MOLECULAR BIOLOGY

بسم الله الرحمن الرحيم

FINAL – Lecture 11

Transcriptional Phenomena in Humans

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُمْ ﴾
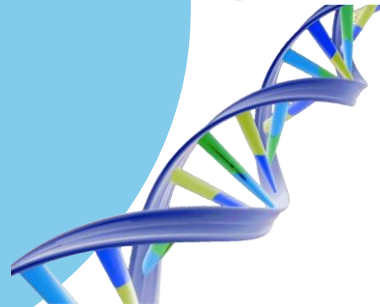
اللهم استعملنا ولا تستبدلنا

Written by :
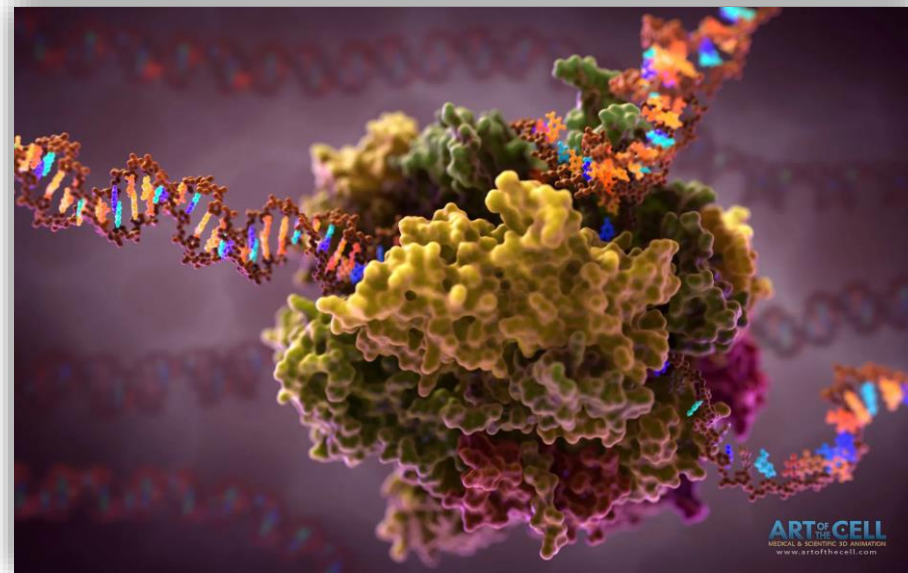
- **Abd Al Rahman Musa**

Reviewed by :

- **Mahmoud Aljunaidi**

Quiz on the previous lecture

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.)

❖ <u>**Transcription**</u> is the process of how the cell makes RNA from DNA.
1. RNA polymerase is the enzyme that builds RNA using one DNA strand as a template.
2. 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.

❖ <u>**Transcription in Eucaryotic genes:**</u>
    - **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.

❖ <u>**RNA Processing in Eukaryotes:**</u>
- **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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Second year, First semester, 2024-2025

وَتَوَكَّلْ عَلَى الْحَيِّ الَّذِي لَا يَمُوتُ وَسَبِّحْ بِحَمْدِهِ ۚ وَكَفَىٰ بِهِ بِذُنُوبِ عِبَادِهِ خَبِيرًا
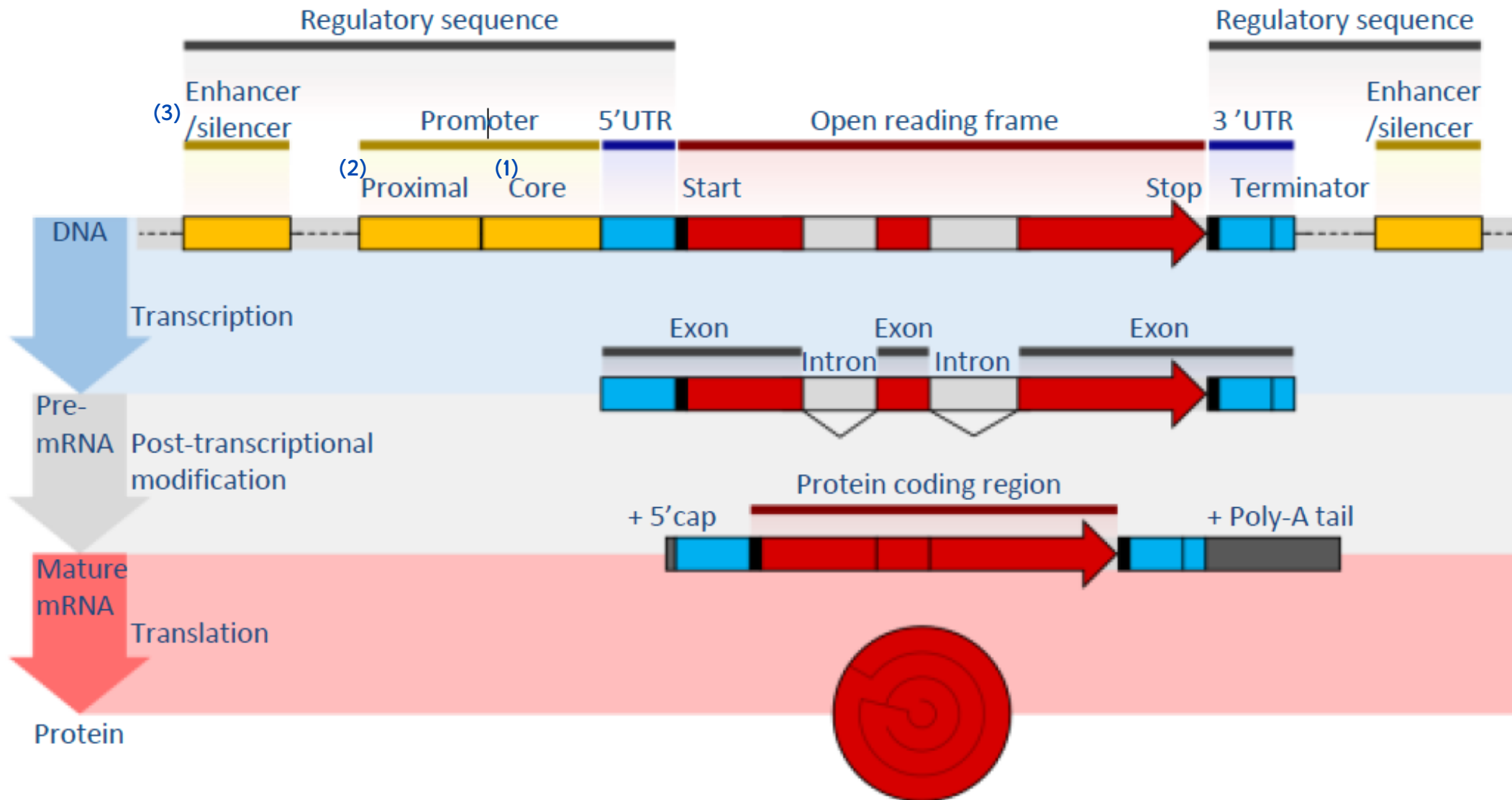
Anatomy of a simple eukaryotic gene

Image Illustration<u>*pt.1*</u>

- Shows ⁽¹⁾core promoter region (ex. TATA box), with a ⁽²⁾proximal promoter element next to it that can control the expression of a certain gene. ⁽¹⁾ Is essential for transcription initiation.

- There's also ⁽³⁾enhancers and silencers that further modulate transcription.

- Enhancers can regulate genes regardless of their location or orientation due to **DNA looping**, which enables proteins bound to enhancers to interact with promoter-bound proteins via the **Mediator** complex.

Remember: for enhancers to function they need to be within the **same domain** as the gene transcribed.

Image Illustration _pt.2_

1. The gene is first **transcribed** (from the transcription site).

2. Primary RNA (**Pre-RNA**) is produced; containing exons and introns.

3. Pre-RNA is **spliced**; by removal of introns and exons (in alternative splicing).

4. **Mature RNA** (mRNA) is produced, with exons only.

5. Mature RNA can be used to produce several *proteins (which can have isoforms) via **translation**.

➢ Alternative splicing increases diversity; we have 20,000 protein coding Genes, but we can have ~80,000 mRNA transcripts.

   * **Protein isoforms**: Variants of proteins with similar functions but differing in their regulation, structure, and binding affinity... etc.
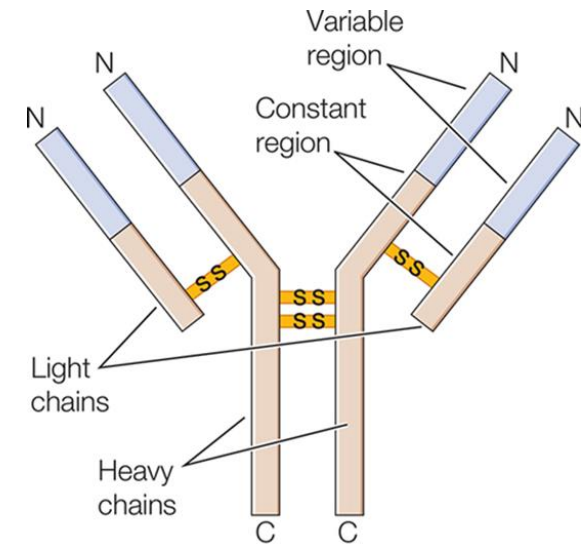
Gene rearrangement

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.
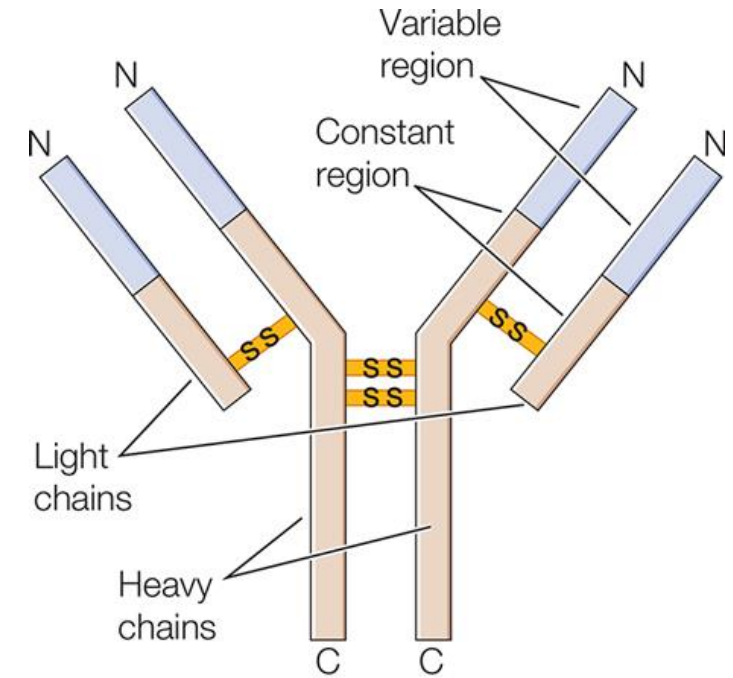
Immunoglobulins- Explanation

- They are **diverse** proteins that interact with **foreign antigens** 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**.
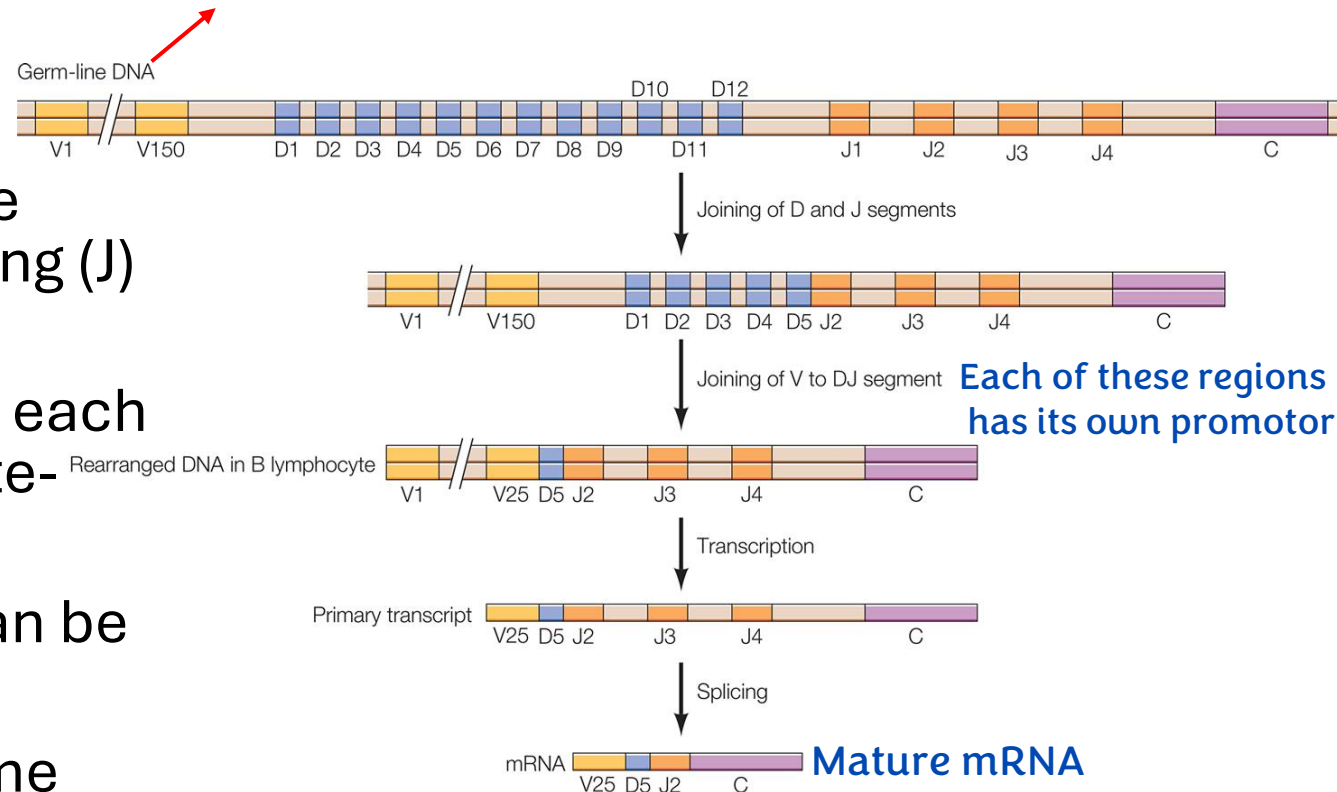
The mechanism

- 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.

Each of these regions has its own promotor

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.
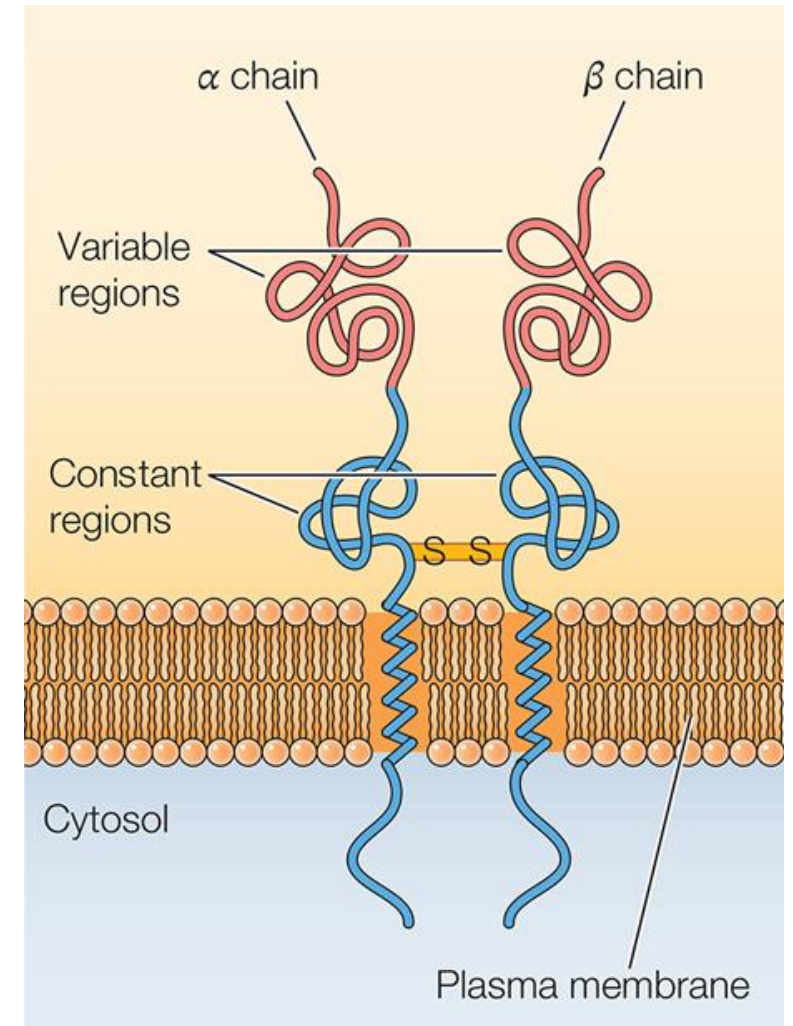
T cells and CART cells

- The T cell receptor on the surface of T lymphocytes is produced by site-specific recombination as well.

- A new type of cancer treatment (CAR-T cell therapy) utilizes a patient's T cells that have been 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.

α chain β chain
Variable regions
Constant regions
S S
Cytosol
Plasma membrane

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

Gene amplification

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 production.

- 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. وكم من نعمة انعمت بها علينا قل لها شكرك عندنا ,الحمدلله عدد خلقك

- Breast tumor cells become amplify the human epidermal growth factor receptor 2 (HER2) making them more aggressive in growth and progression.
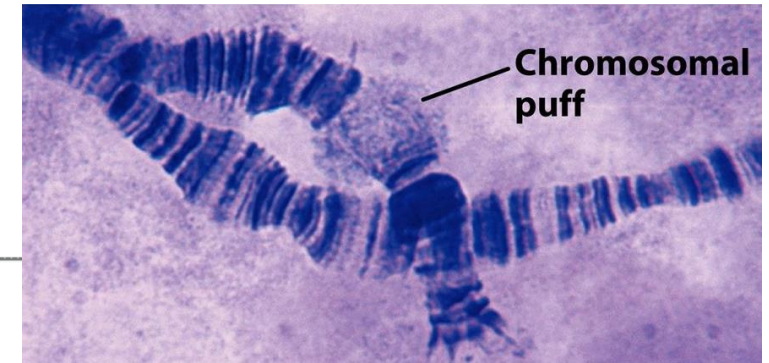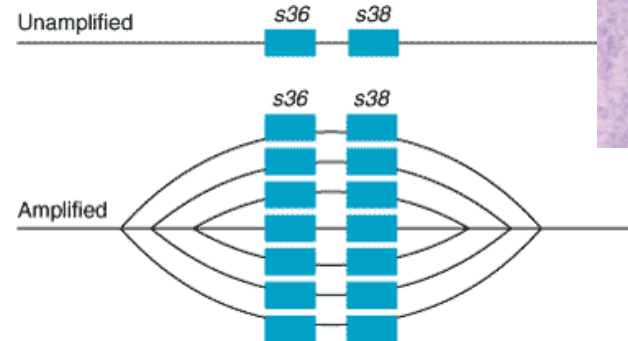

Normal breast **cell**

Abnormal HER2+ breast cancer cell

Signal

Nucleus
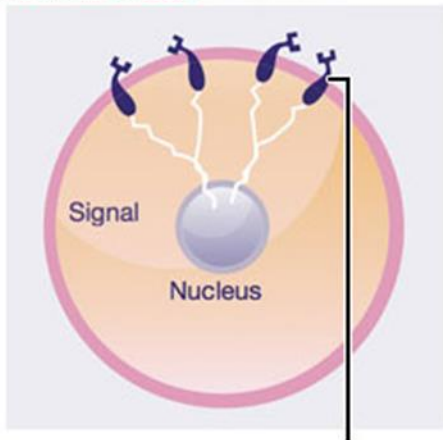
Normal amount of HER2 receptors send signals telling cells to grow and divide.[1]
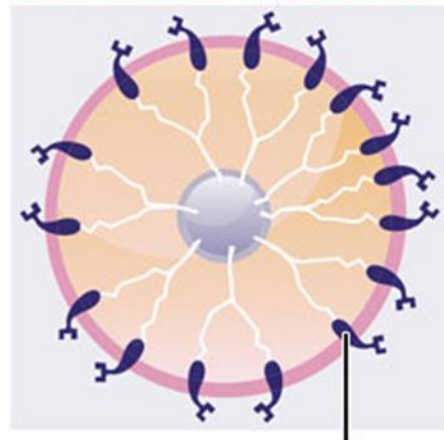
Too many HER2 receptors send more signals, causing cells to grow too quickly.[1]

Unamplified   s36   s38

Amplified   s36   s38

Chromosomal puff

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.


0   1   2   3
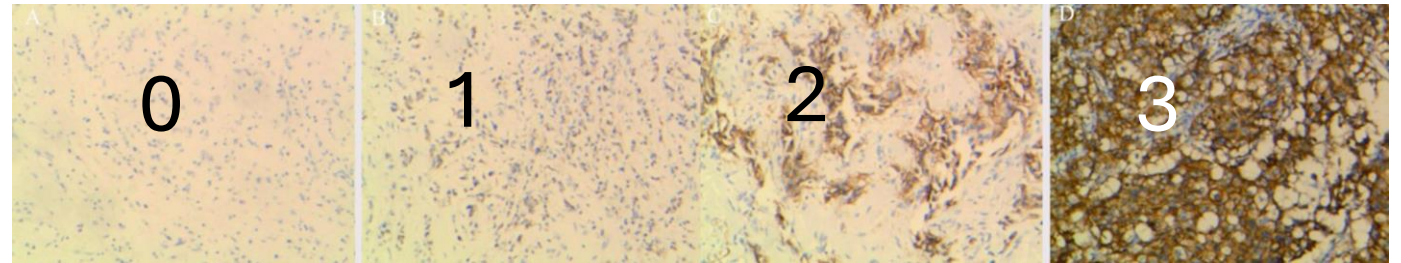
Different scoring for determining the gene amplification using immunohistochemistry
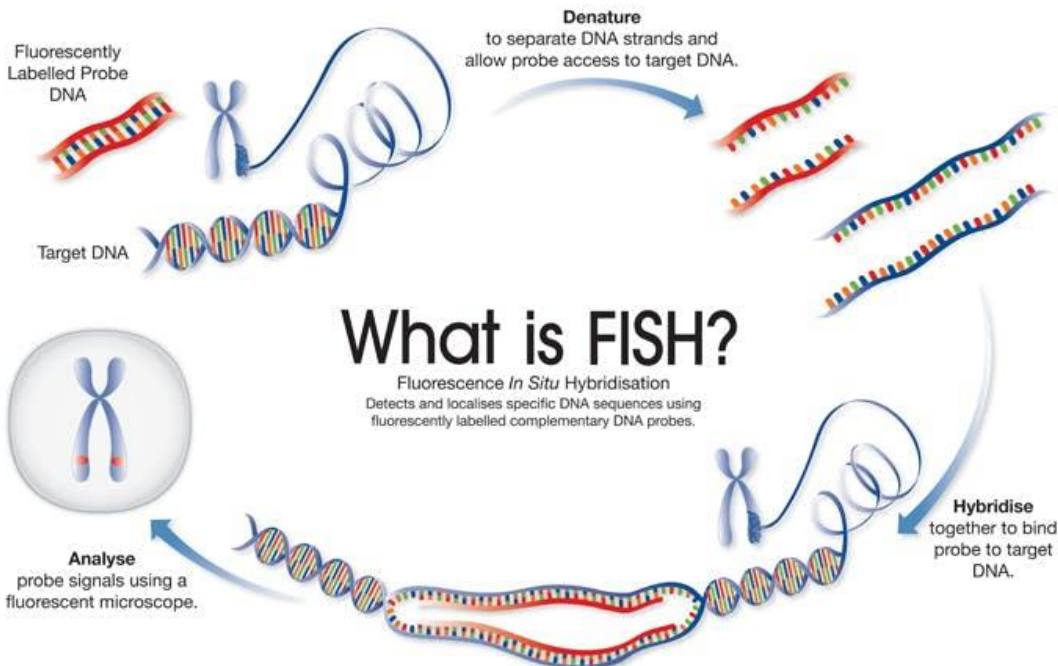
What is FISH?
Fluorescence *In Situ* Hybridisation
Detects and localises specific DNA sequences using fluorescently labelled complementary DNA probes.

Fluorescently Labelled Probe DNA

Target DNA

Denature to separate DNA strands and allow probe access to target DNA.

Hybridise together to bind probe to target DNA.

Analyse probe signals using a fluorescent microscope.

The high amount of red/pink dots indicate the presence of gene amplification (a- normal state / b- gene amplification)
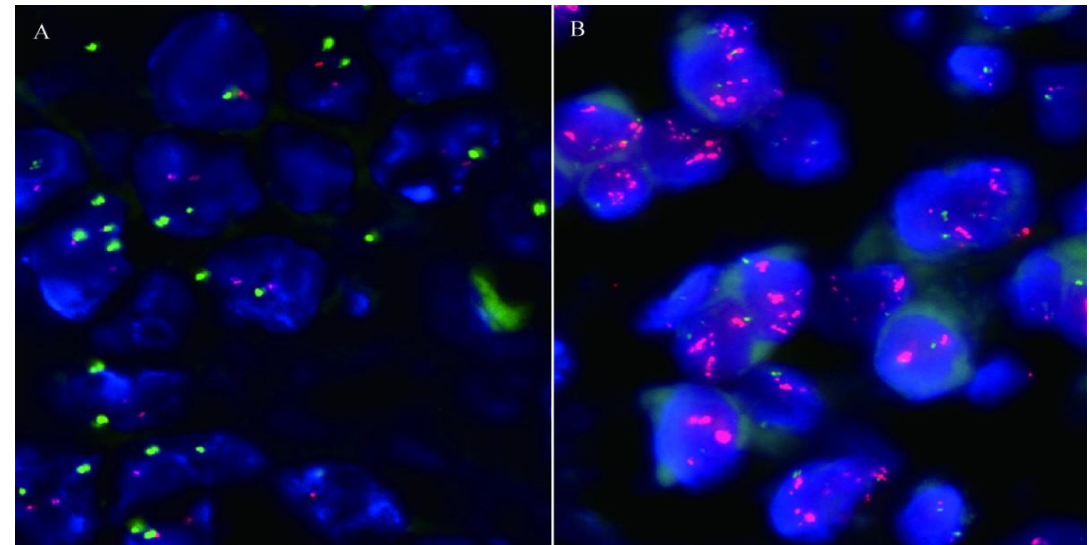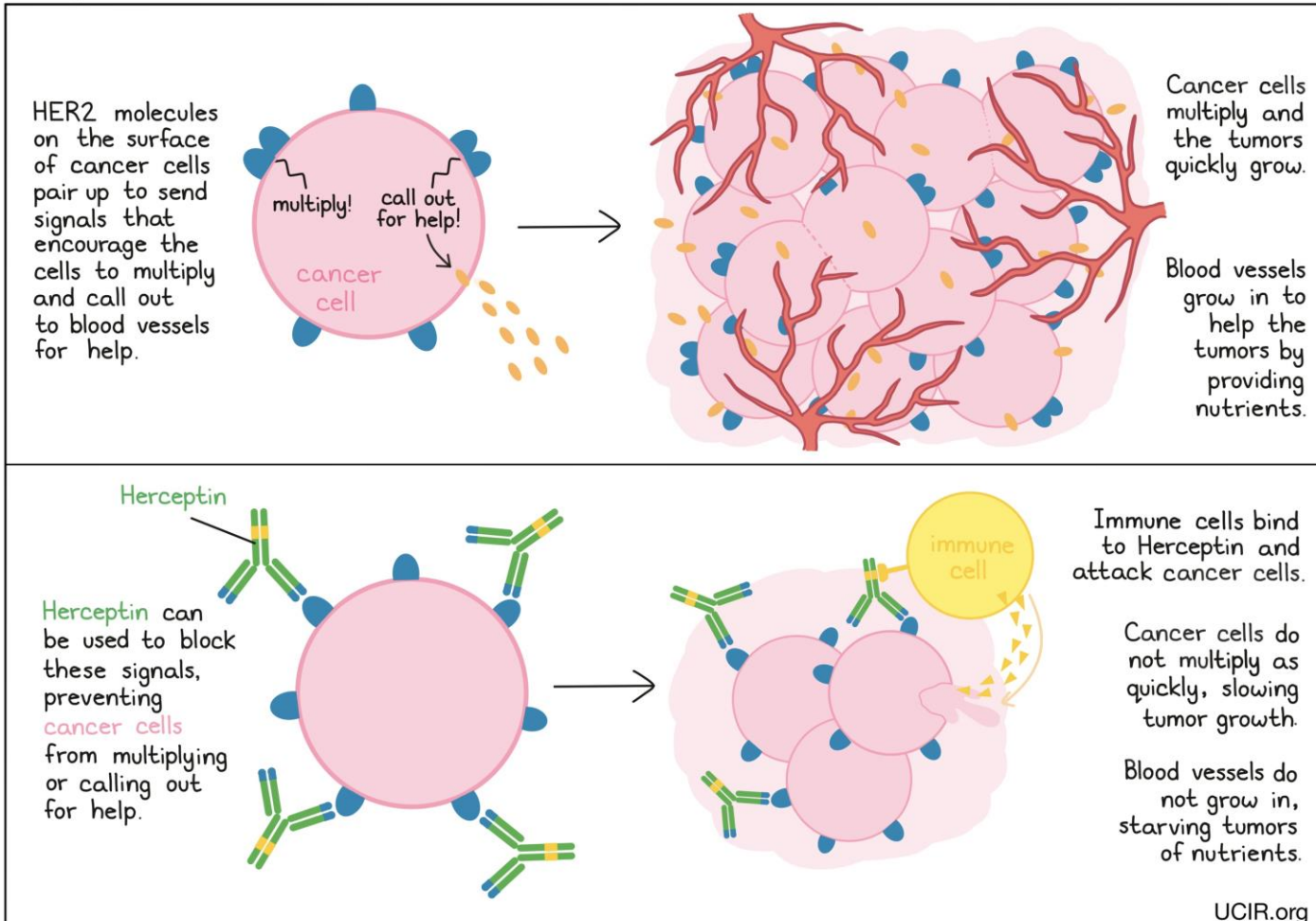
How are HER2-enriched cancers treated?

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.

Herceptin: how it works

HER2 molecules on the surface of cancer cells pair up to send signals that encourage the cells to multiply and call out to blood vessels for help.

multiply!   call out for help!

cancer cell

Cancer cells multiply and the tumors quickly grow.

Blood vessels grow in to help the tumors by providing nutrients.

Herceptin

Herceptin can be used to block these signals, preventing cancer cells from multiplying or calling out for help.

immune cell

Immune cells bind to Herceptin and attack cancer cells.

Cancer cells do not multiply as quickly, slowing tumor growth.

Blood vessels do not grow in, starving tumors of nutrients.
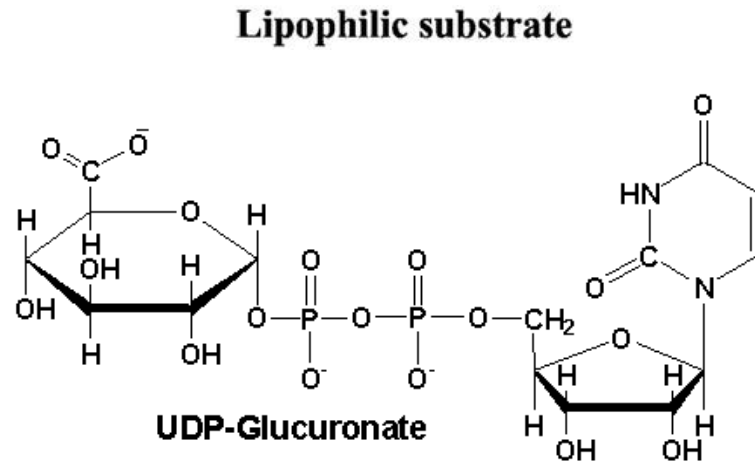
UCIR.org

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

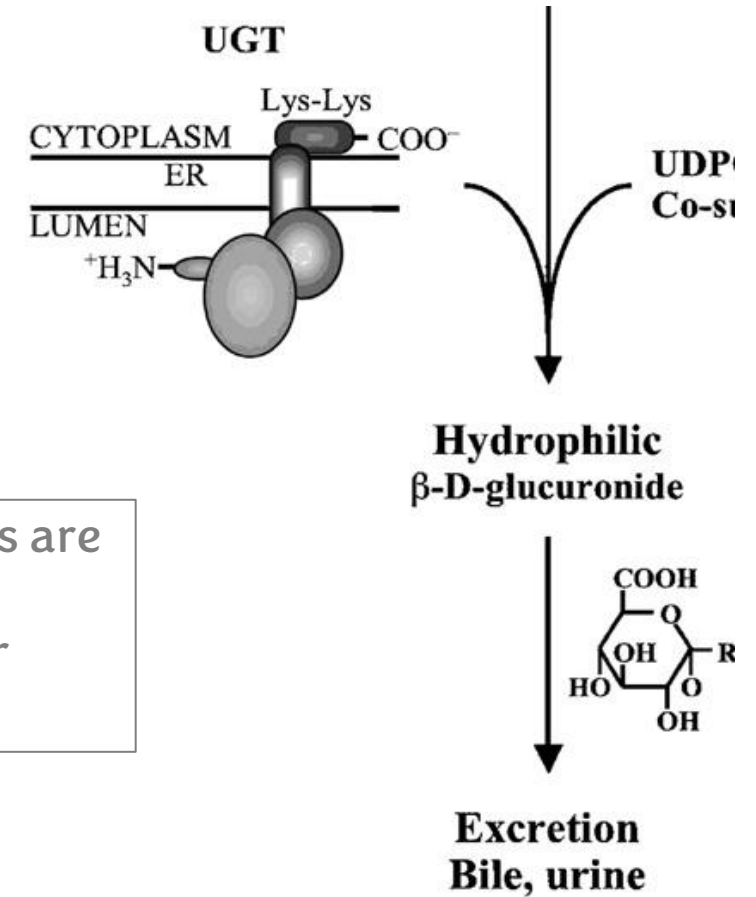# Multiple promotors, multiple exon 1s

It's common

An example of alternative splicing:
UDP-glucuronosyltransferase (UGT)

Lipophilic substrate

UDP-Glucuronate

UGT

Lys-Lys

CYTOPLASM — COO$^-$

ER

LUMEN

$^+$H$_3$N—

Hydrophilic
β-D-glucuronide

UDP
Co-s

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

COOH

R

HO

OH

Excretion
Bile, urine

The uridine diphosphate glucuronosyltransferase (UGT) enzymes transfer glucuronic acid onto xenobiotics and other endogenous compounds making them water soluble and allowing for their biliary or renal elimination.
This process happens at the liver.

The enzyme(s) has many heterogenous substrates

Lipophilic substrate

Therapeutic drugs
Carcinogens
Environmental toxicants
Dietary constituents
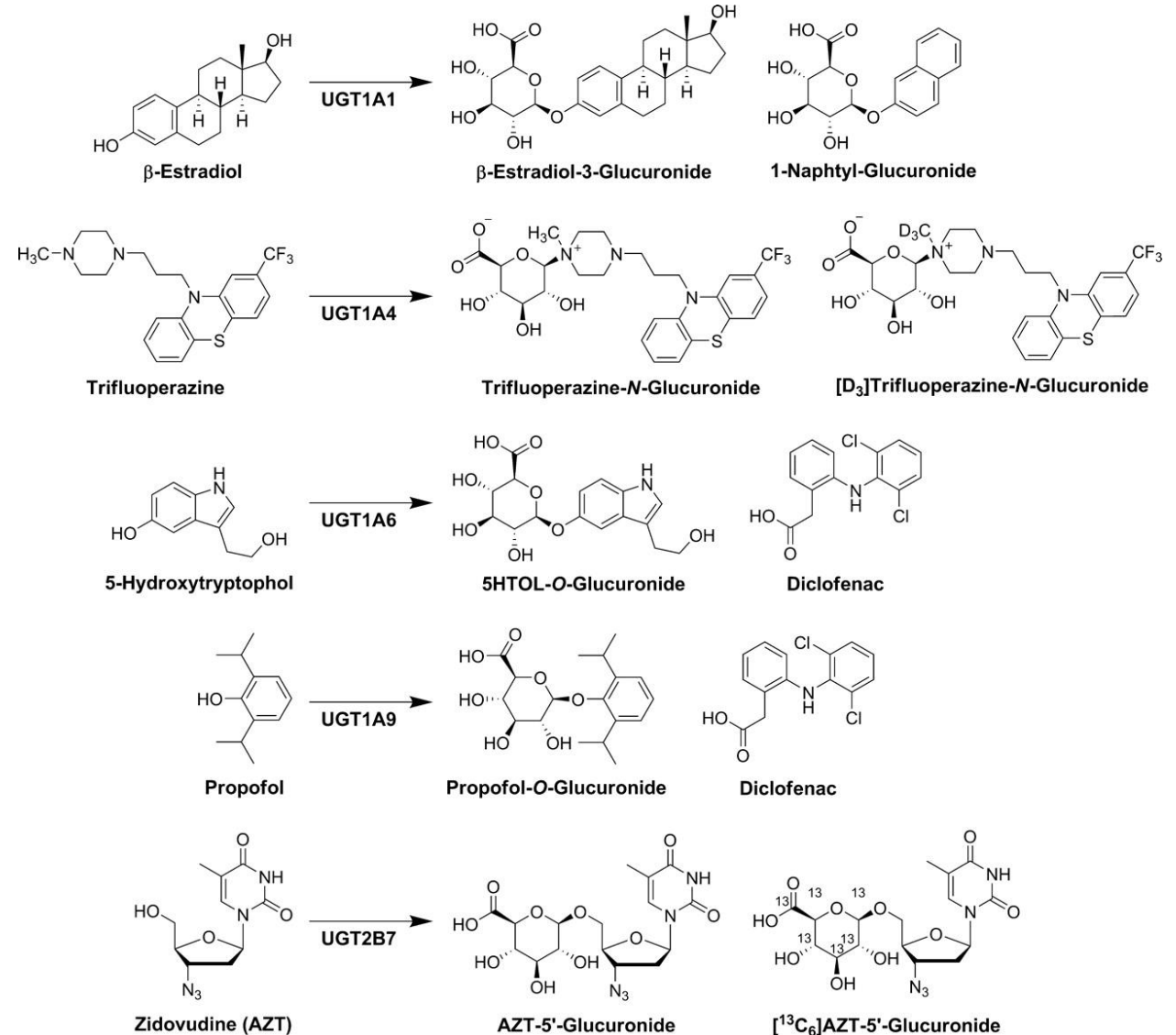Bilirubin

Biliary acids
Steroïds
Retinoic acids
Fatty acids

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.

Then this...

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.

Variable exon 1A, human

Human

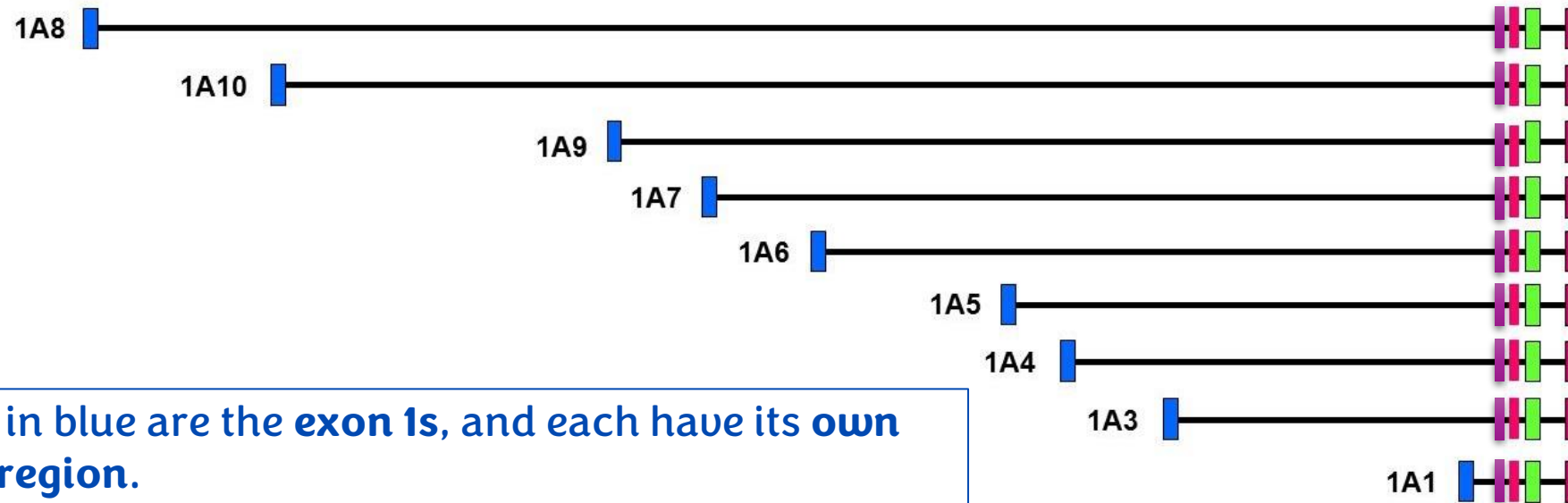Shared and constant exons 2-5, human
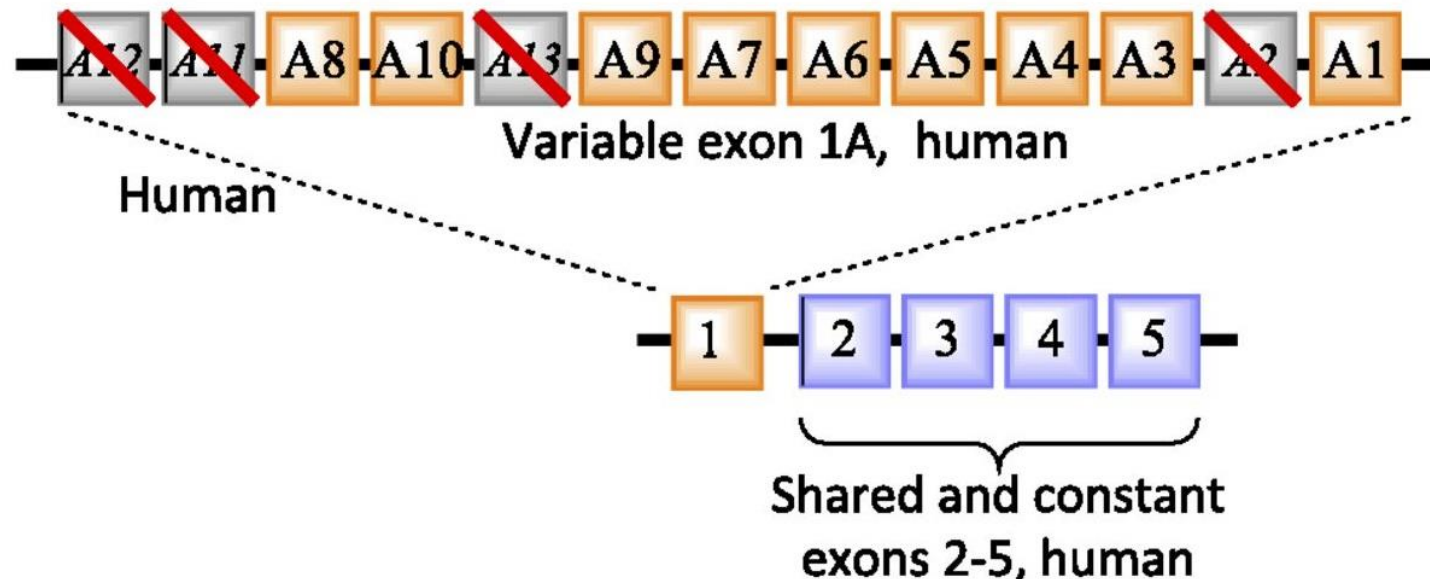
So, each sub exon can be transcribed independently of others → which will lead to 9 different binding sites that can react with 9 different substrates.

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
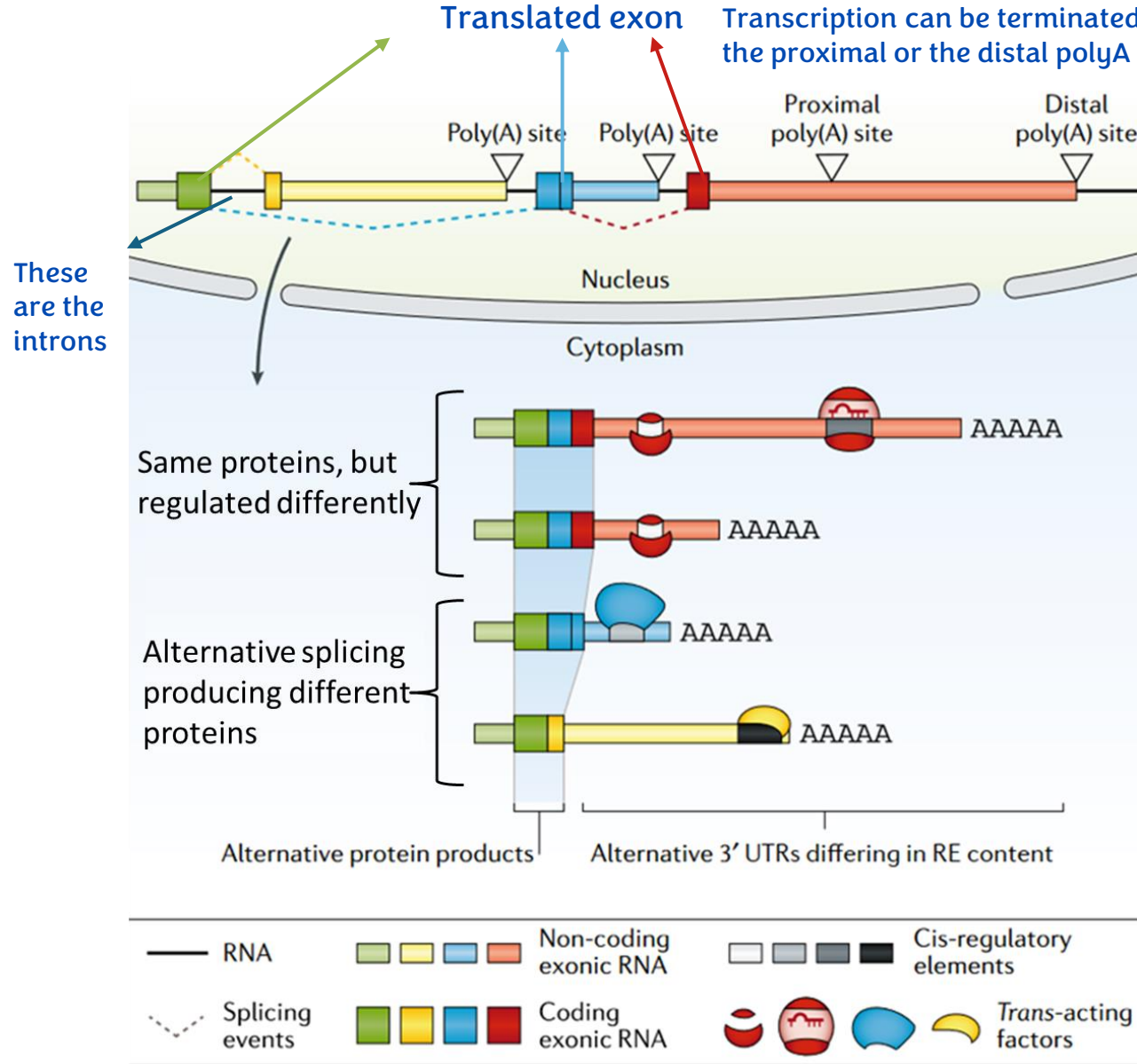is the reason behind the diversity of humans

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

# Alternative splicing and alternative polyadenylation

The advantage of polyadenylation

Translated exon

Transcription can be terminated either in the proximal or the distal polyA site

Poly(A) site   Poly(A) site   Proximal poly(A) site   Distal poly(A) site

These are the introns

Nucleus

Cytoplasm

Same proteins, but regulated differently

Alternative splicing producing different proteins

Alternative protein products   Alternative 3' UTRs differing in RE content

— RNA   Non-coding exonic RNA   Cis-regulatory elements

Splicing events   Coding exonic RNA   Trans-acting factors

- Transcription can be terminated at different poly-A sites generating short and long mature mRNAs.
  - The long mRNA is regulated differently than the short mRNA (*stay tuned for the microRNA part of this course*)
- The pre-mRNA can also be spliced differently.

  - This mechanism is important for regulating mRNA and translation.

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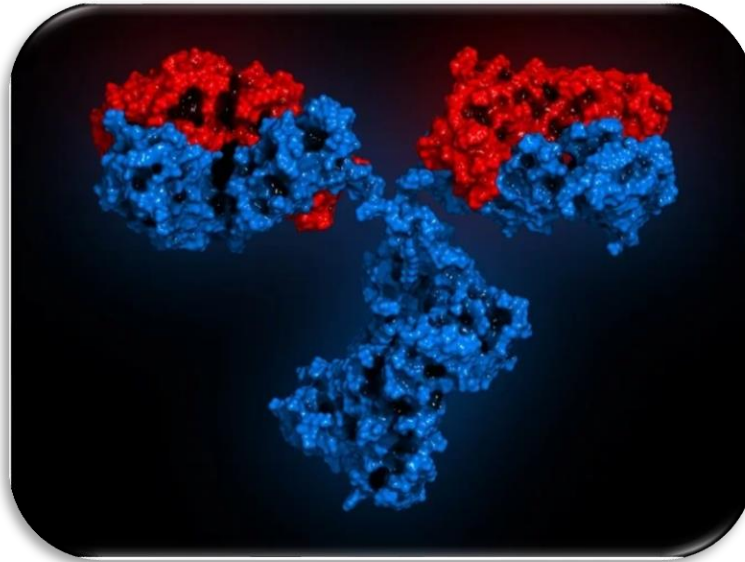
For any feedback, scan the code or click on it.

Corrections from previous versions:

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

Additional Resources: رسالة من الفريق العلمي:

فَإِنَّ مَعَ الْعُسْرِ يُسْرًا ۝

سُورَةُ الشَّرْح

بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِيمِ

#نطالب بارجاع طاولة الهوكي