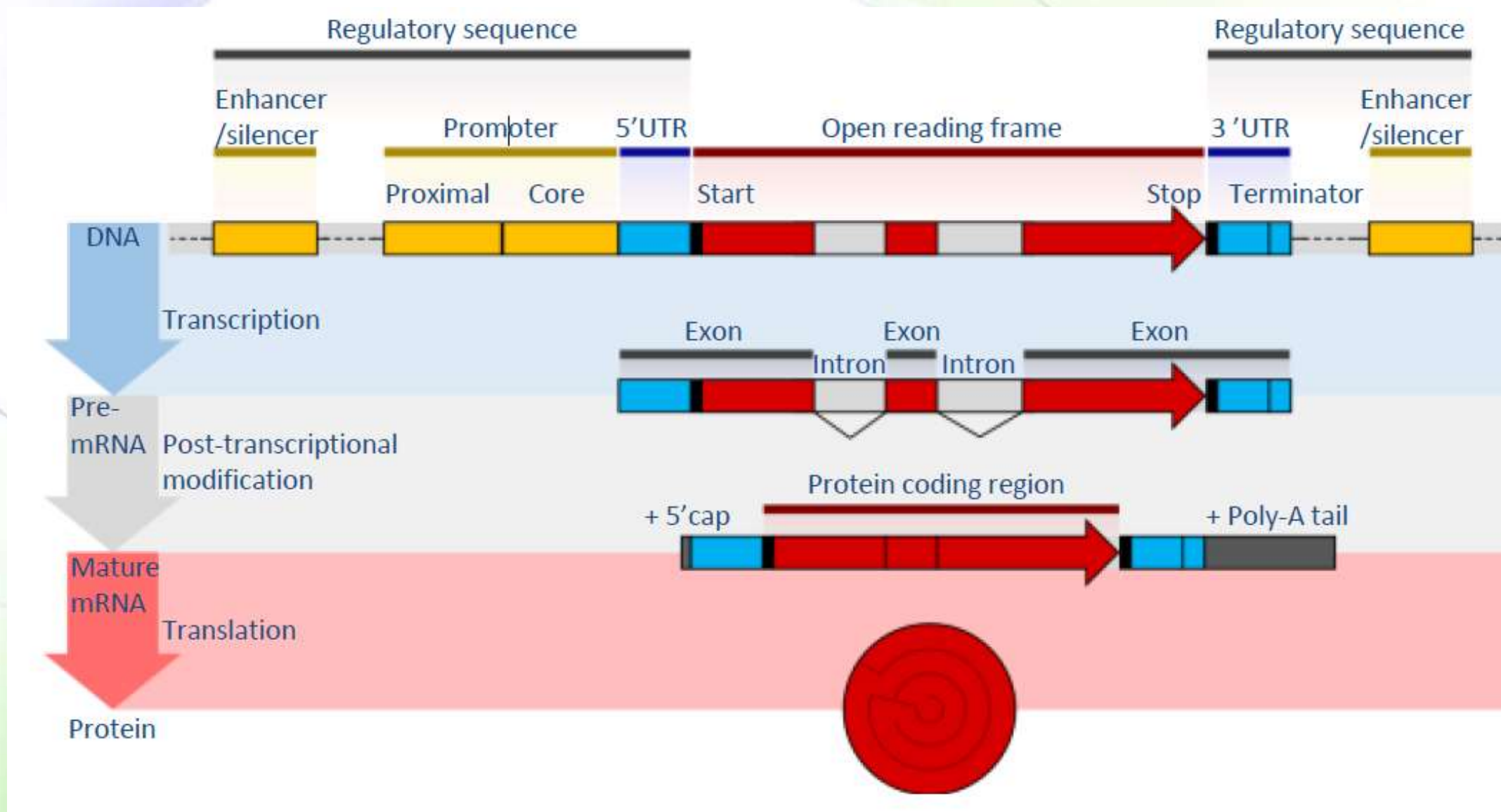




# Transcriptional phenomena in humans

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School of Medicine  
Second year, First semester, 2024-2025

# Anatomy of a simple eukaryotic gene



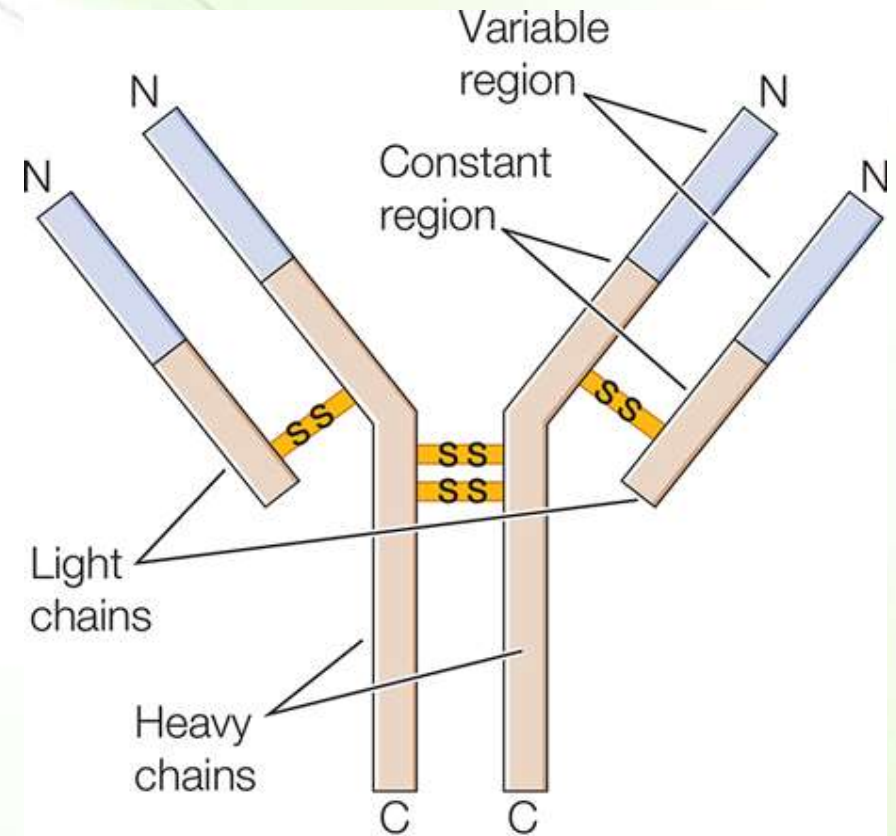


# Gene rearrangement

# Immunoglobulins



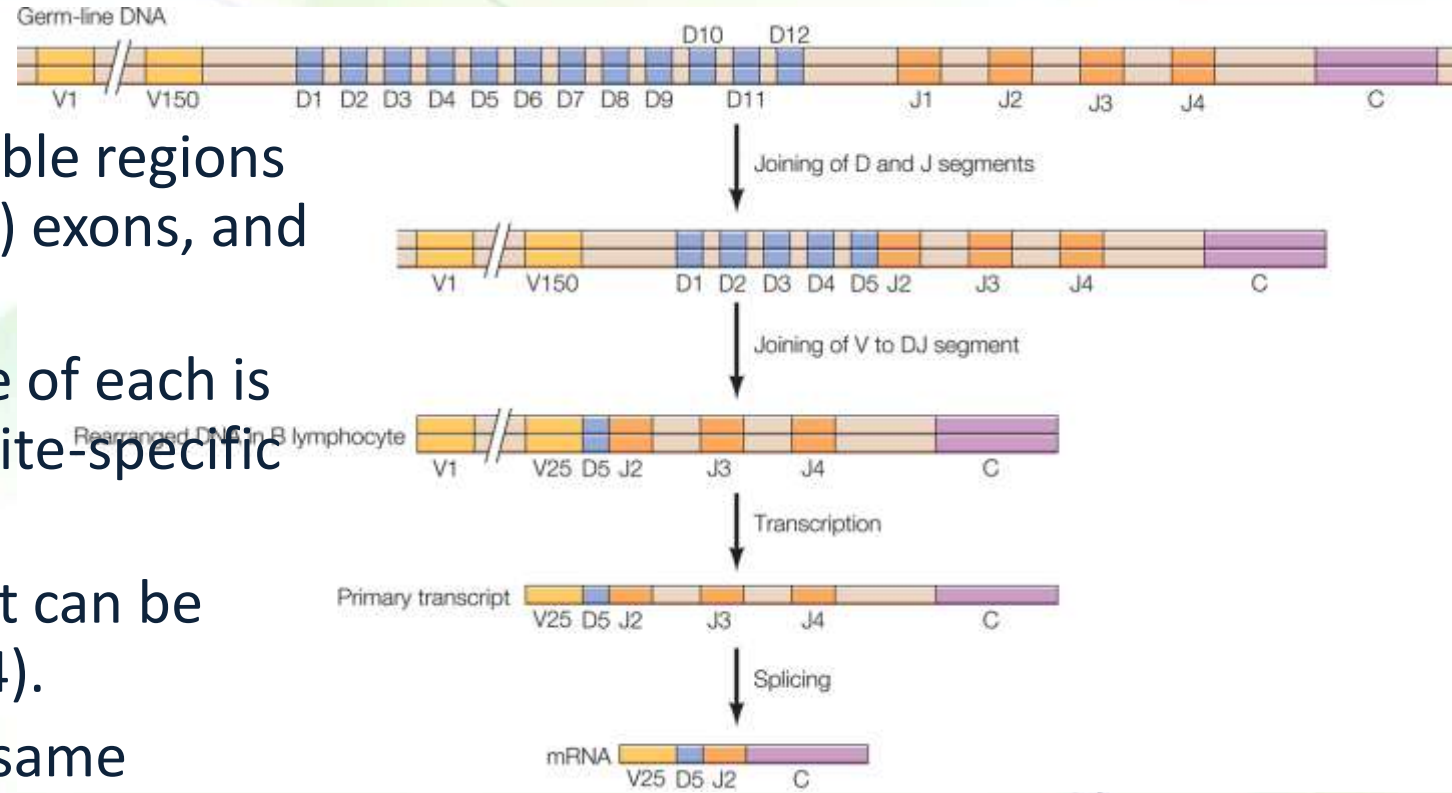
- The human body can possess a population of approximately  $10^{12}$  B lymphocytes that can produce and release immunoglobulins (antibodies), but each cell can produce one type of an immunoglobulin.
- Each antibody has a unique antigen-binding variable region that is encoded by unique genes formed by **site-specific recombination** during B-lymphocyte development.



# 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 ( $150 \times 12 \times 4$ ).
- 600 light chains are produced by the same mechanism resulting in a possible  $4 \times 10^6$  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.

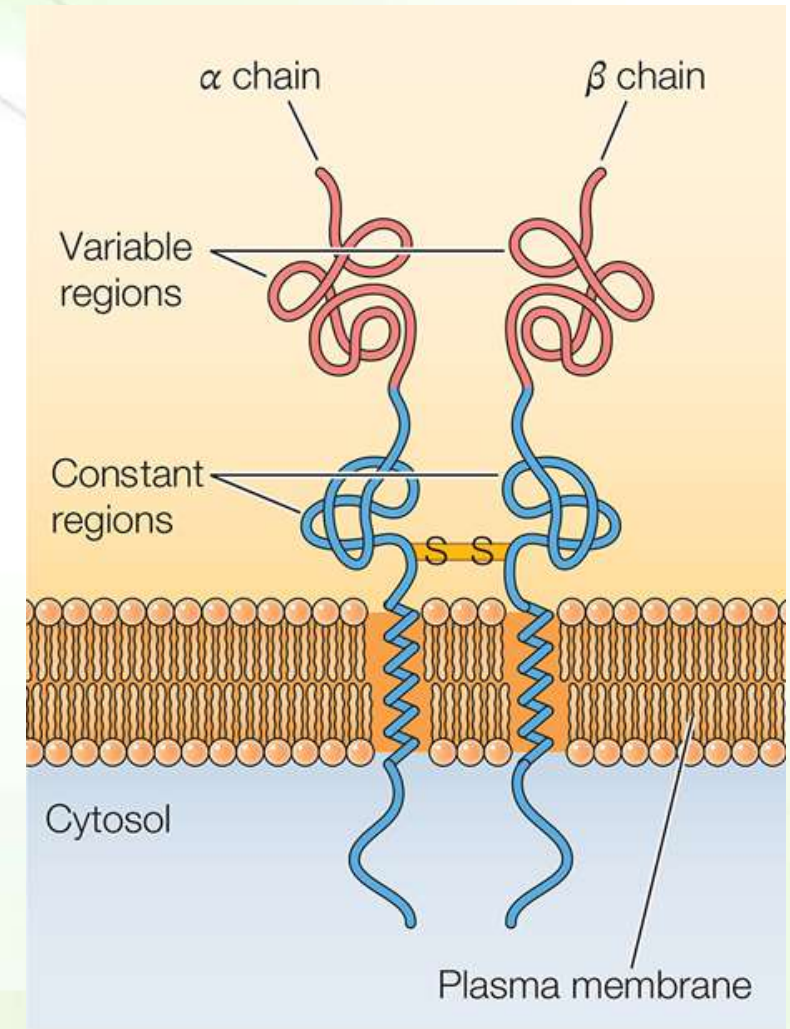


Somatic hypermutation is an additional mechanism where multiple mutations are introduced during DNA replication within the rearranged immunoglobulin variable regions.

# 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.





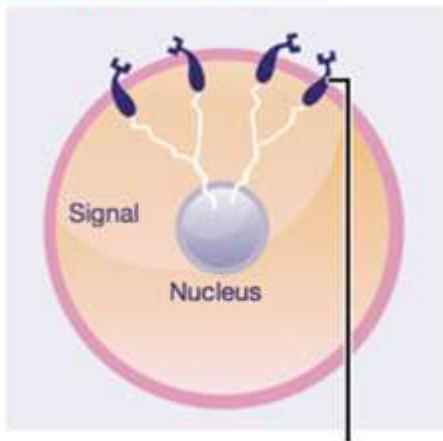
# Gene amplification

# Gene amplification



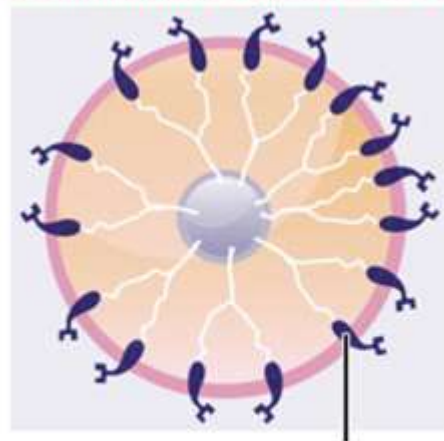
- It is an increase in copy number of a restricted chromosome region increasing the quantity of DNA in these regions and, hence, increasing RNA and protein production.
- Cancer cells use it to develop resistance from methotrexate whereby the target gene, dihydrofolate reductase, is amplified.
- Breast tumor cells become amplify the human epidermal growth factor receptor 2 (HER2) making them more aggressive in growth and progression.

Normal breast cell

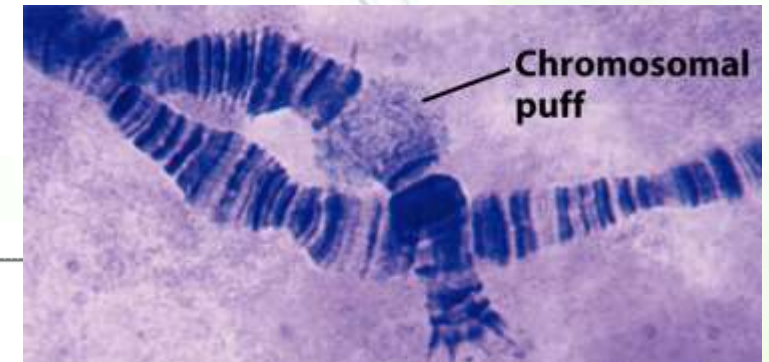
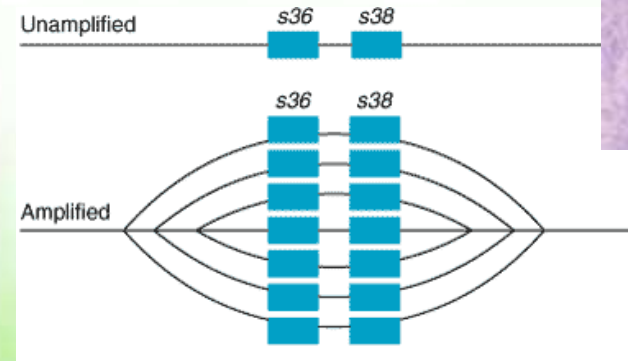


Normal amount of HER2 receptors send signals telling cells to grow and divide.<sup>1</sup>

Abnormal HER2+ breast cancer cell



Too many HER2 receptors send more signals, causing cells to grow too quickly.<sup>1</sup>

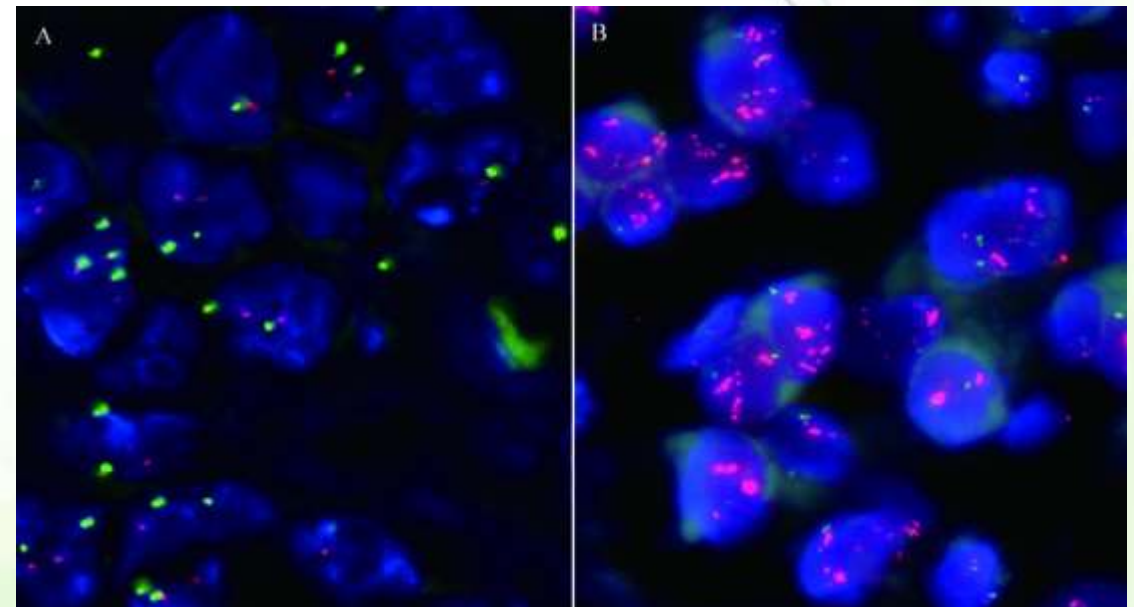
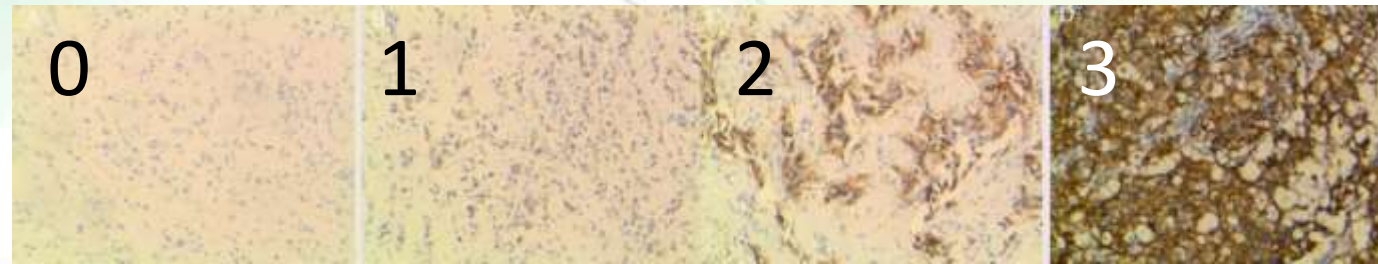
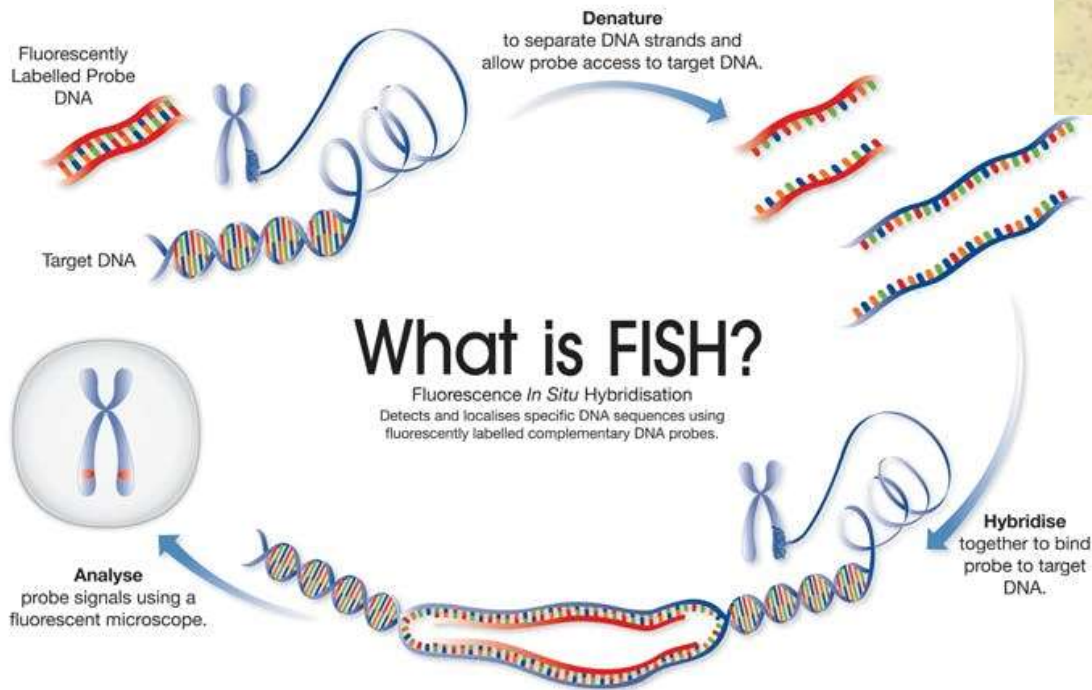




# How is it detected?



- If immunohistochemistry shows unequivocal staining, then FISH is done.

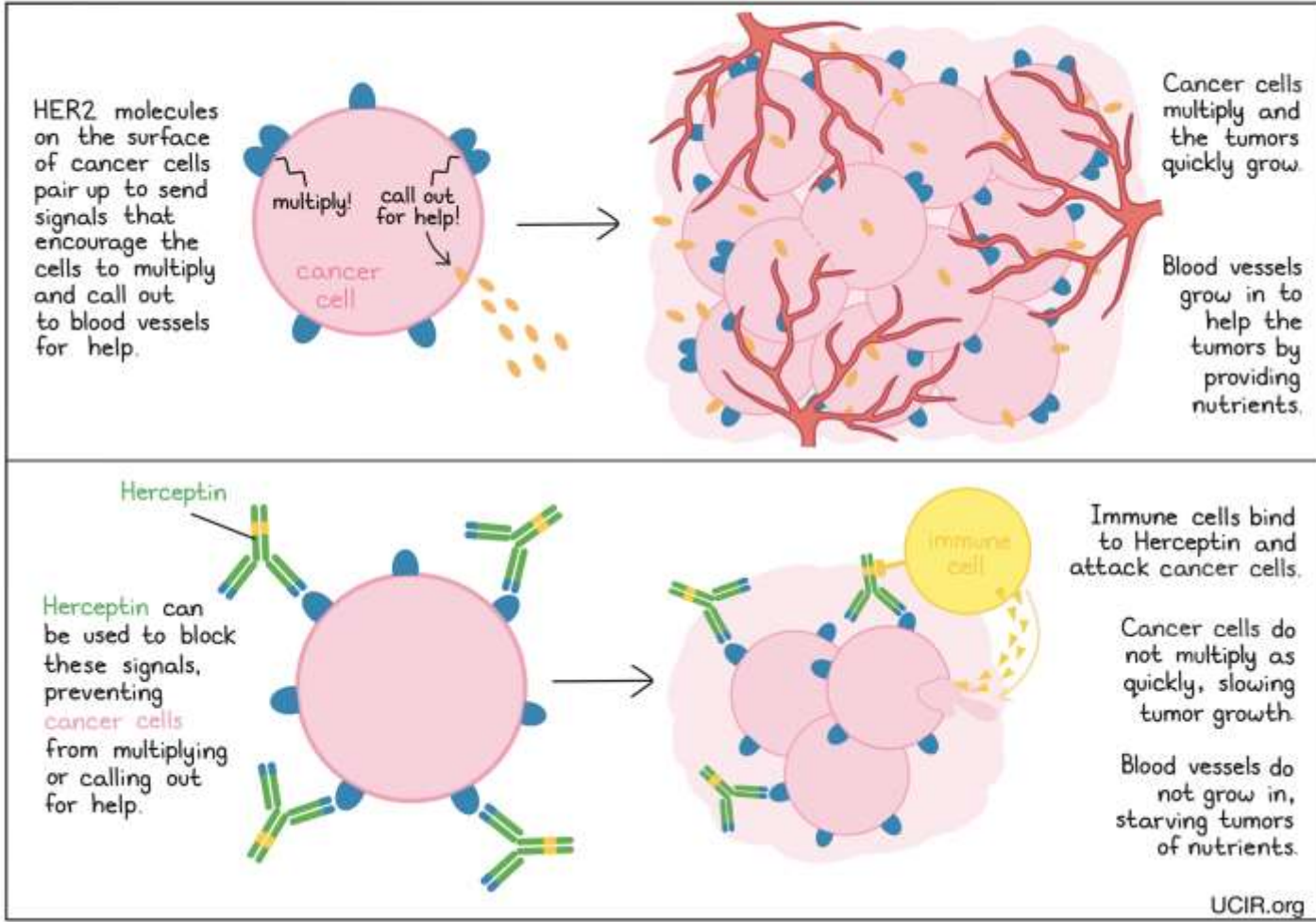


# How are HER2-enriched cancers treated?



## *Herceptin (trastuzumab)*

Herceptin: how it works



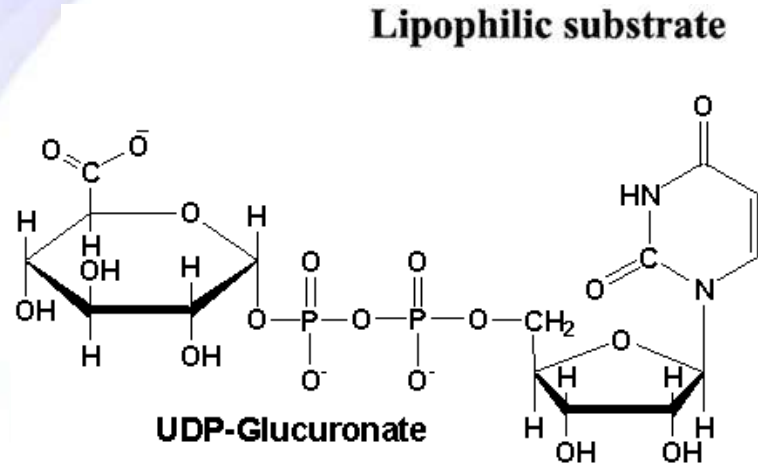


# Multiple promoters, multiple exon 1s

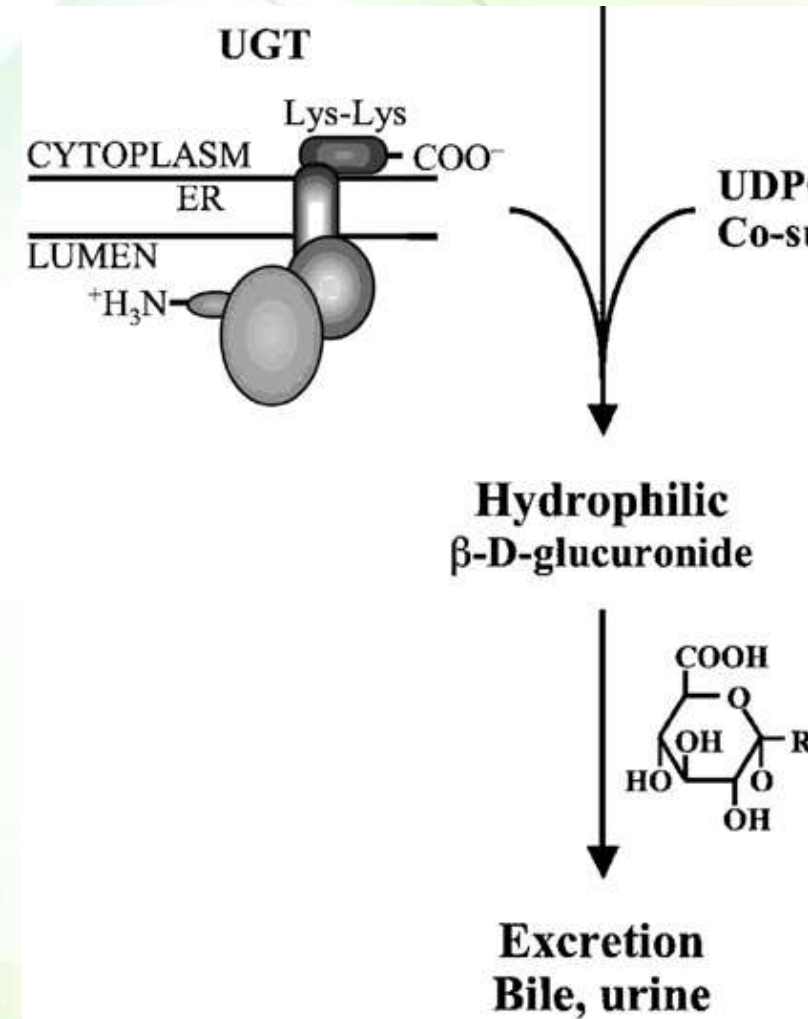
# An example of alternative splicing:



## UDP-glucuronosyltransferase (UGT)



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.



# The enzyme(s) has many heterogenous substrates



## Lipophilic substrate

Therapeutic drugs

Carcinogens

Environmental toxicants

Dietary constituents

Bilirubin

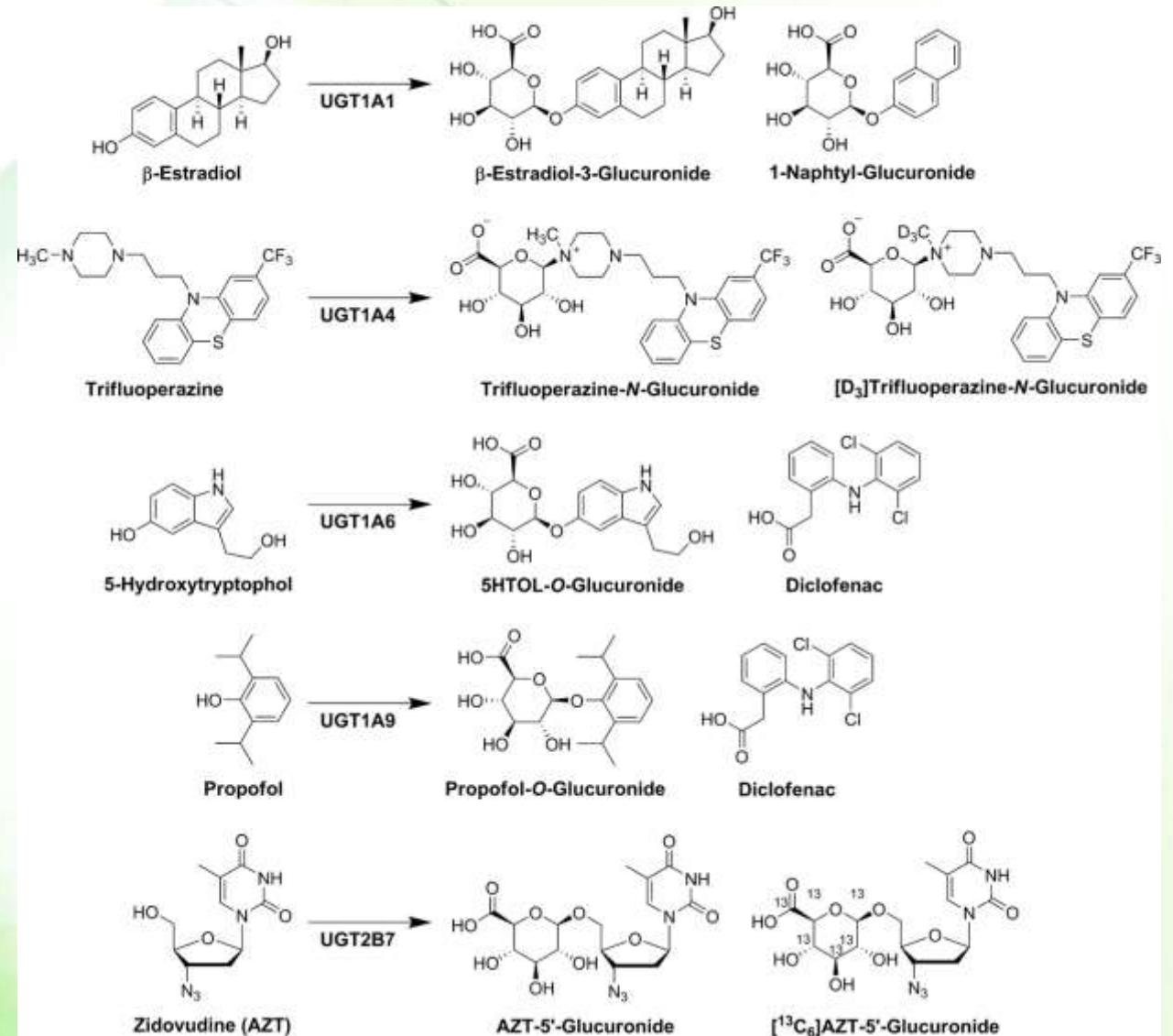
Biliary acids

Steroids

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.



# and different reactions are catalyzed in different tissues



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

# Get this concept, first...



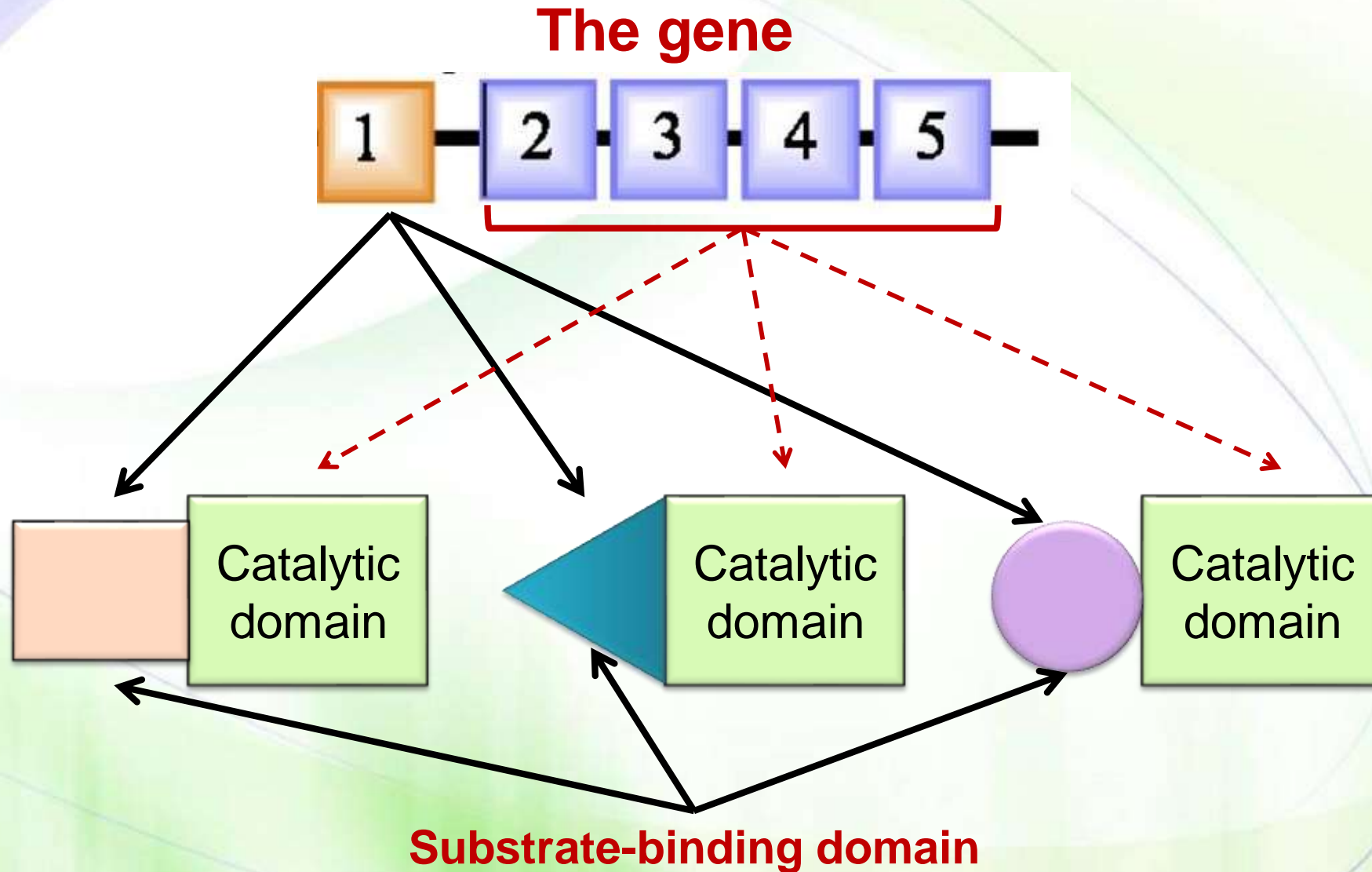
## One drill, many flutes



## One head, many hats



# Then this...

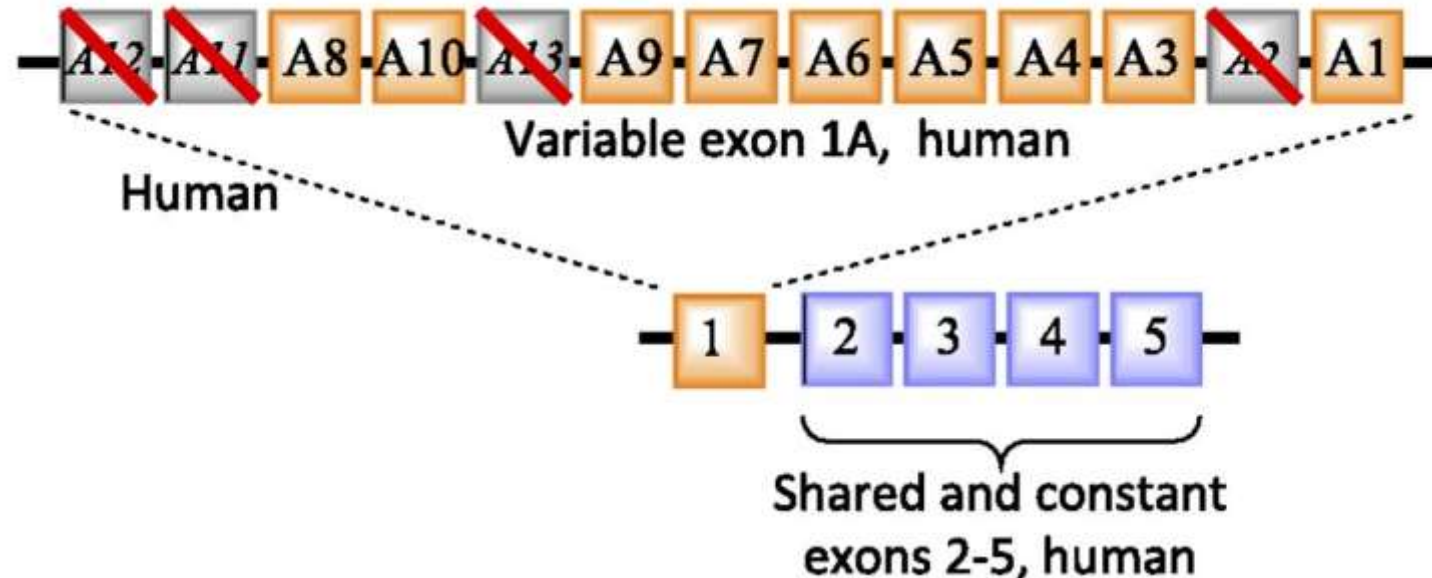




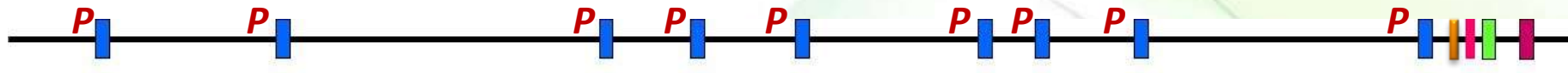
# How does UGT1A do this?



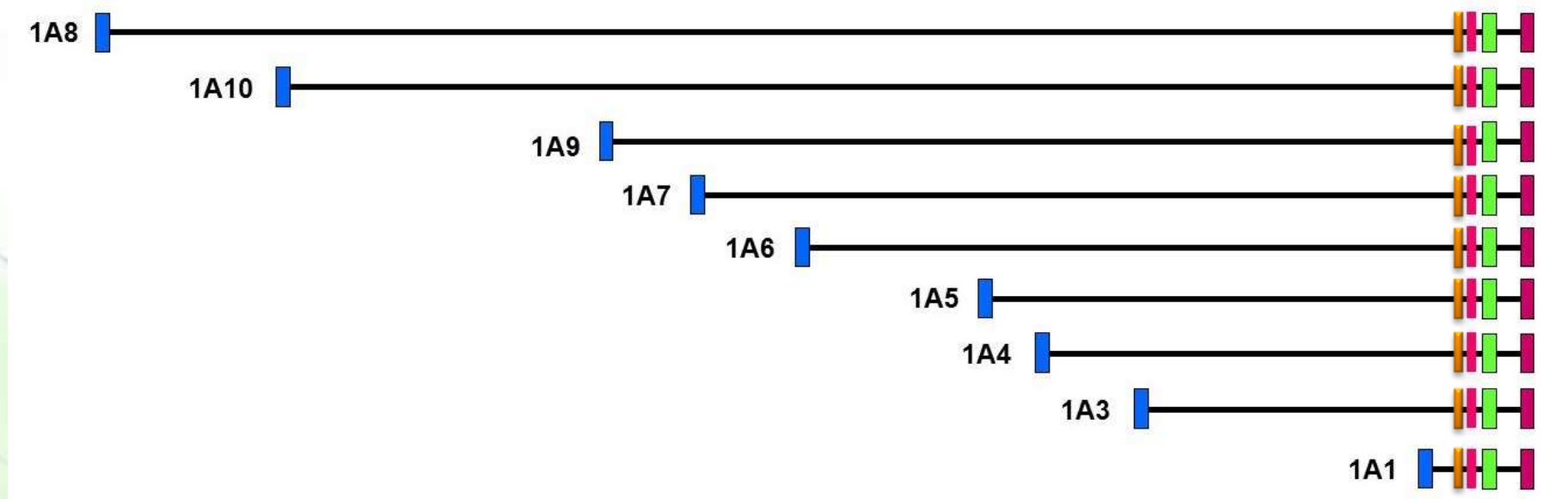
- Exons 2, 3, 4, and 5 encode the catalytic domain that interacts with UDP-glucuronic acid, and exon 1 determines substrate specificity, **but**...
- Exon 1 contains **NINE** tandemly arrayed first exons and each one has its own promoter.
- The 9 exons determine substrate specificity and one of them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



# Splice variants for UGT1A



## The possible transcripts



# Explaining the substrate specificity and tissue distribution

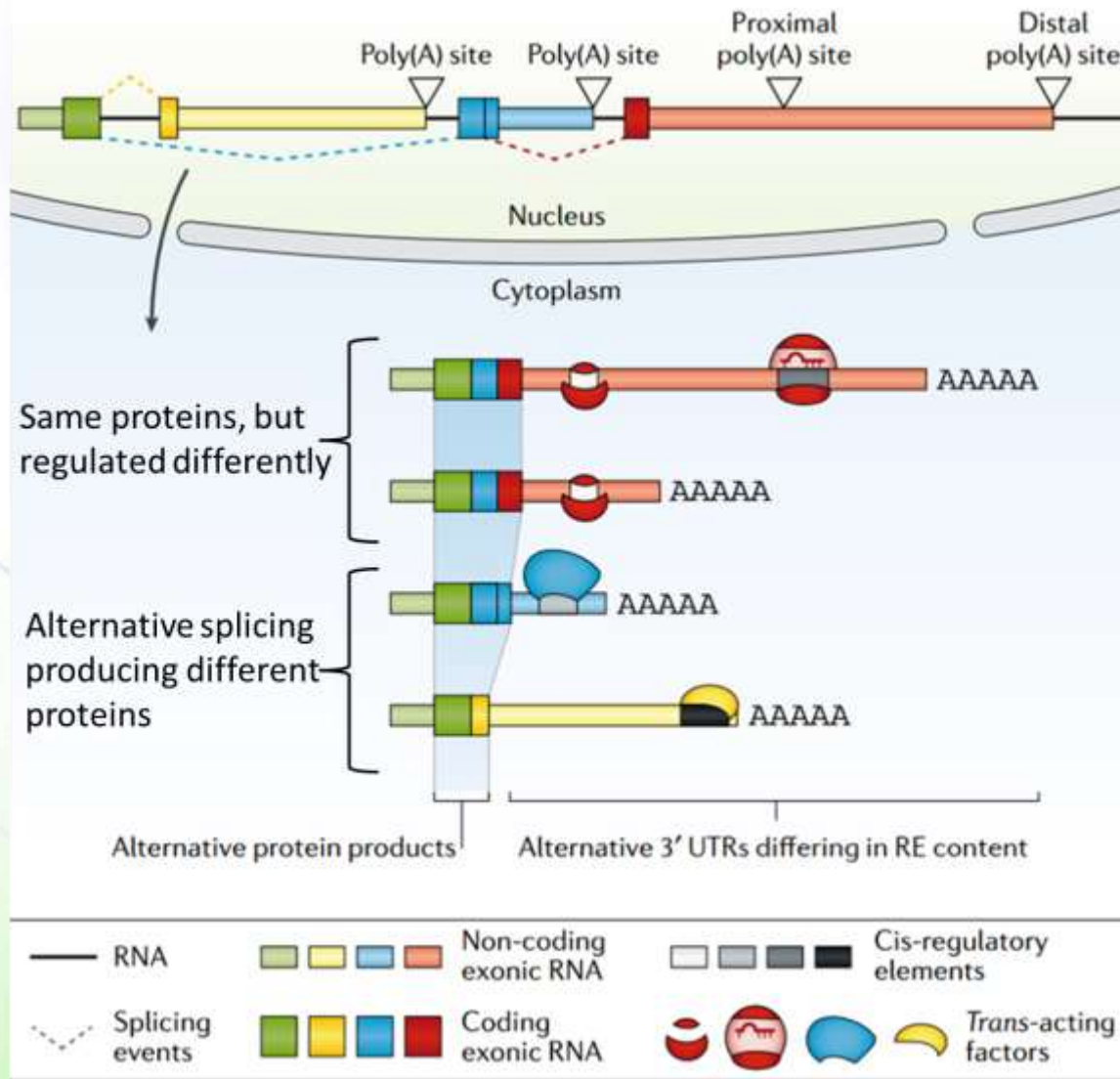


<b>Gene</b>	<b>Where expressed</b>	<b>Substrates</b>
<b>UGT1A1</b>	<b>Biliary tissue, colon, intestine, liver, stomach</b>	<b>Etoposide</b>
<b>UTG1A3</b>	<b>Biliary tissue, colon, liver, stomach</b>	<b>Genistein</b>
<b>UGT1A4</b>	<b>Biliary tissue, colon, intestine, liver</b>	<b>Tamoxifen</b>
<b>UGT1A6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



# Alternative splicing and alternative polyadenylation

# The advantage of polyadenylation



- 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.