى السلف رضي الله عنهم وصيّة نبيّهم ﷺ، فجعلوا الموت أمام أعينهم، فقصرت آمالُهم، وصلحت أعمالُهم وقلوبُهم.

انظر إلى الربيع بن خثيم رضي الله عنه وهو مَن هو صلاحًا وعلمًا وزهدًا يقول: لوغفل قلبي عن ذكر الموتِ ساعةً واحدةً لفسد قلبي. وقال أبو الدرداء رضي الله عنه: كفى بالموت واعظًا، وكفى بالدهر مُفرّقًا، اليوم في الدور، وغدًا في القبور.

عندما دخلت فاطمة الزّهراء رضي الله عنها على أبيها رسول الله ﷺ وهو يجود بنفسه (أي قاربَ أن يموت) فقالت : "واكَربَ أباهُ ! " وعندما سمعها ﷺ قال لها : **"لا كَرْبَ على أبيك بعد اليومِ، إنه قد حضر من أبيك ما ليس بتاركٍ منه أحدًا، الموافاةُ يومَ القيامةِ .."**

ويقول الحافظ ابن رجب رحمه الله: يا من تمرُّ عليه سنةٌ بعد سنةٍ وهو مُستثقل في نوم الغفلةِ والسّنة، يا من يأتي عليه عامٌ بعد عامٍ وقد غَرِق في بحر الخطايا.. يا من يشاهد الآيات والعبر كلّما توالت عليه الأعوام والشهور ولا ينتفع بما يسمع ولا بما يرى من عظائم الأمور.

فالموتُ نهاية كلّ حيٍّ لا محالة، طوبى لمن كان للموت ذاكرًا، وله عاملًا، لم تشغله دنياه عن آخرته. اللّهمّ أيقظنا من غفلتنا، ووفّقنا للاستعداد للموت، والعمل له.

الدقيقة 5:00

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			