Transcriptional phenomena in humans

Prof. Mamoun Ahram
School of Medicine
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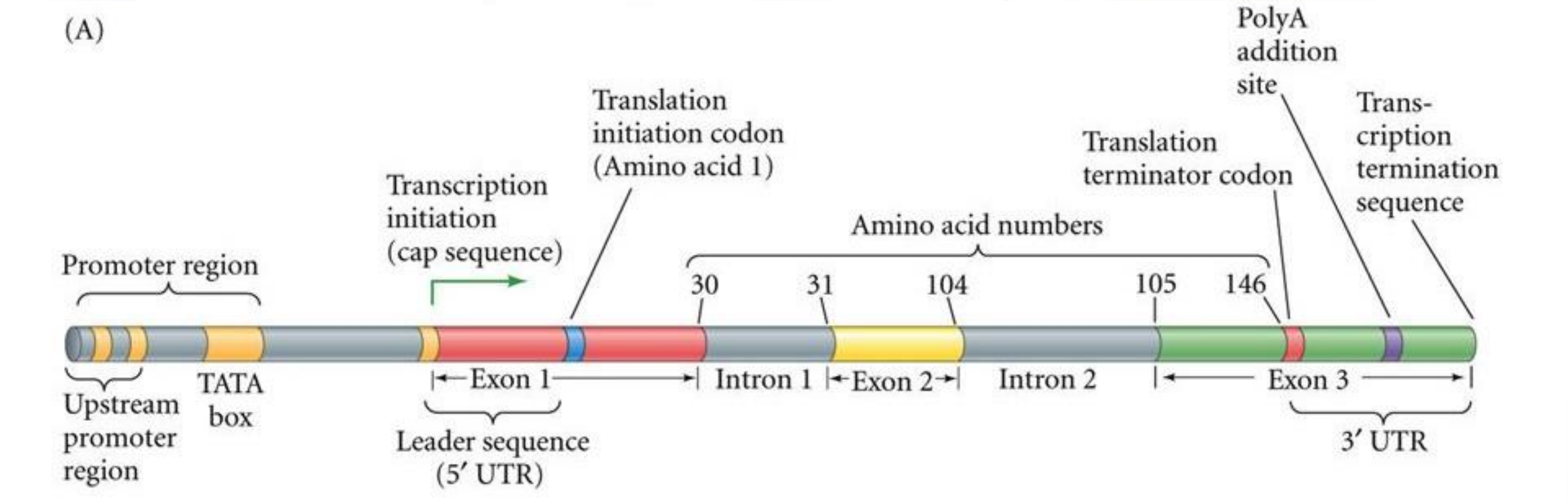
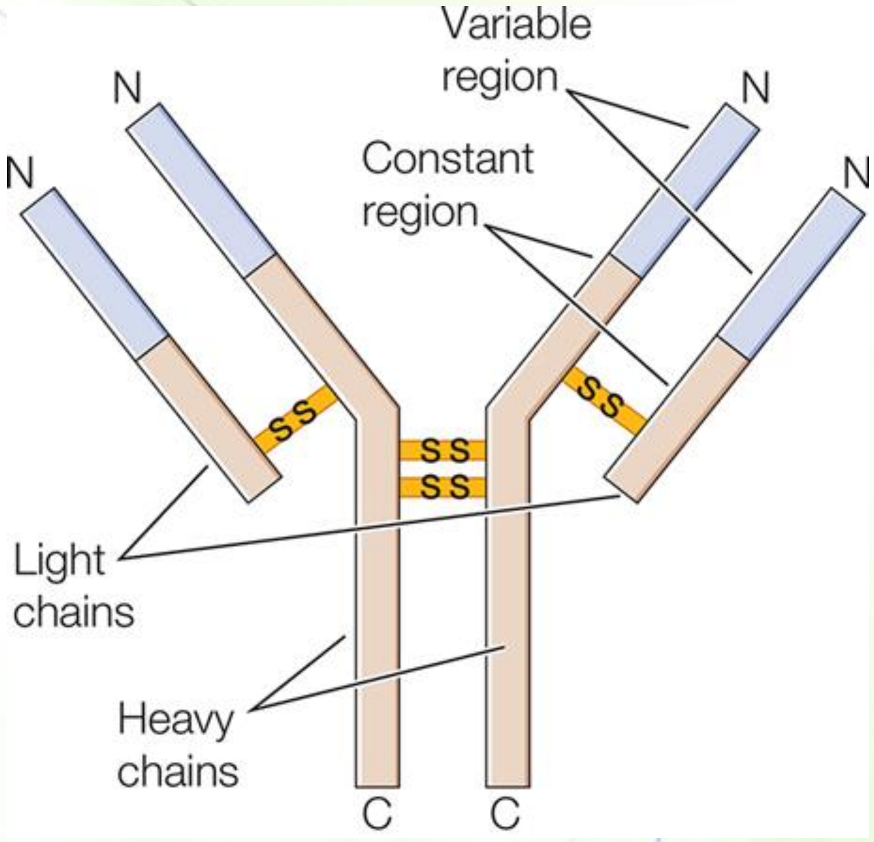
Anatomy of a eukaryotic gene



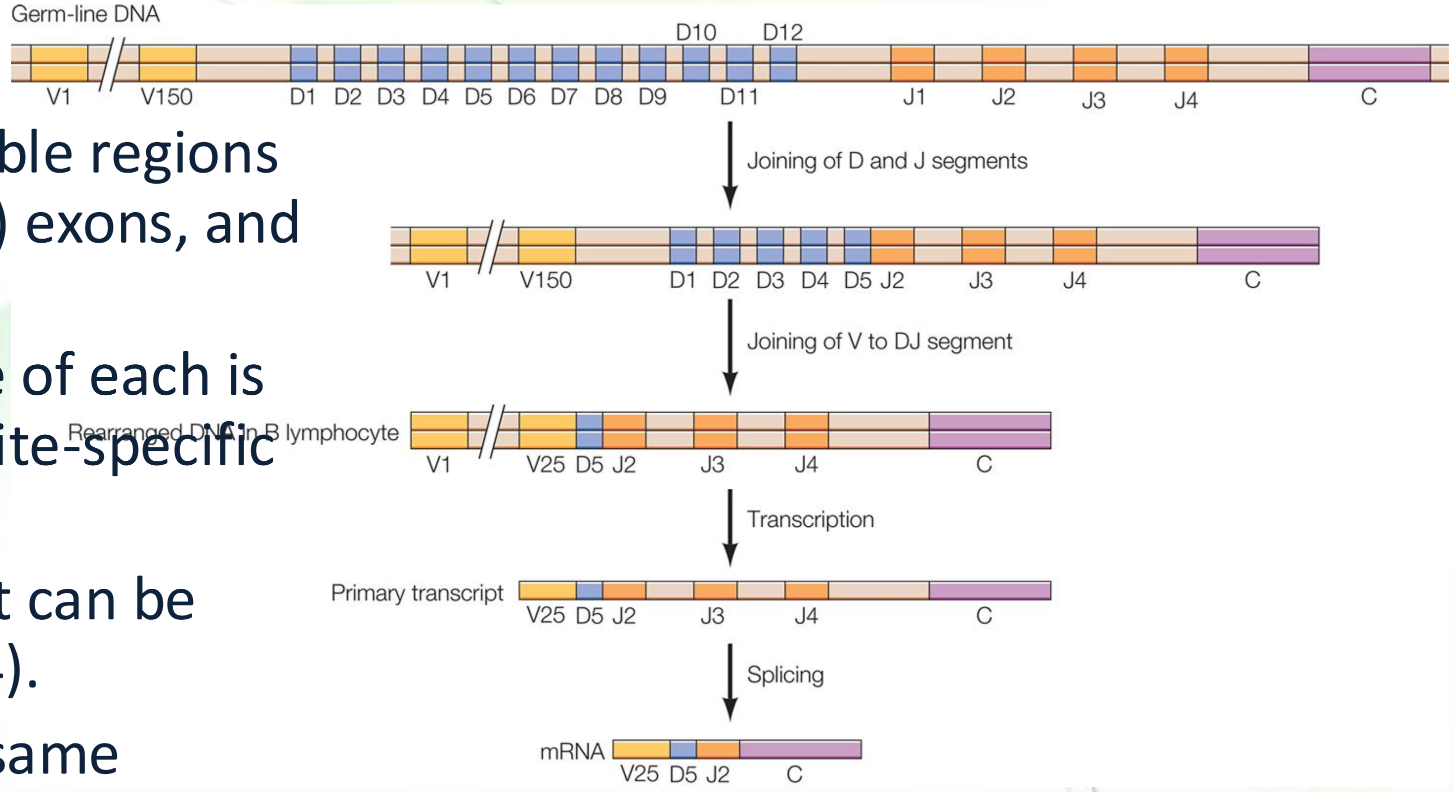
Gene rearrangment

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

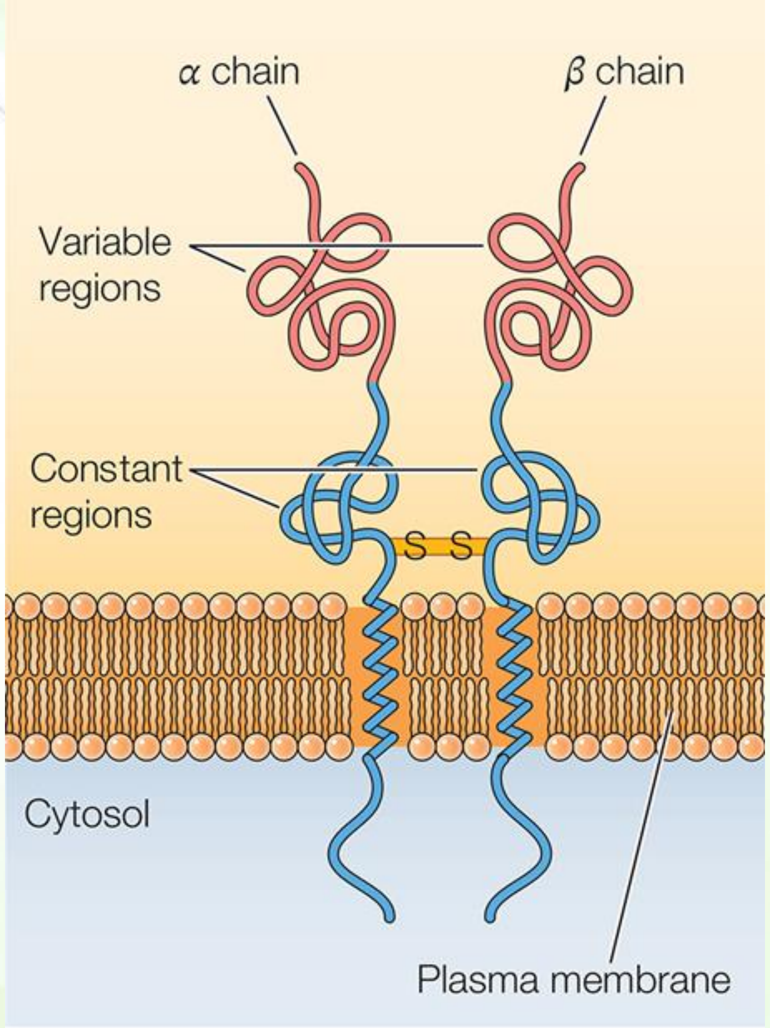


- Each heavy gene consists of 150 variable regions (V), 12 diversity exons (D), 4 joining (J) exons, and one constant exon (C).
- During lymphocyte development, one of each is combined with one of the others by site-specific recombination.
- The total number of heavy chains that can be generated is about 7200 ($150 \times 12 \times 4$).
- 600 light chains are produced by the same mechanism resulting in a possible 4×10^6 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.

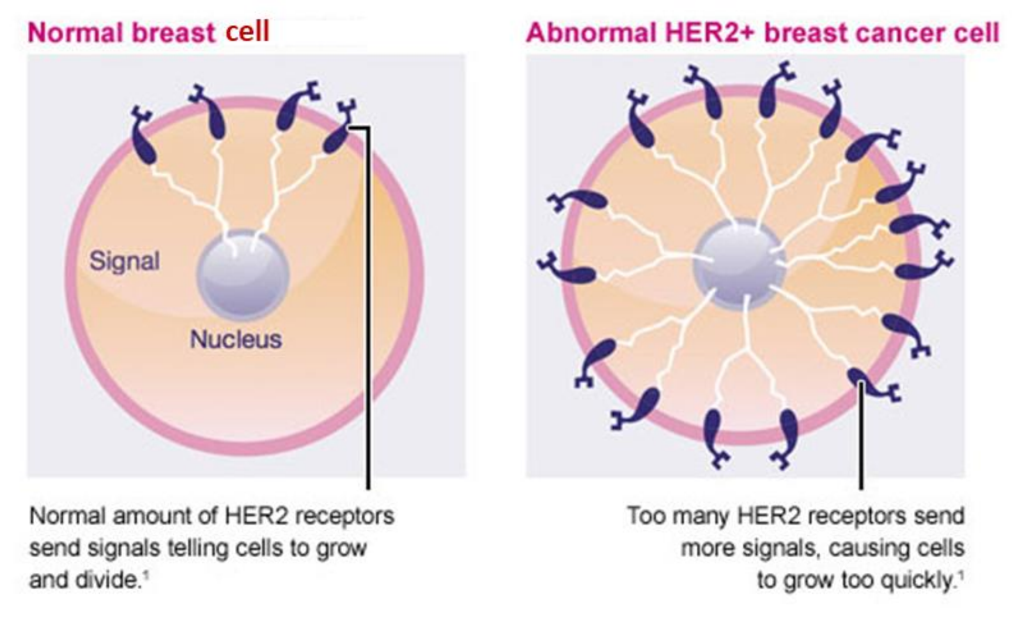


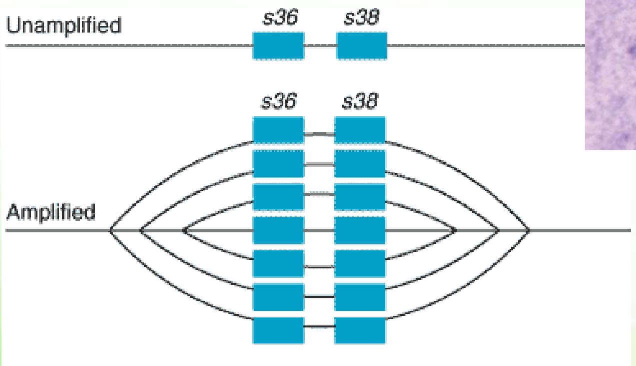
https://www.cancer.gov/about-cancer/treatment/research/car-t-cells

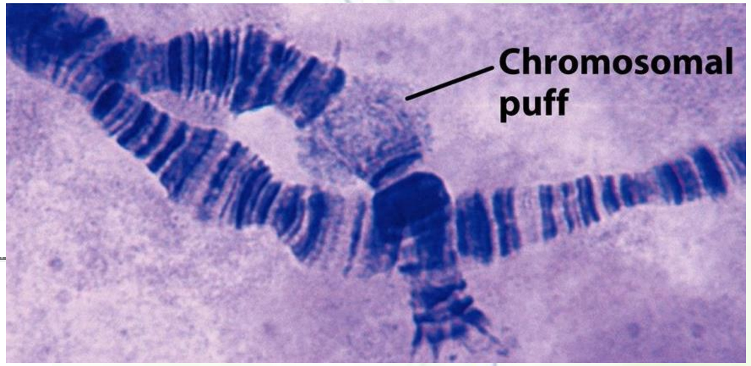
Gene amplification

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.

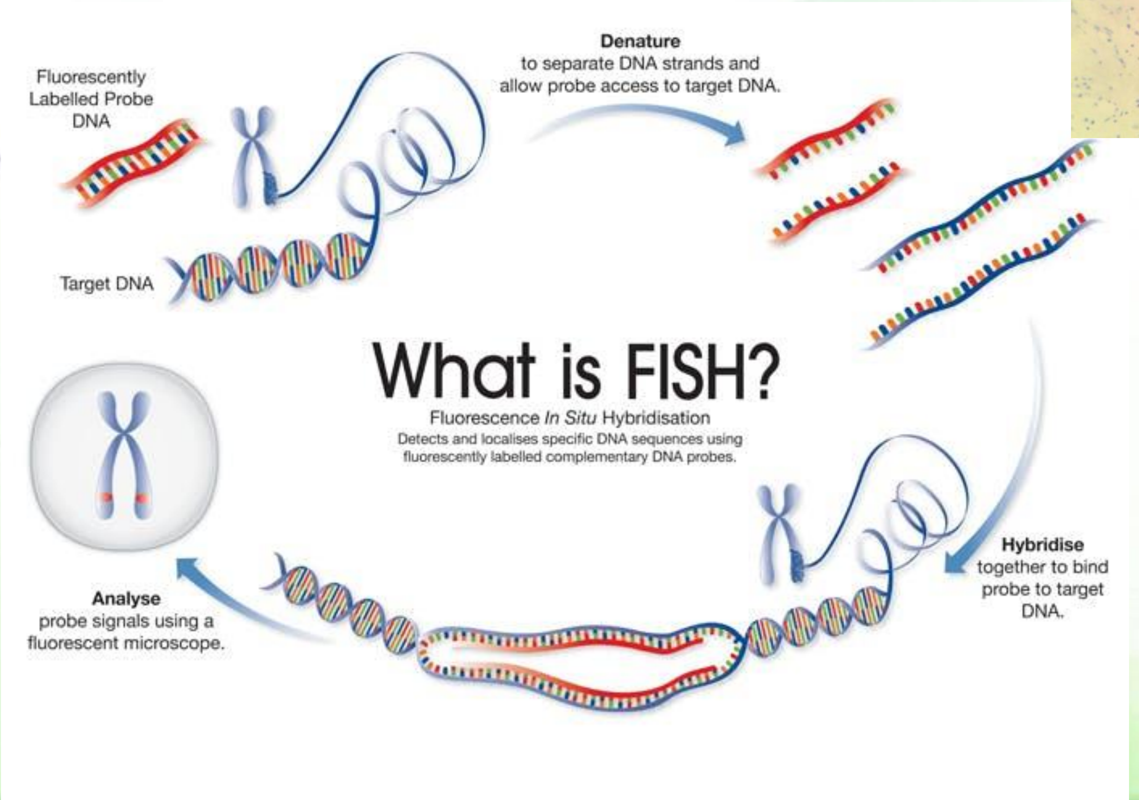


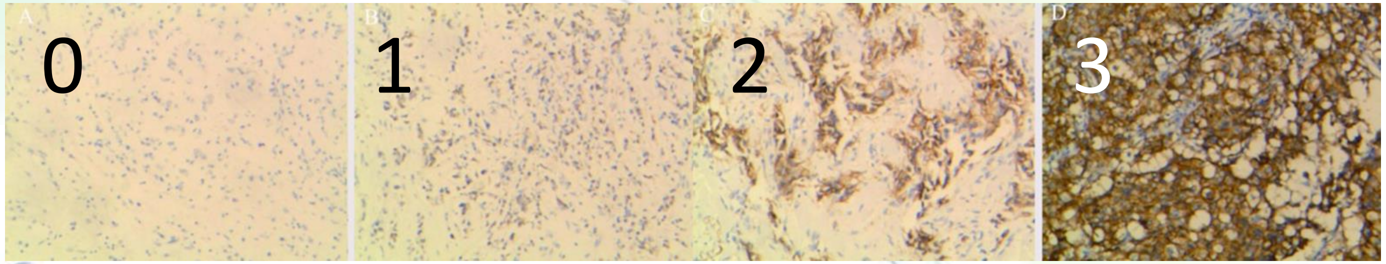


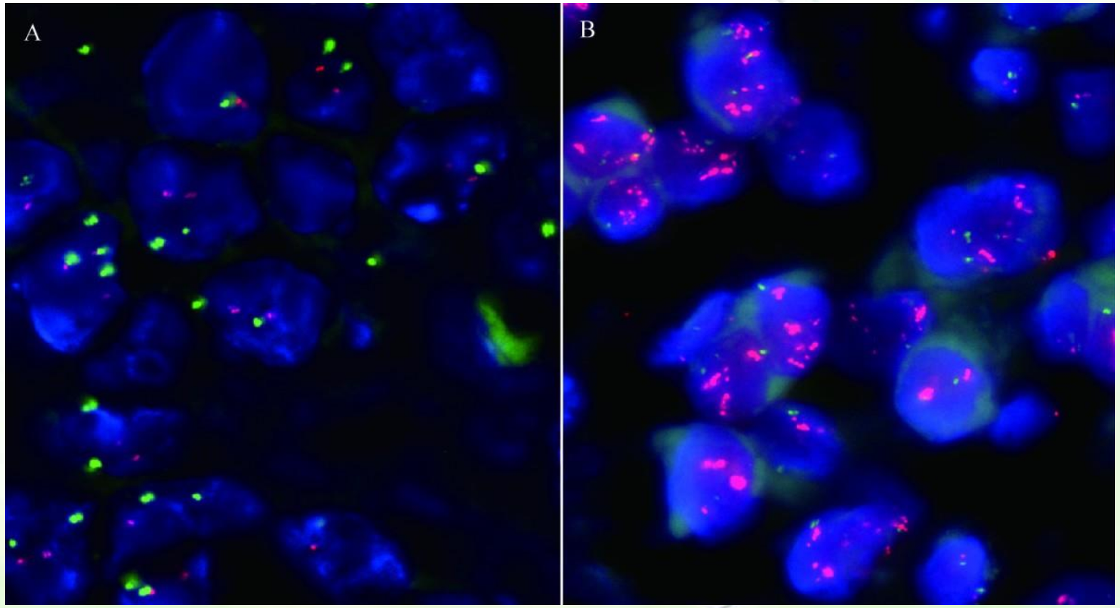


How is it detected?

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

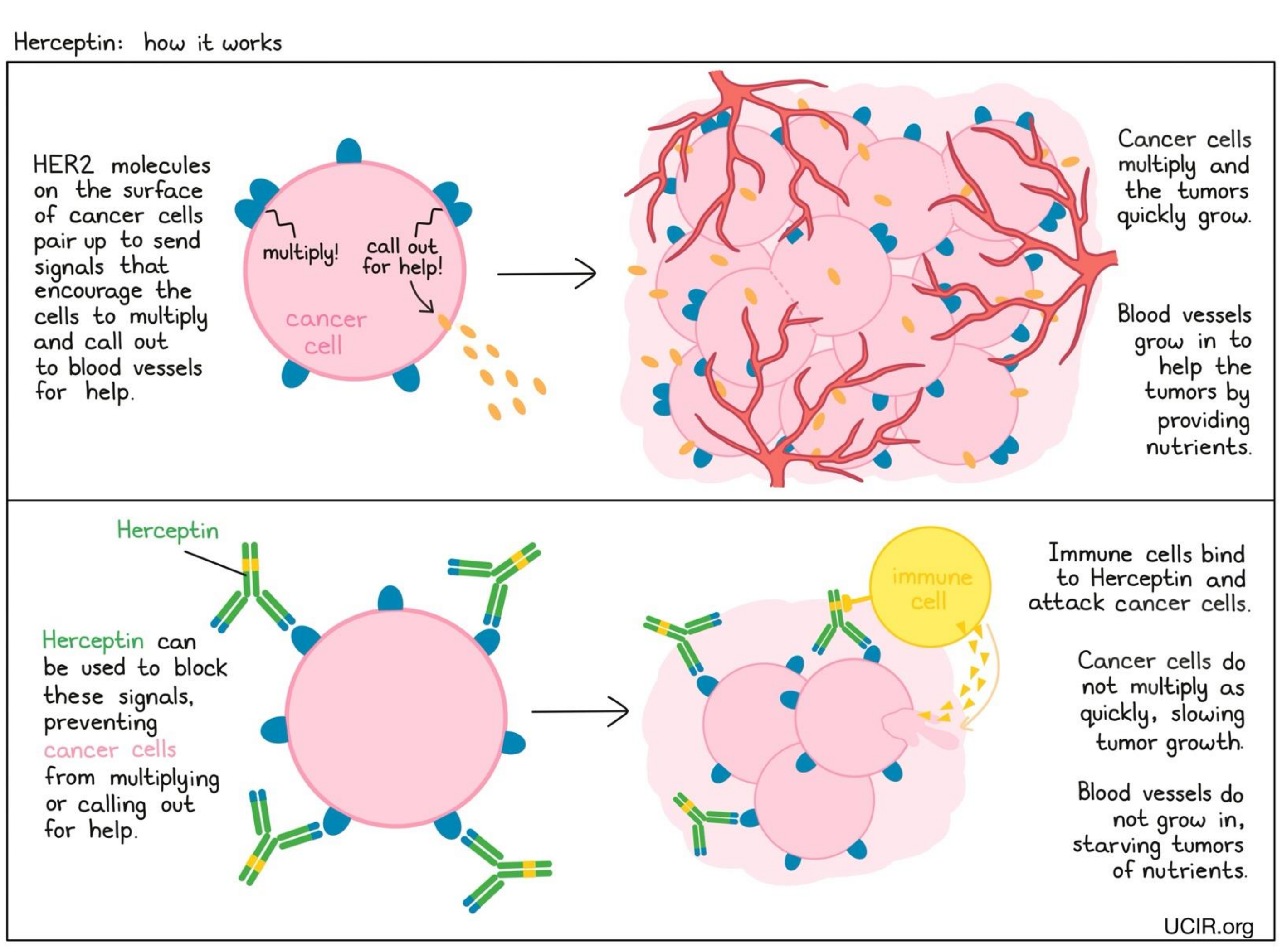






How are HER2-enriched cancers treated?

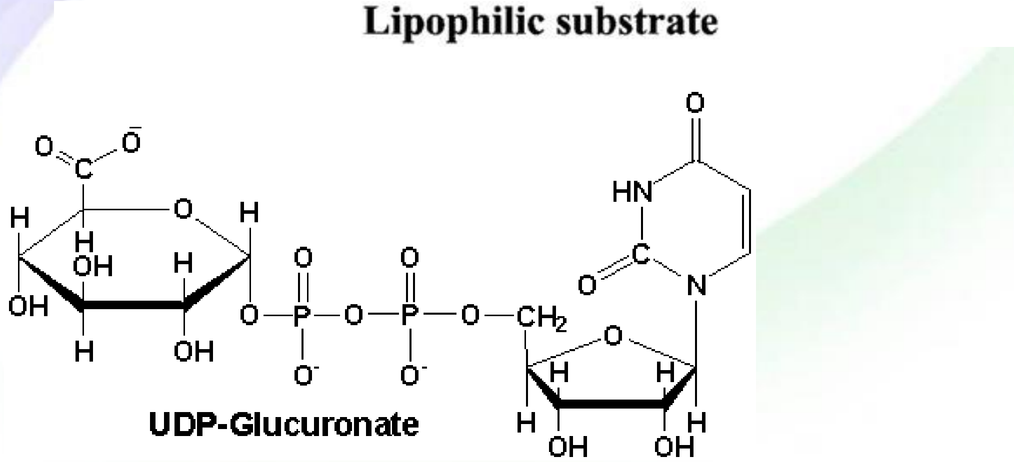
Herceptin (trastuzumab)

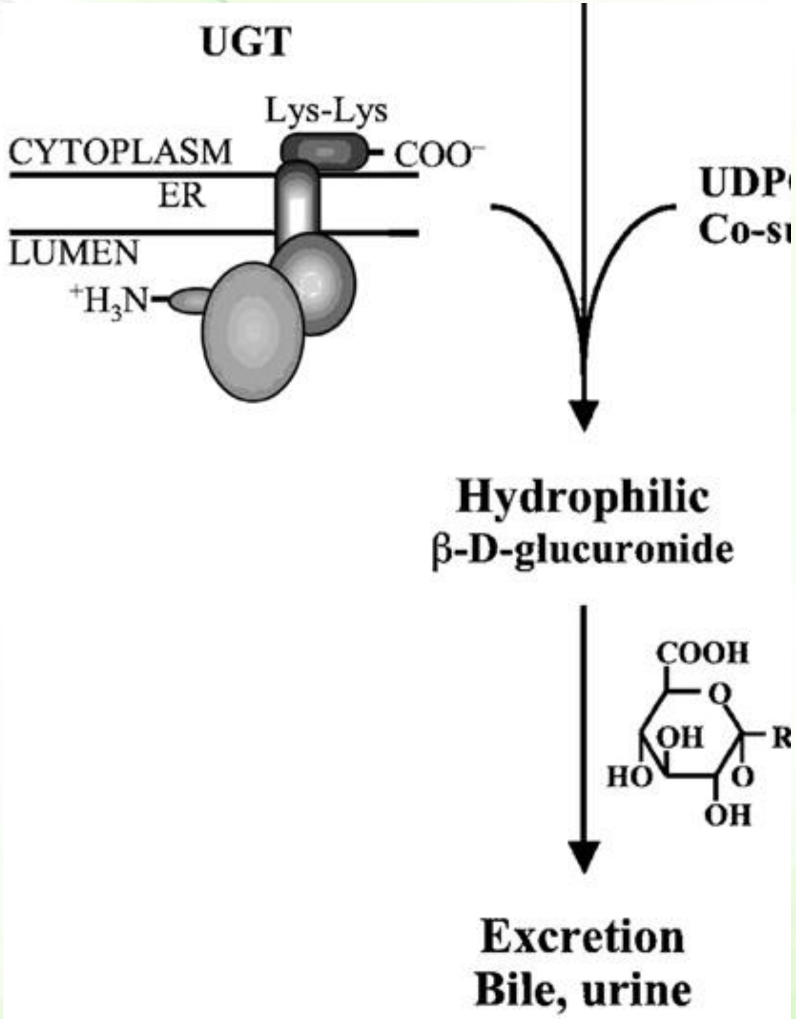


Multiple promotors, multiple exon 1s

An example of alternative splicing:

UDP-glucuronosyltransferase (UGT)





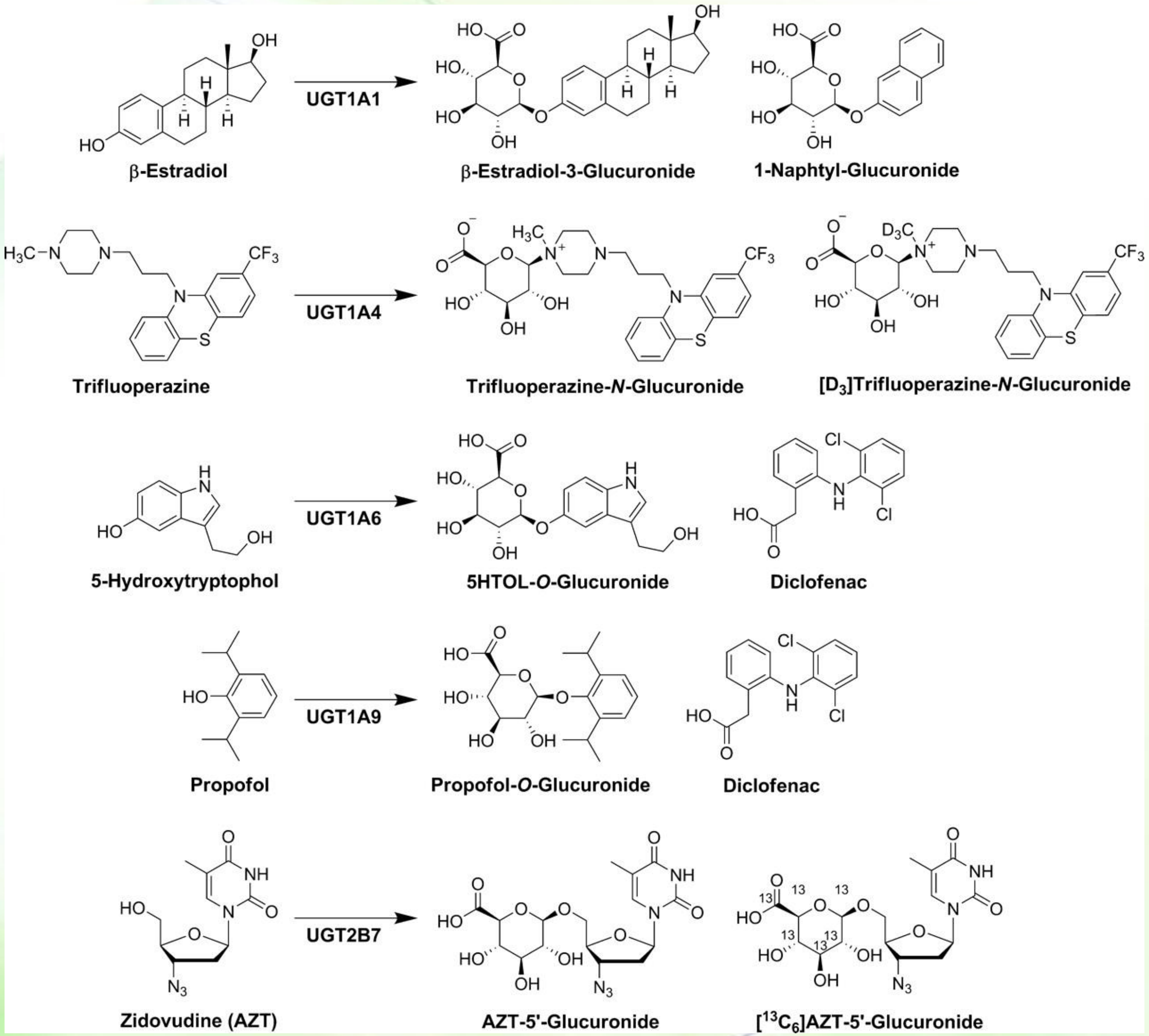
The uridine diphosphate glucuronosyltransferase (UGT) enzymes transfer glucuronic acid onto xenobiotics and other endogenous compounds making them water soluble and allowing for their biliary or renal elimination.

The enzyme(s) has many heterogenous substrates

Lipophilic substrate

Therapeutic drugs	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.



and different reactions are catalyzed in different tissues

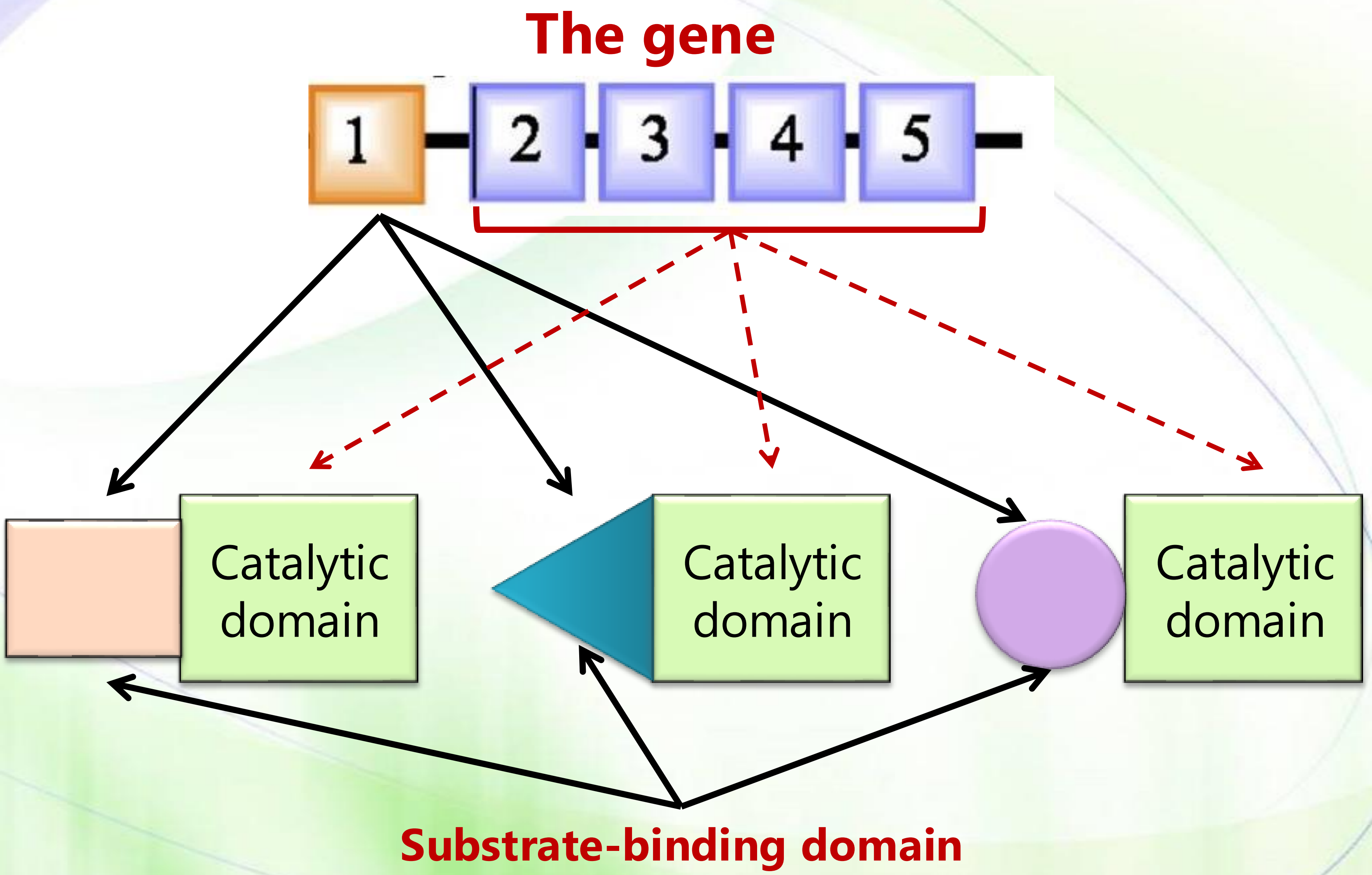
Substrates	Place of reaction
Etoposide	Biliary tissue, colon, intestine, liver, stomach
Genistein	Biliary tissue, colon, liver, stomach
Tamoxifen	Biliary tissue, colon, intestine, liver
PCBs	Biliary tissue, brain, colon, kidney, larynx, liver, lung, stomach
Heterocyclic amines	Esophagus, intestine, kidney, 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

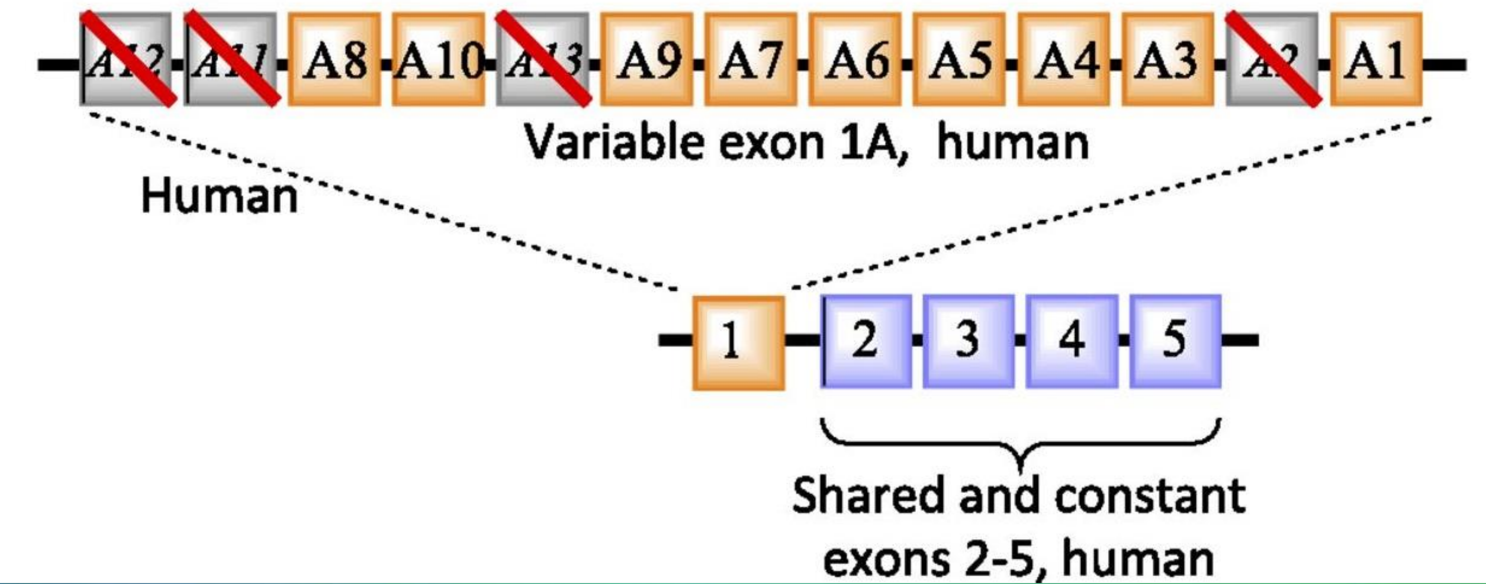
One head, many hats

Then this...

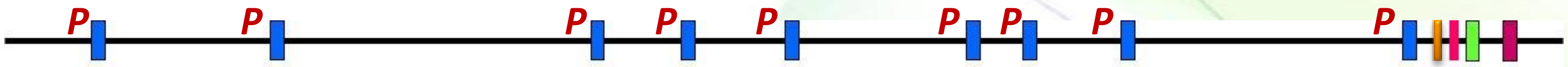

The gene
1
2
3
4
5
Catalytic domain
Catalytic domain
Catalytic domain
Substrate-binding domain

How does UGT1A do this?

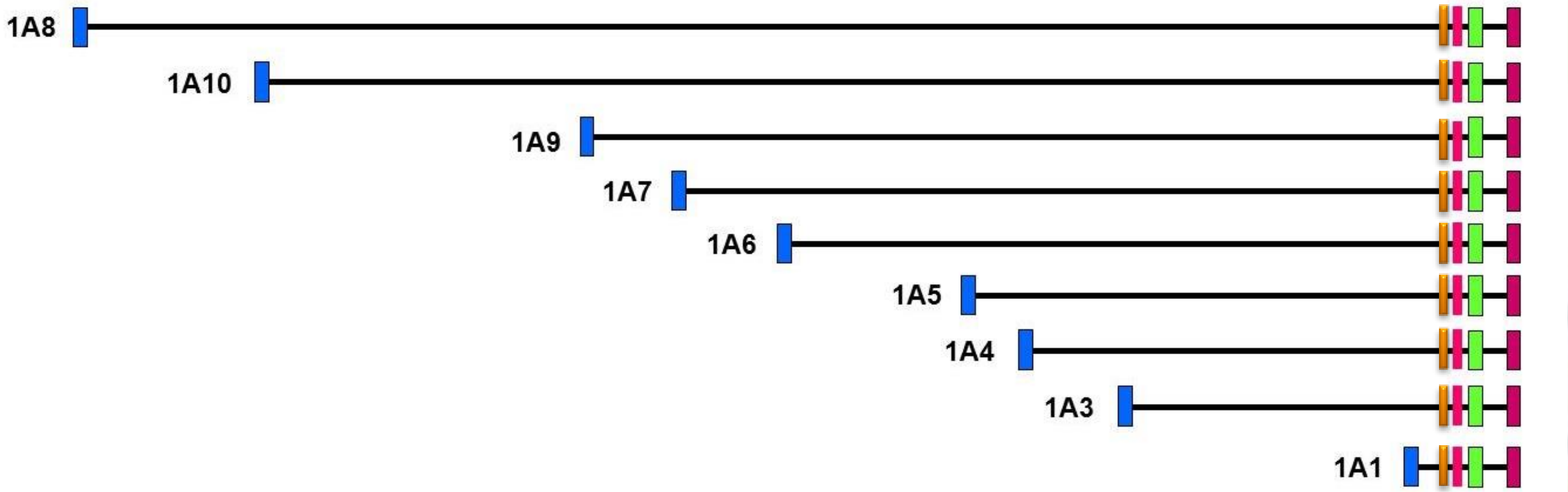
- Exons 2, 3, 4, and 5 encode the catalytic domain that interacts with UDP-glucuronic acid, and exon 1 determines substrate specificity, but…
- Exon 1 contains NINE tandemly arrayed first exons and each one has its own promoter.
- The 9 exons determine substrate specificity and one of them is spliced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



Splice variants for UGT1A



The possible transcripts

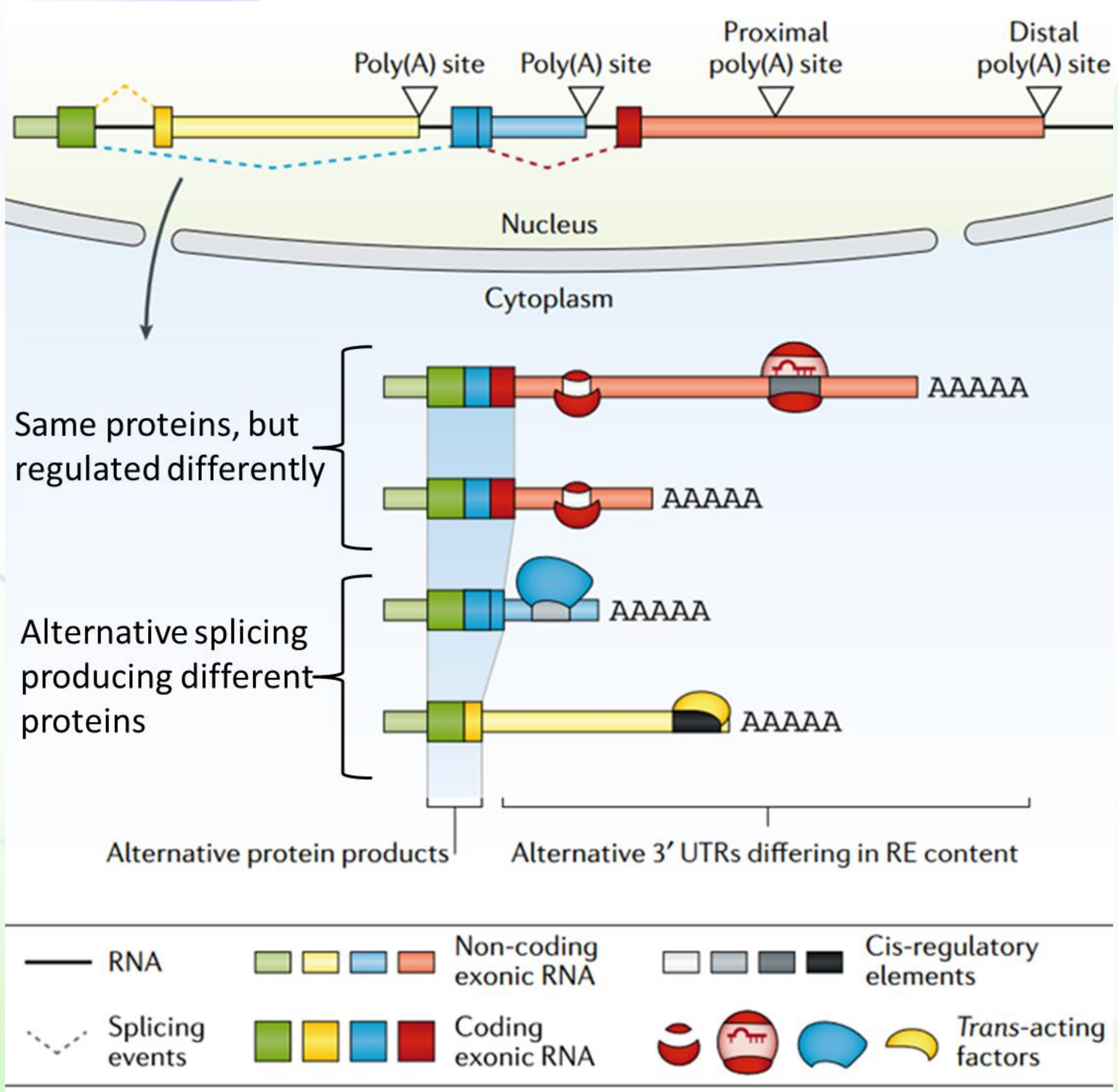


Explaining the substrate specificity and tissue distribution

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



- 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.

Regulation of mRNA stability

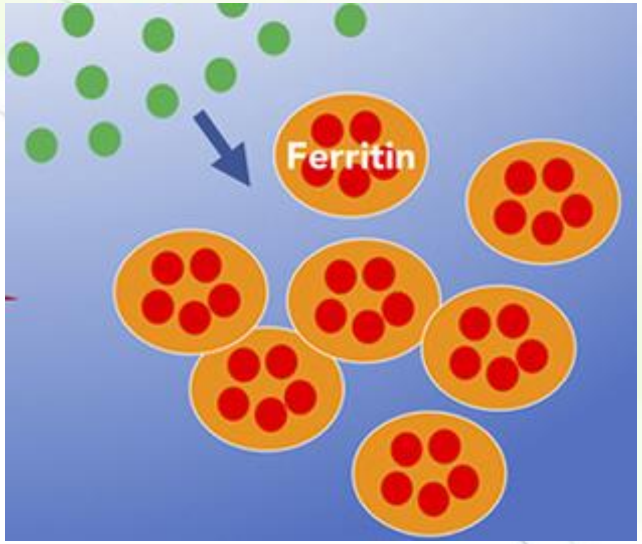
Physiology of iron

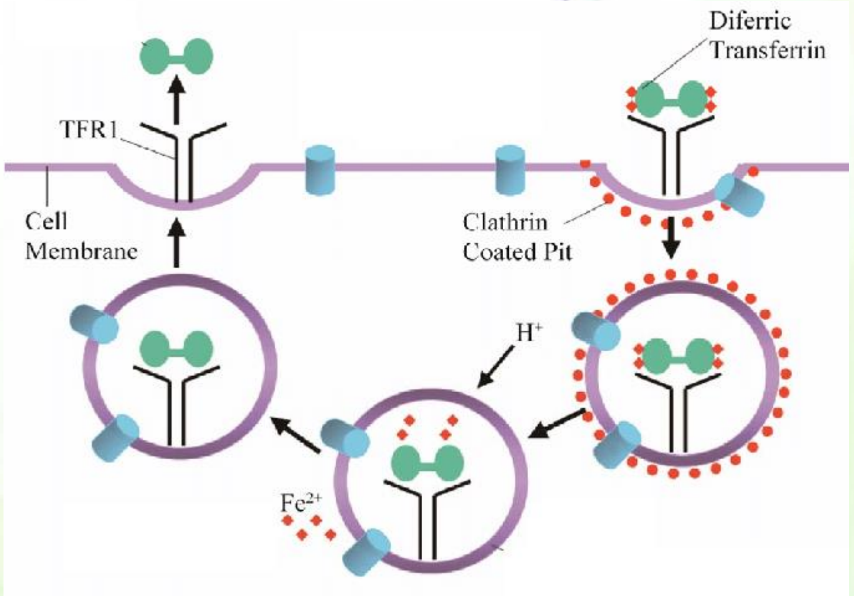
- Iron is an essential metal for the human body.
  - Oxygen transport
  - Enzyme function
- But, too much iron can be toxic.
  - Organ failure
  - Bacterial infection
- The level of iron is intricately maintained.

The players

- Liver ferritin protein stores iron when abundant (in the liver).
- Transferrin receptor mediates iron entry via receptor-mediated endocytosis into peripheral cells when needed.

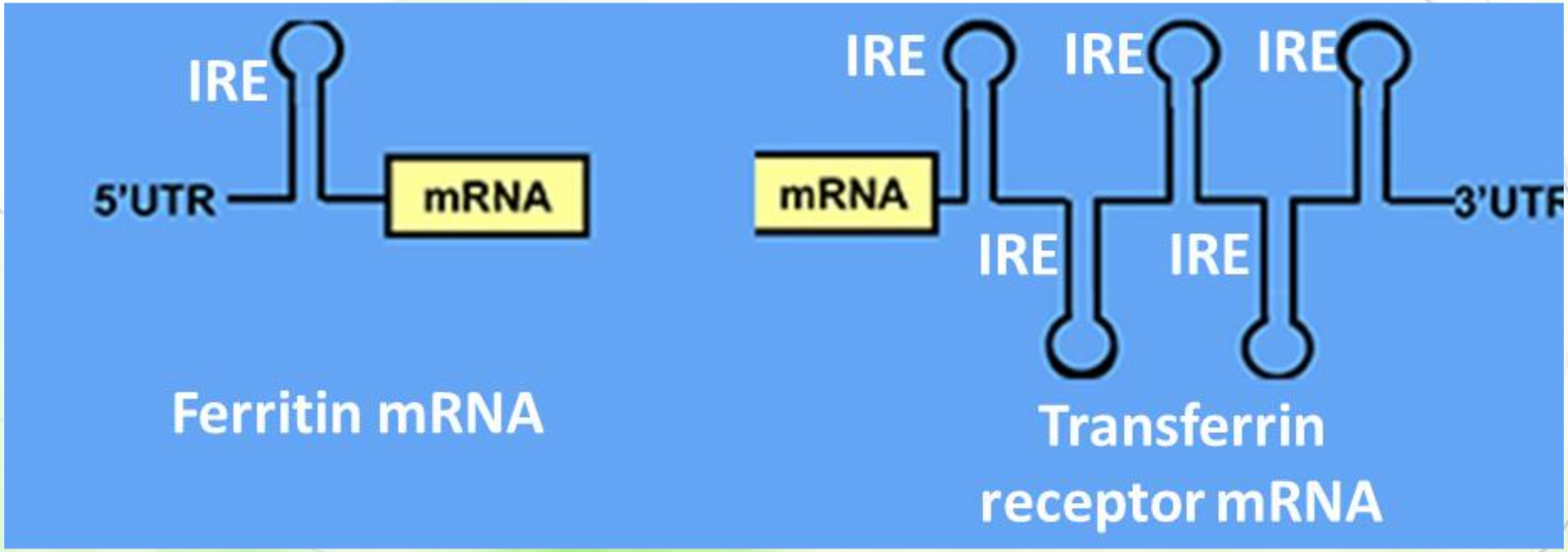
- When iron is high, expression of ferritin should be up-regulated and expression of transferrin receptor should be down-regulated, and vice versa.





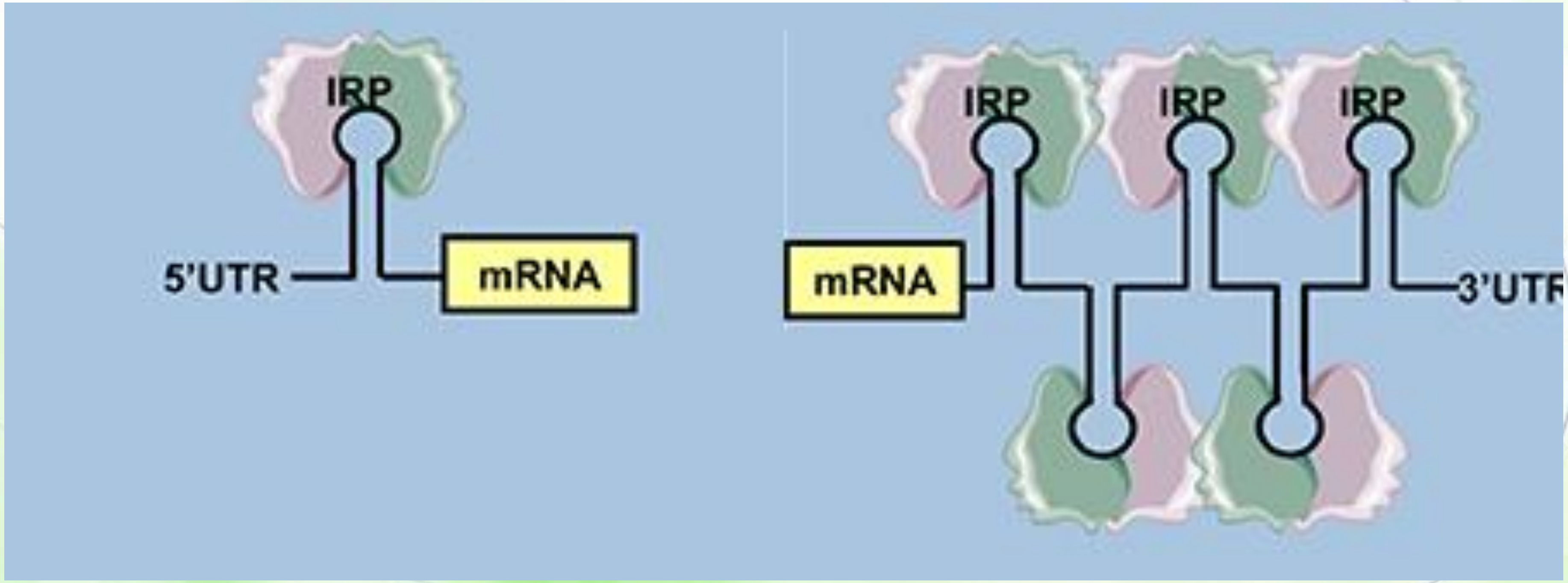
Iron-response elements

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



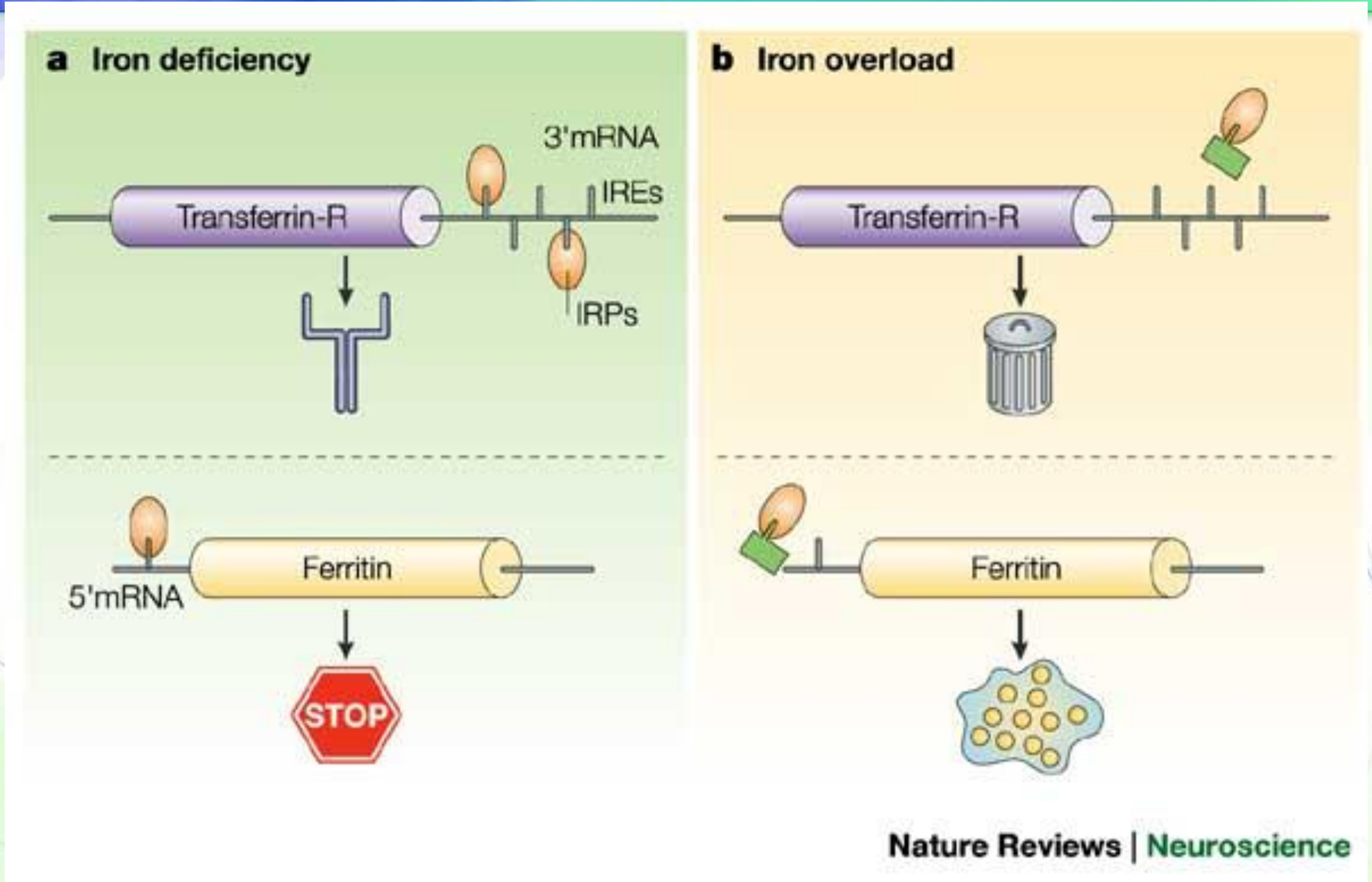
Iron regulatory protein

- When iron is low, the iron regulatory protein (IRP) 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.

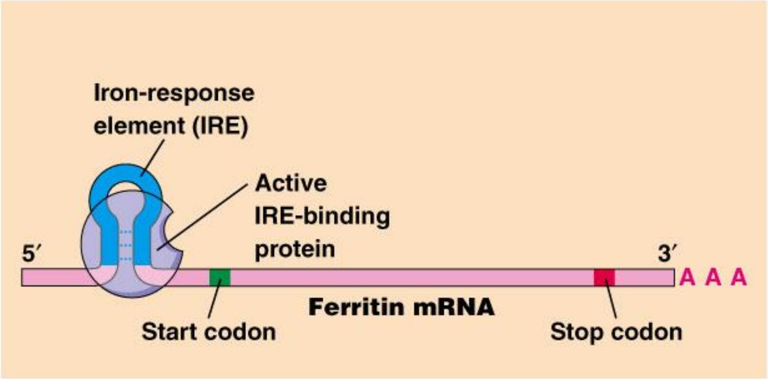


Effect on expression

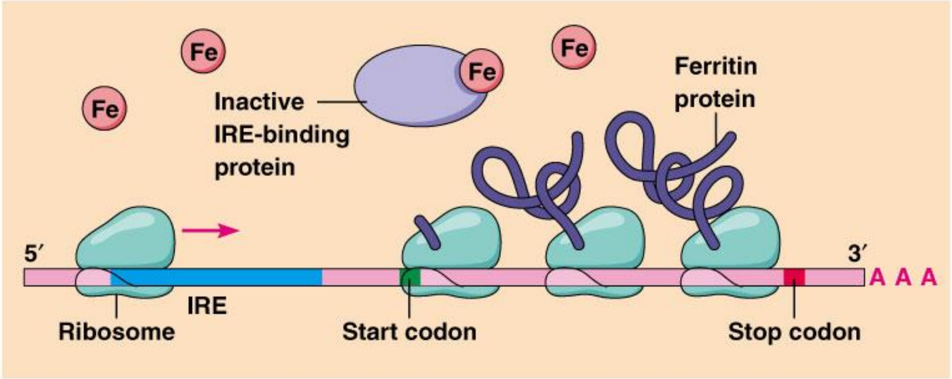
- When iron is abundant (high) in the cells, it binds to IRP, disabling the binding of IRP to the mRNAs of transferrin receptor and ferritin.
  - Transferrin receptor: mRNA is destabilized and is degraded, lowering protein level, and, hence, iron uptake.
  - Ferritin: Translation is activated and storage 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.


a Iron deficiency
3'mRNA
IREs
Transferrin-R
IRPs
Ferritin
5'mRNA
STOP
b Iron overload
Transferrin-R
Ferritin
Nature Reviews | Neuroscience

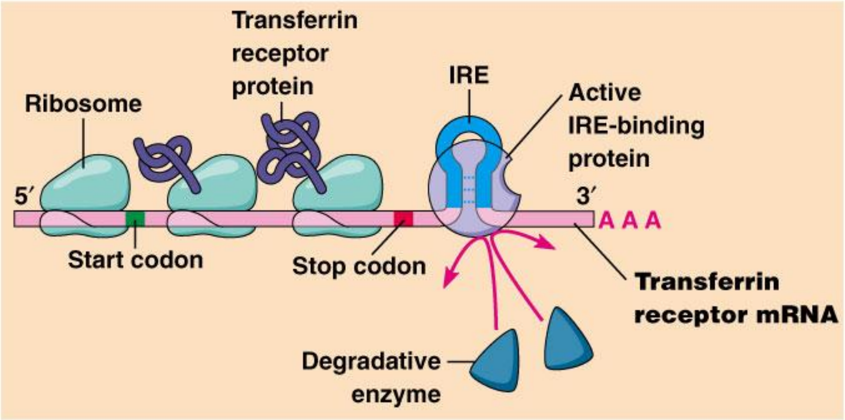
(a) Low iron concentration. IRE-binding protein binds to IRE, so translation of ferritin mRNA is inhibited.



(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.