



Molecular Biology (11)

Transcriptional phenomena in humans

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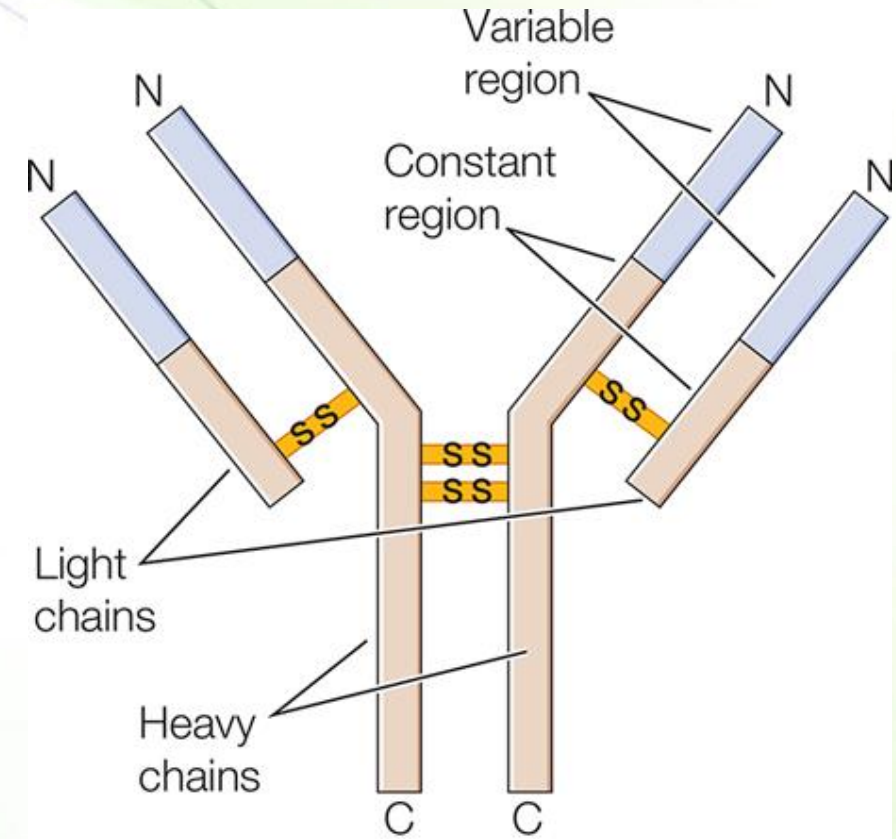


Gene rearrangement

Immunoglobulins



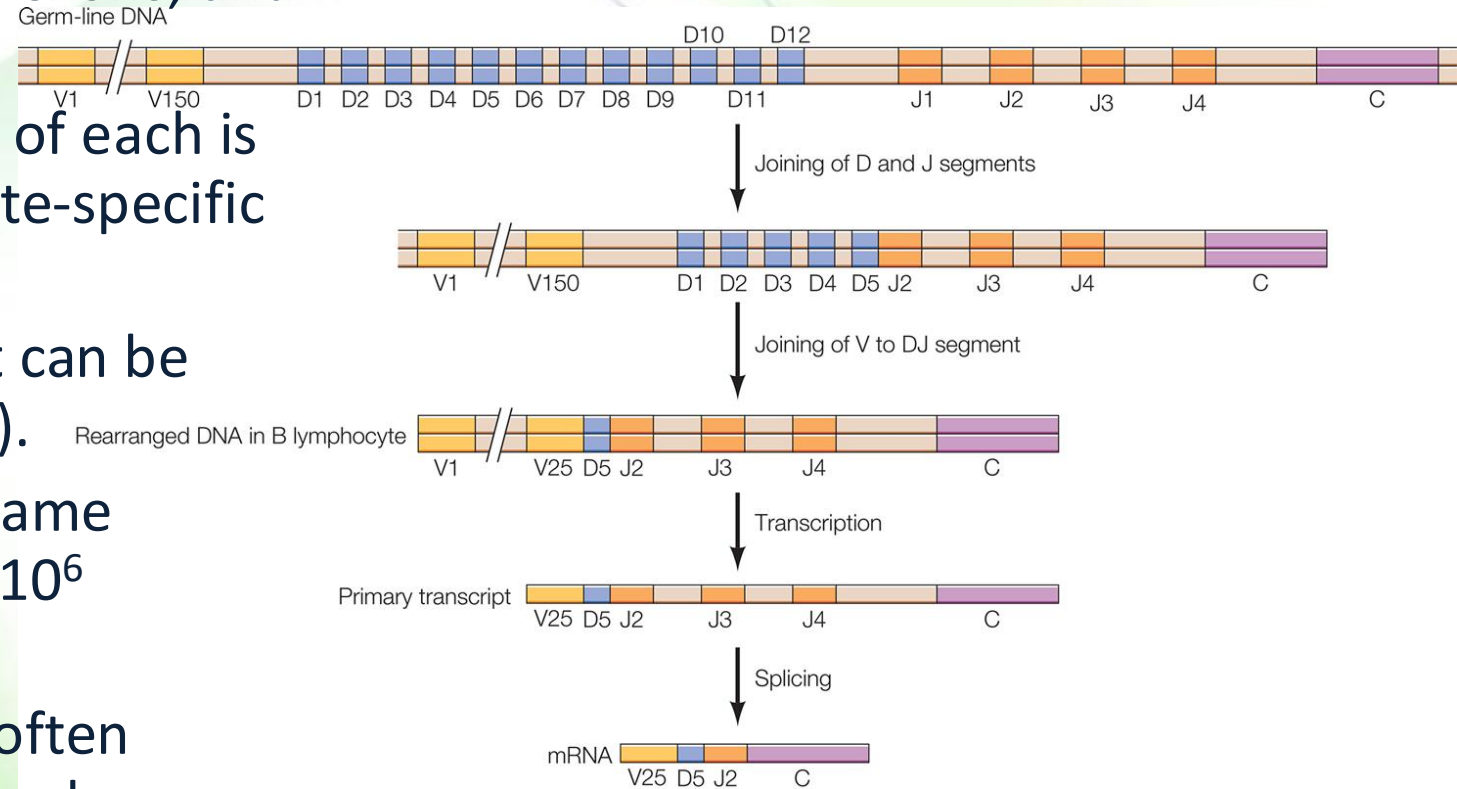
- The human body can possess a population of approximately 10^{12} B lymphocytes that can produce and release immunoglobulins (antibodies), but each cell can produce one type of an immunoglobulin.
- Each antibody has a unique antigen-binding variable region that is encoded by unique genes formed by **site-specific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



The mechanism



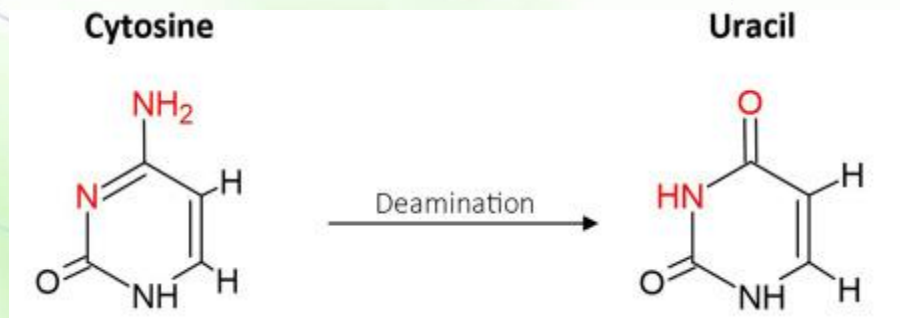
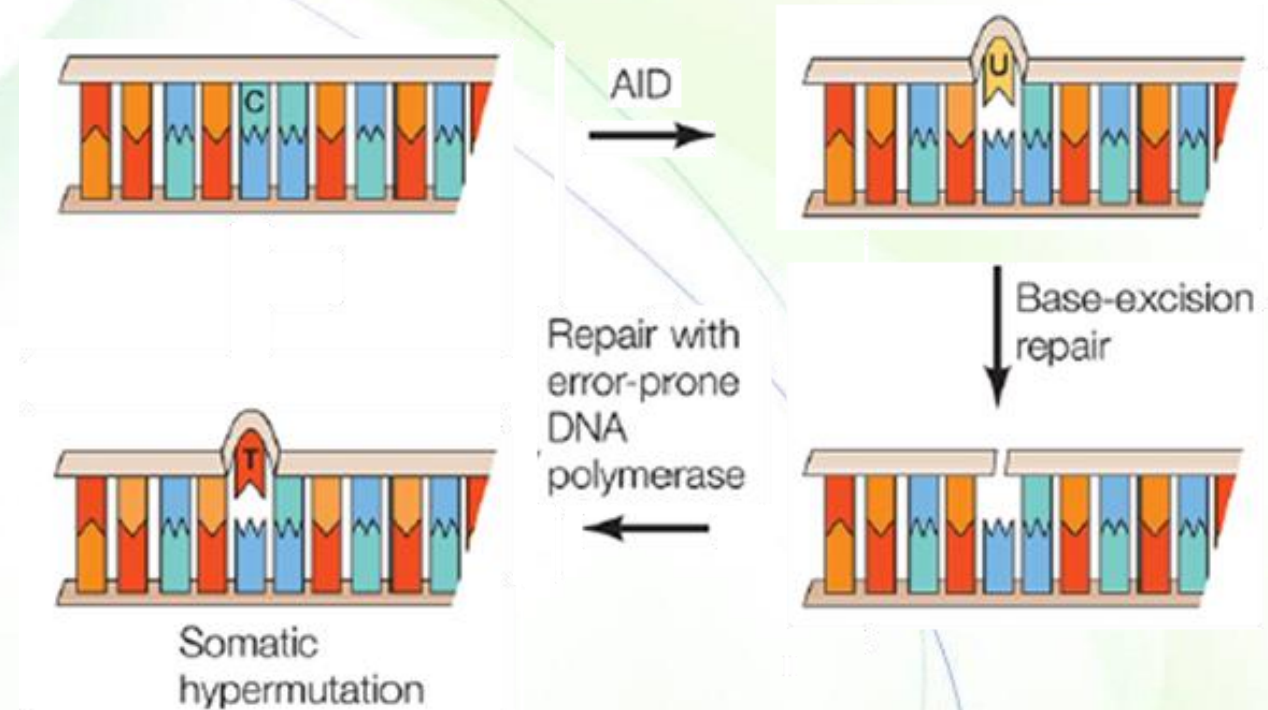
- Each heavy gene consists of 150 variable regions (V), 12 diversity exons (D), 4 joining (J) exons, and one constant exon (C).
- During lymphocyte development, one of each is combined with one of the others by site-specific recombination.
- The total number of heavy chains that can be generated is about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



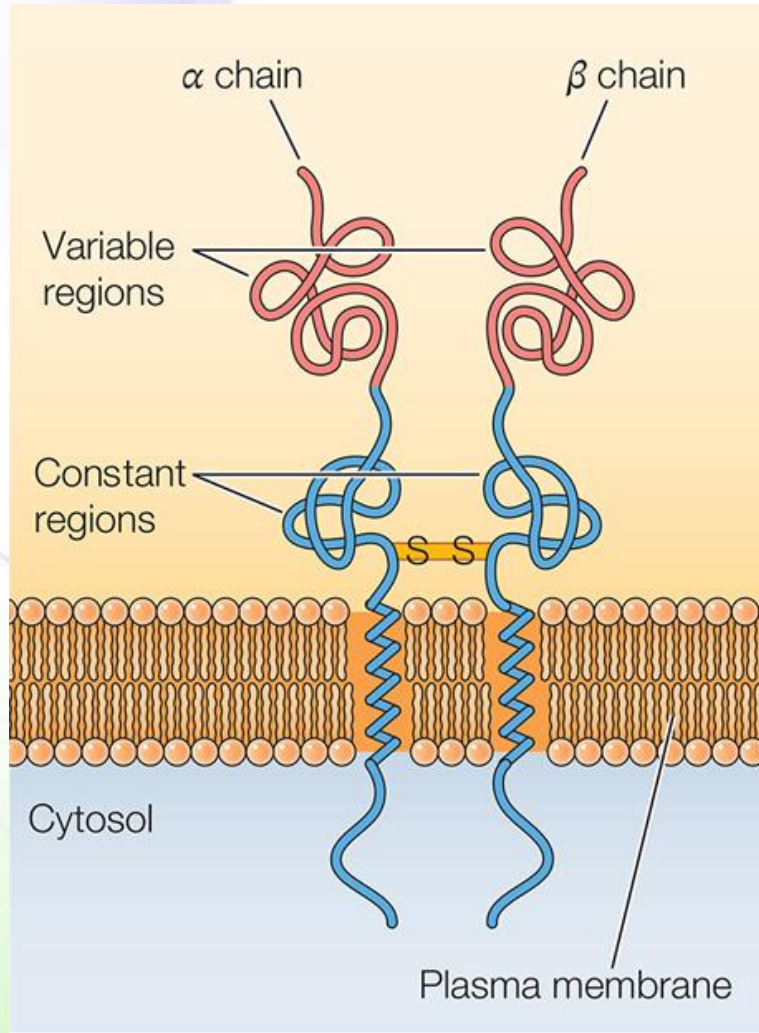
Somatic hypermutation



- In the variable region, the activation-induced deaminase enzyme (AID) converts C's to U's in the DNA.
- The U's are removed, 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



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



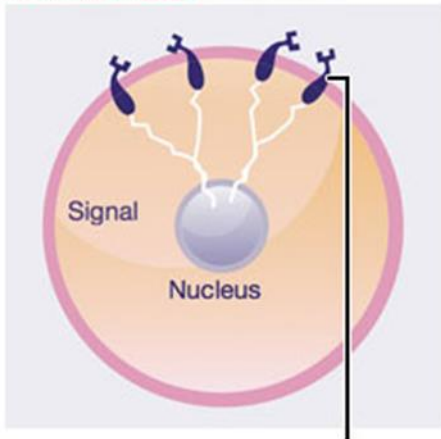
Gene amplification

Gene amplification



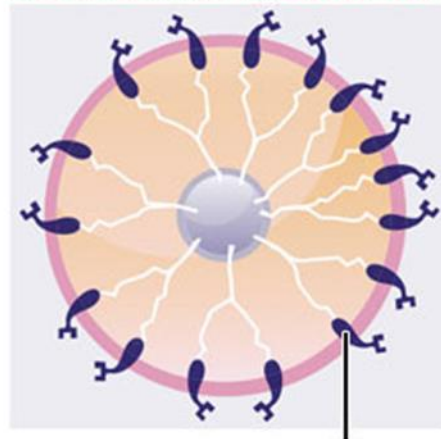
- It is an increase in copy number of a restricted chromosome region increasing the quantity of DNA in these regions and, hence, increasing RNA and protein levels.
- Cancer cells use it to develop resistance from methotrexate whereby the target gene, dihydrofolate reductase, is amplified.
- Breast tumor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

Normal breast cell

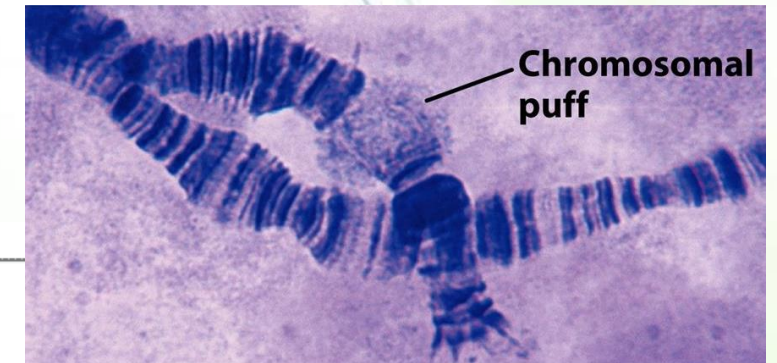
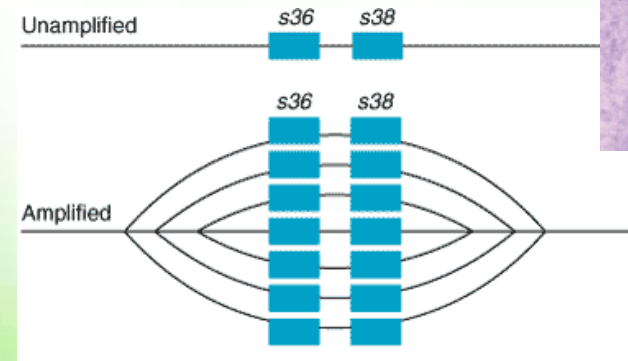


Normal amount of HER2 receptors send signals telling cells to grow and divide.¹

Abnormal HER2+ breast cancer cell



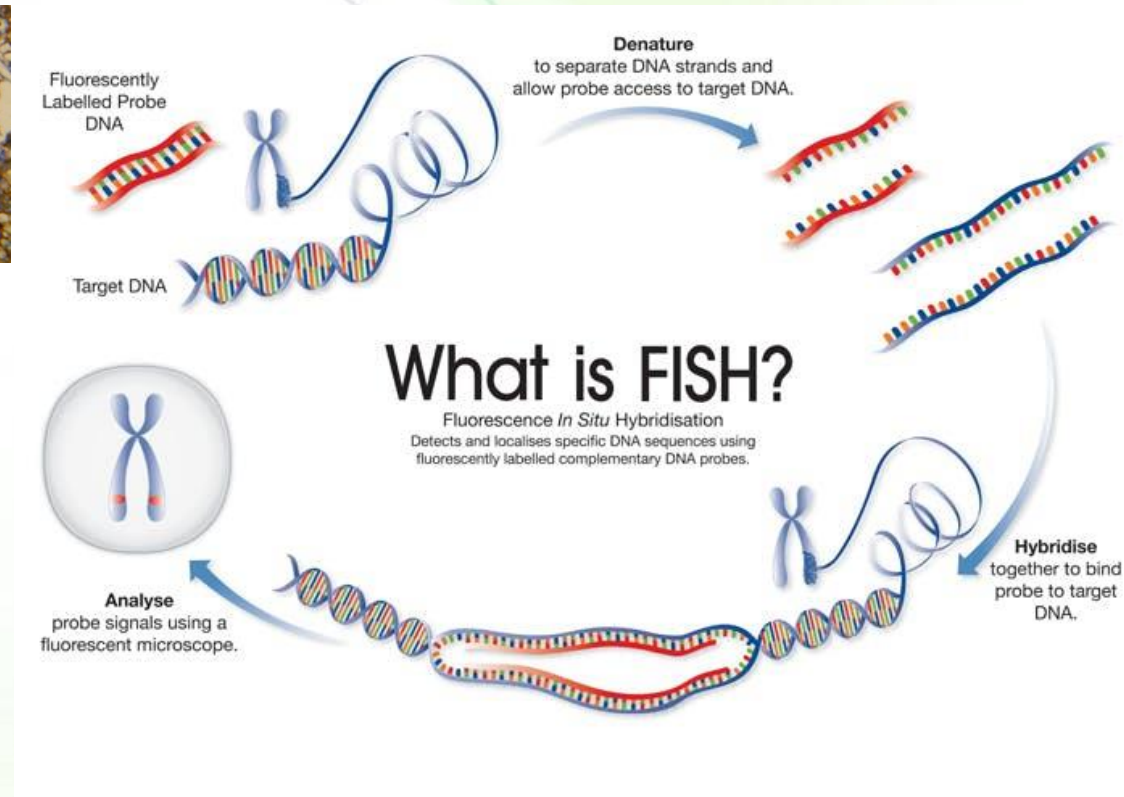
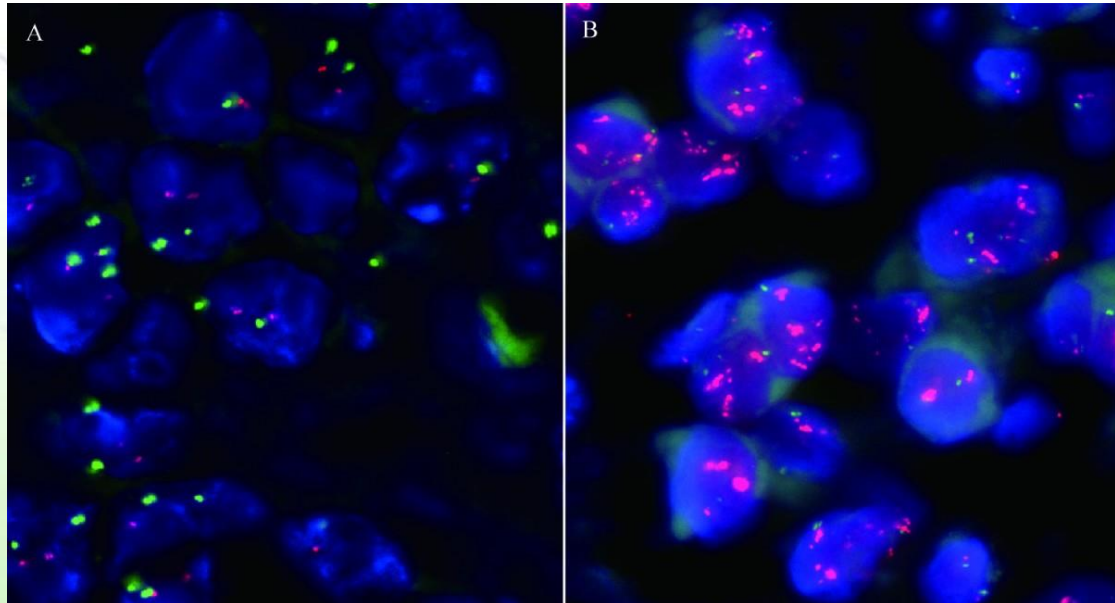
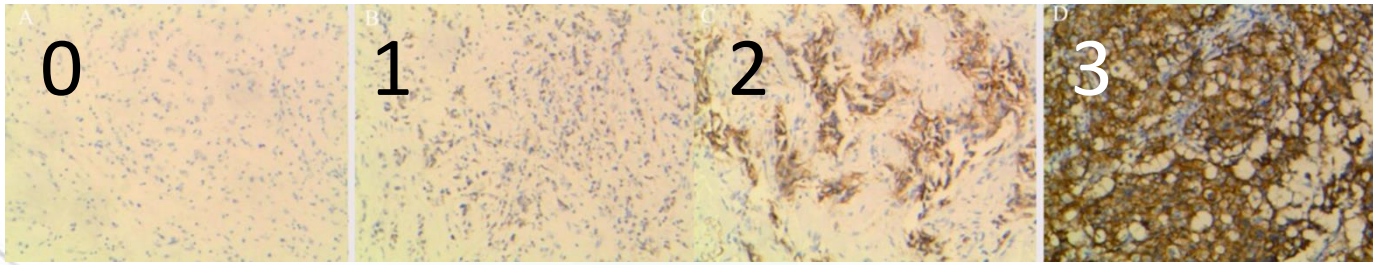
Too many HER2 receptors send more signals, causing cells to grow too quickly.¹



How is it detected?



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

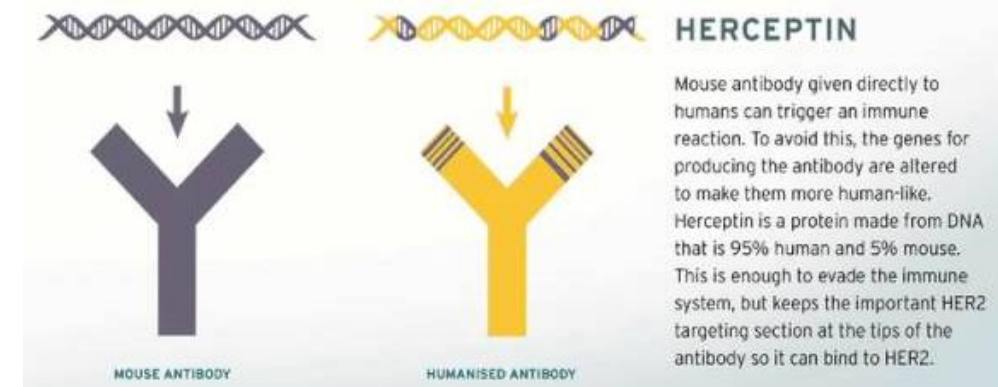
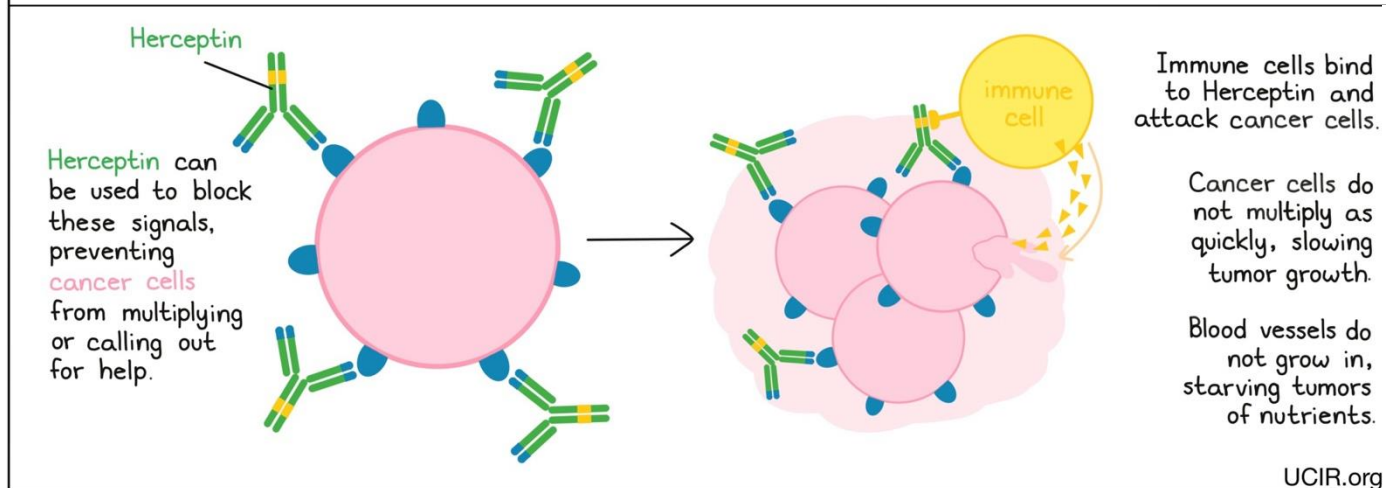
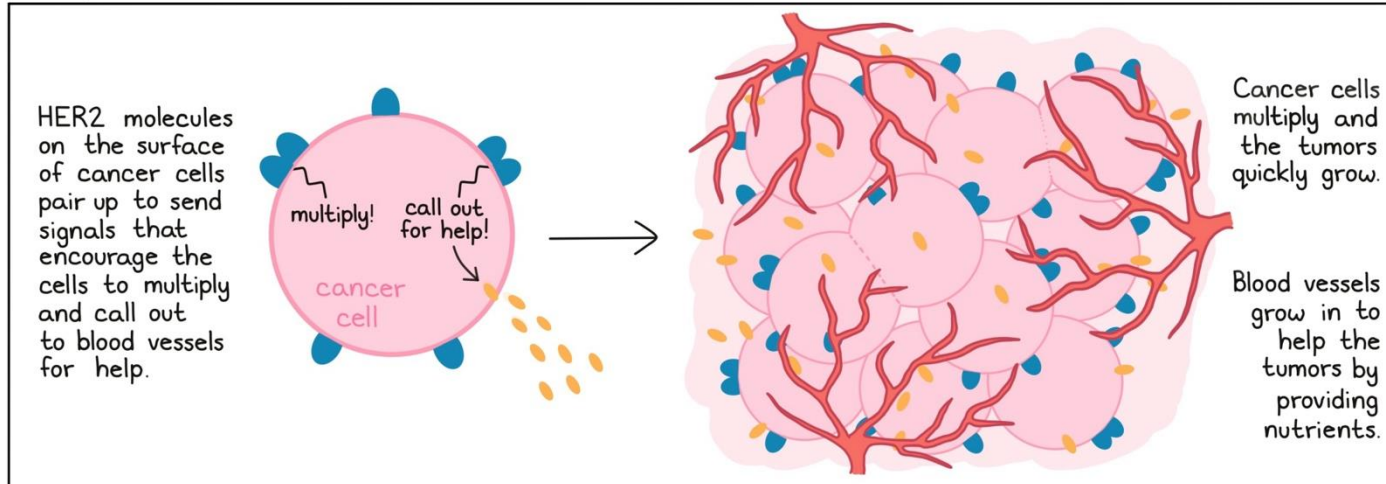


How are HER2-enriched cancers treated?



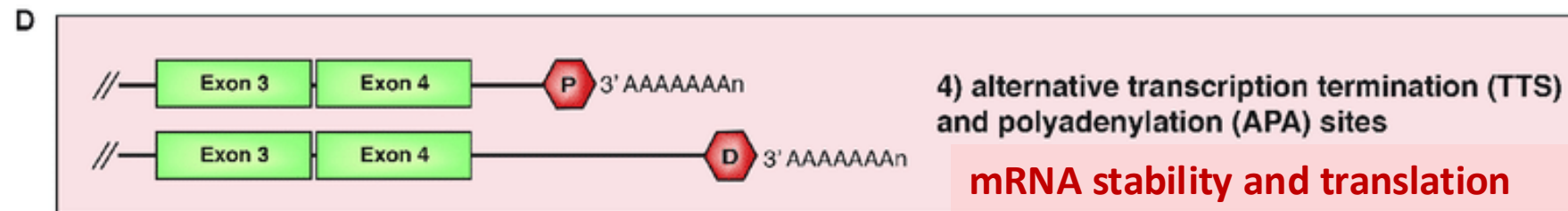
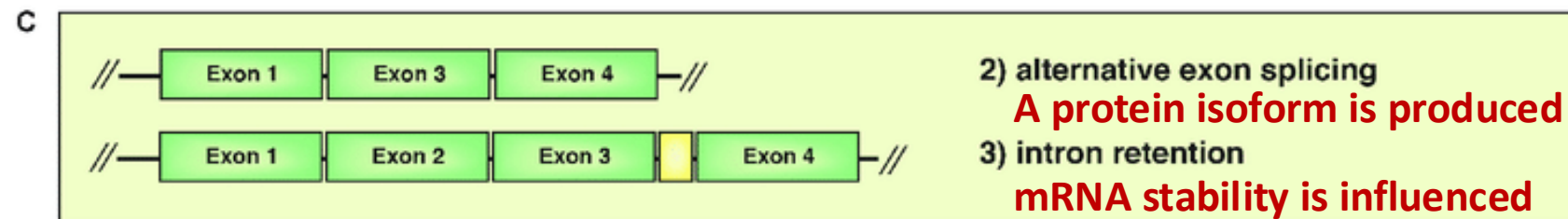
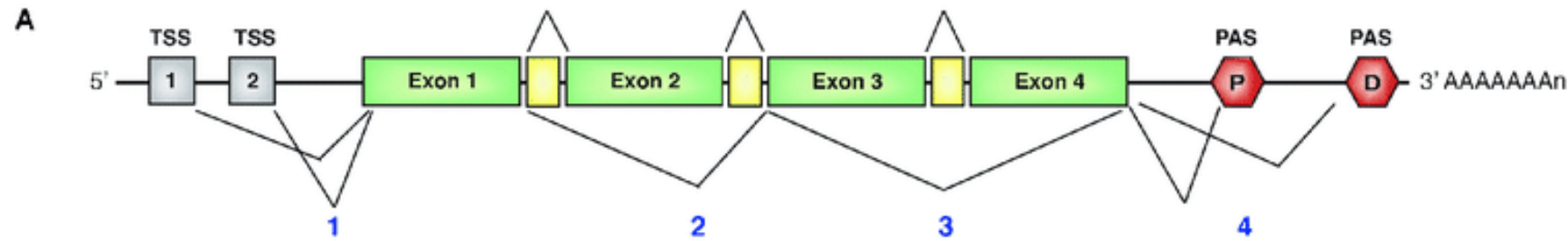
Herceptin (trastuzumab)

Herceptin: how it works



The alternatives:

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



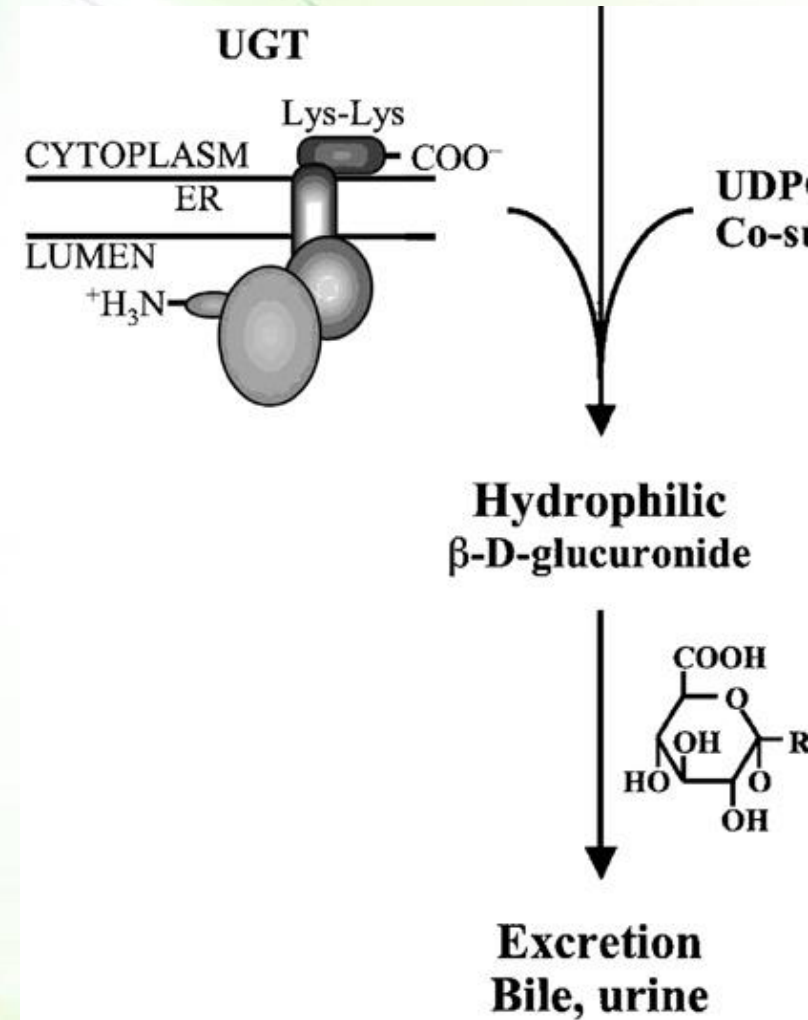
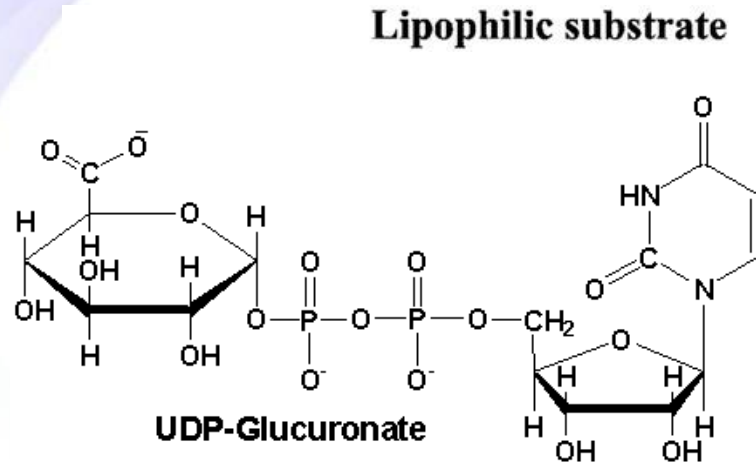
mRNA stability and translation efficiency are regulated by microRNA

One gene, multiple promoters, multiple exons

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

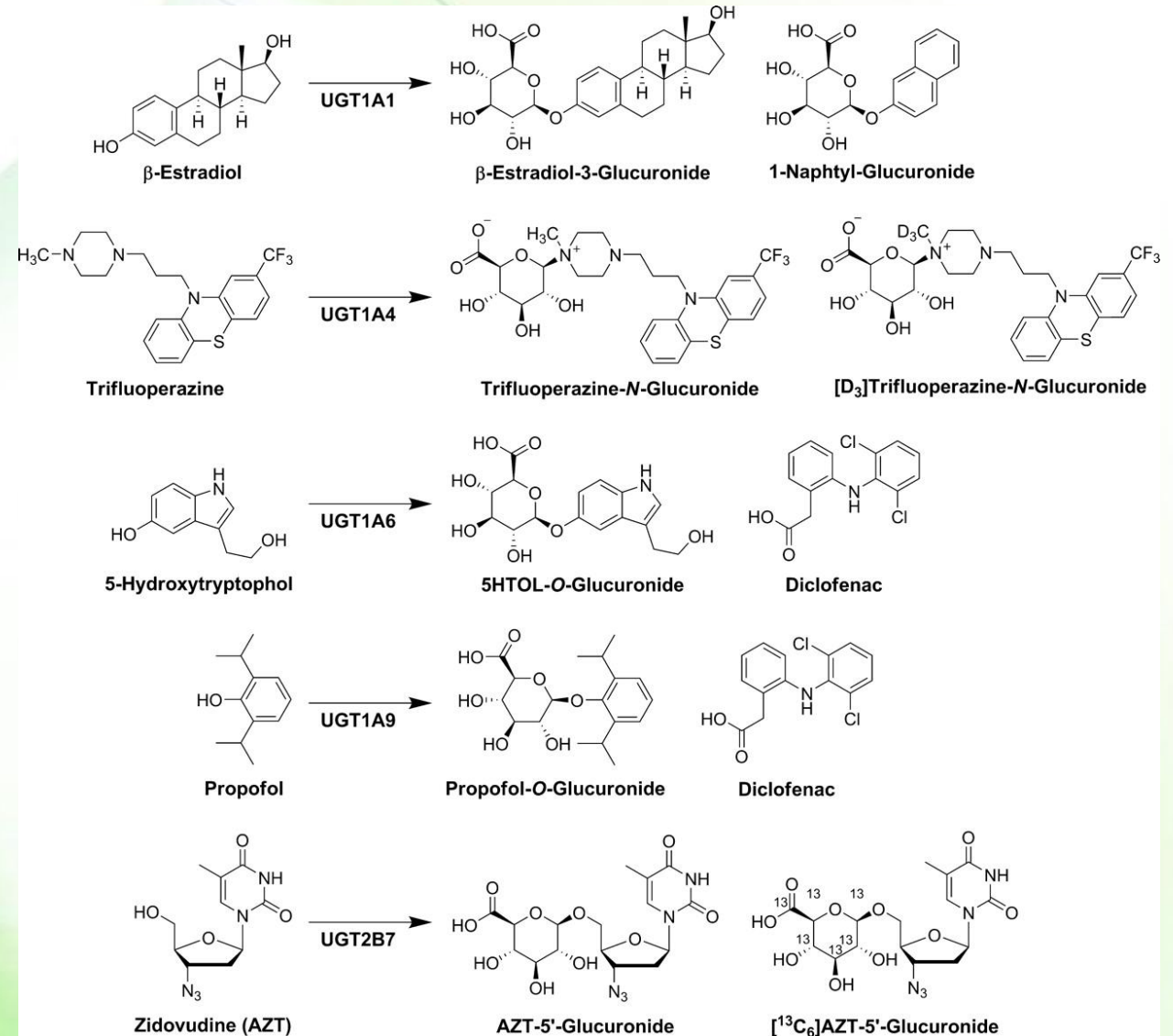
Biliary acids

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



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...



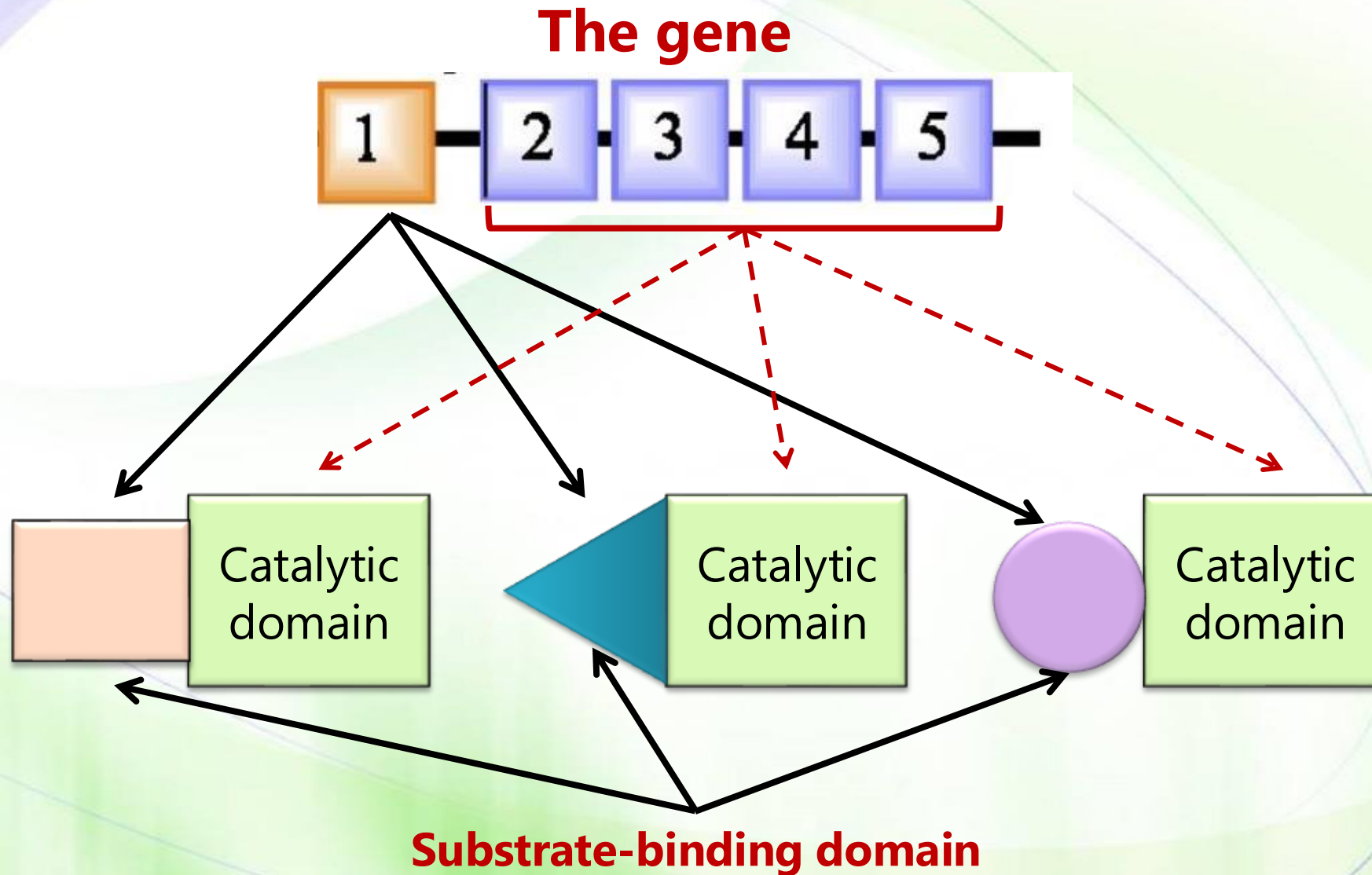
One drill, many flutes



One head, many hats



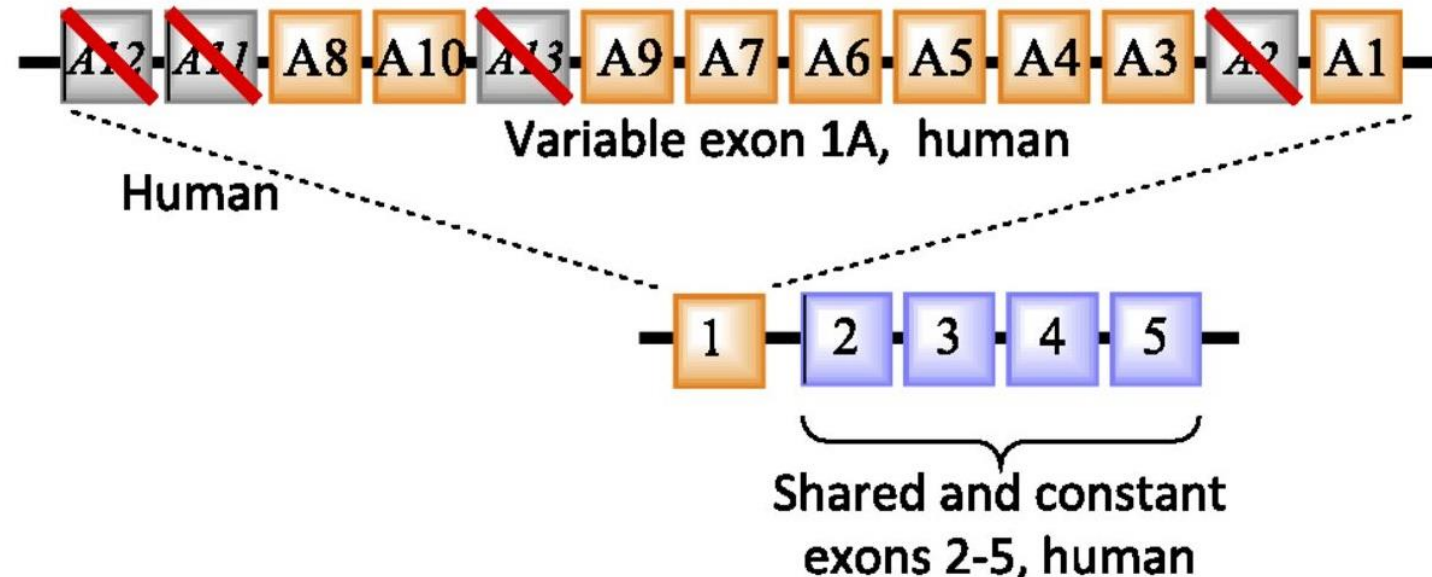
Then this...



How does UGT1A do this?



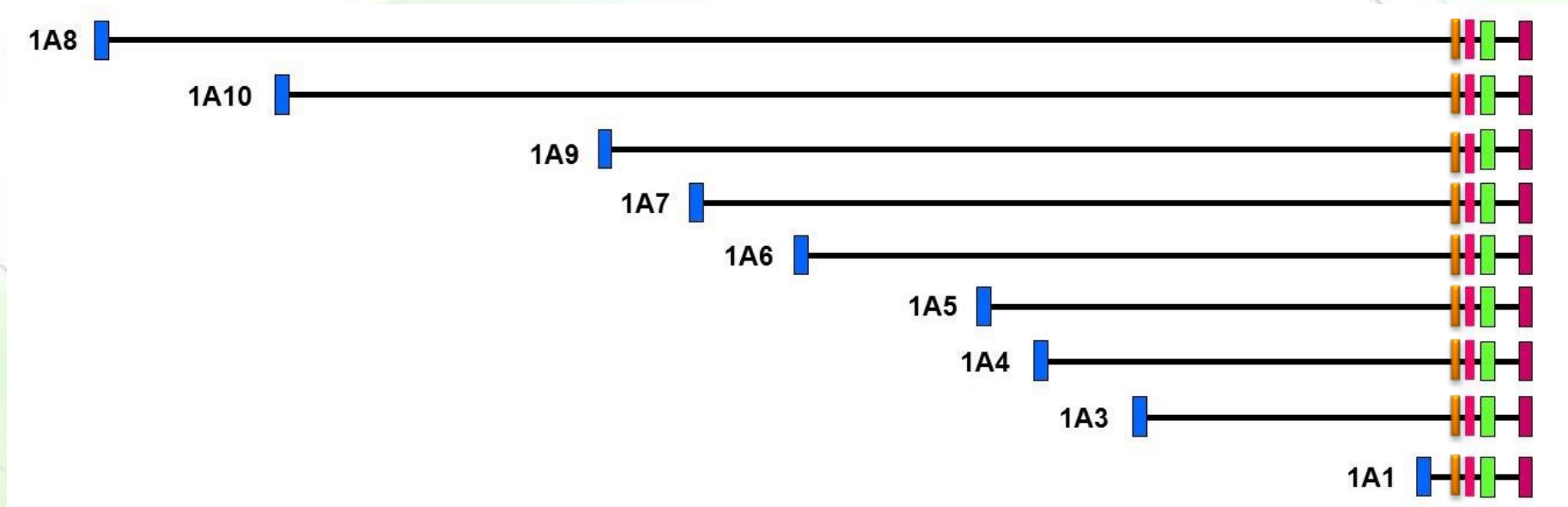
- Exons 2, 3, 4, and 5 encode the catalytic domain that interacts with UDP-glucuronic acid, and exon 1 determines substrate specificity, **but**...
- Exon 1 contains **NINE** tandemly arrayed first exons and each one has its own promoter.
- The 9 exons determine substrate specificity and one of them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



Regulation of mRNA stability

Physiology of iron

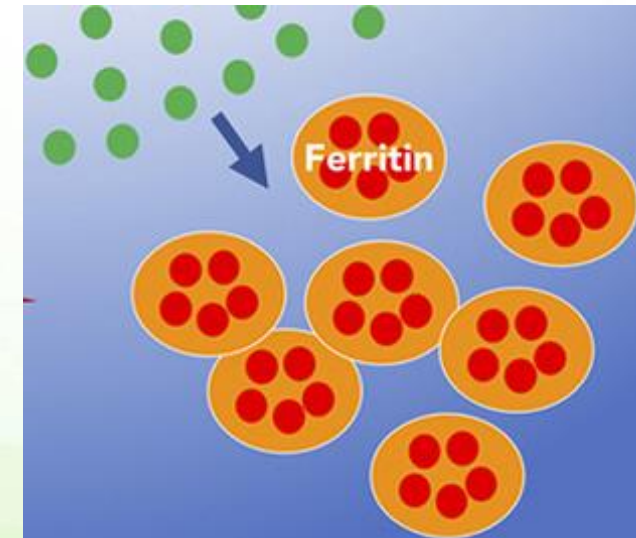
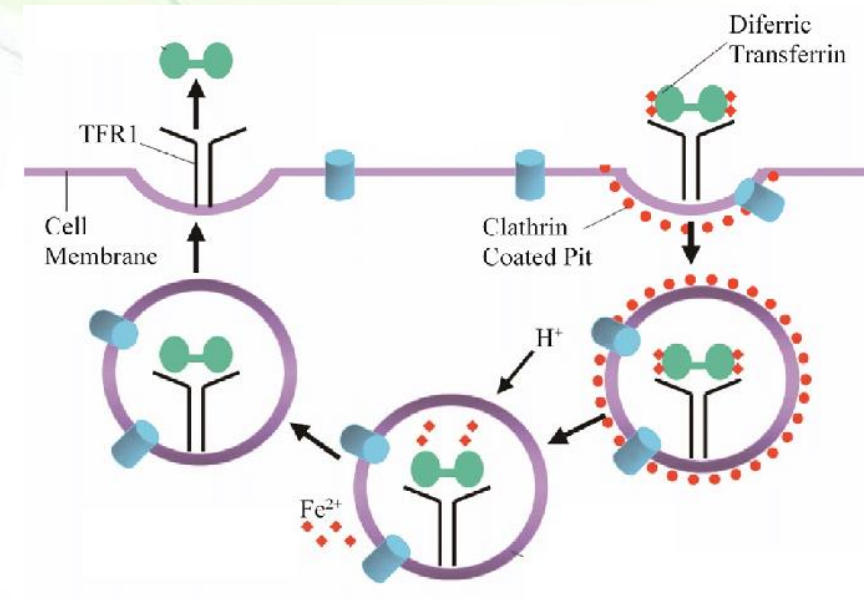


- Iron is an essential metal for the human body.
 - Oxygen transport
 - Enzyme function
- But, too much iron can be toxic.
 - Organ failure
 - Bacterial infection
- The level of iron is intricately maintained.

The players



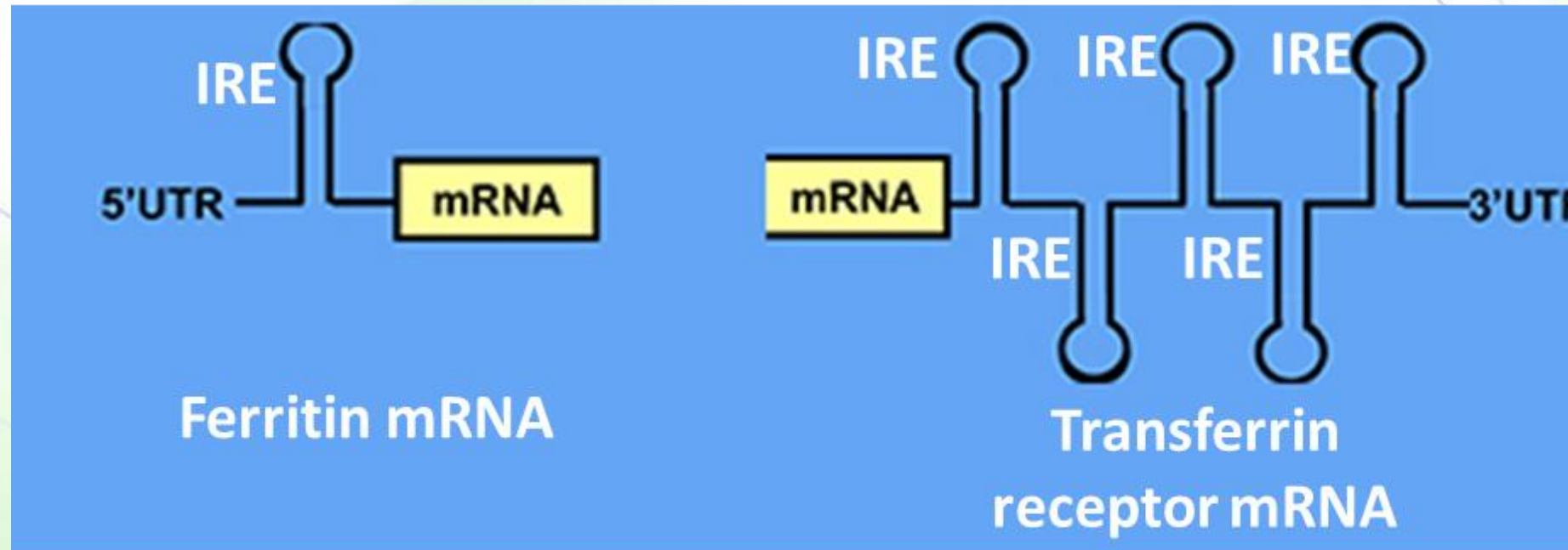
- Liver ferritin protein stores iron when abundant (in the liver).
- Transferrin receptor mediates iron entry via receptor-mediated endocytosis into peripheral cells when needed.
- When iron is high, expression of ferritin should be up-regulated and expression of transferrin receptor should be down-regulated, and vice versa.



Iron-response elements



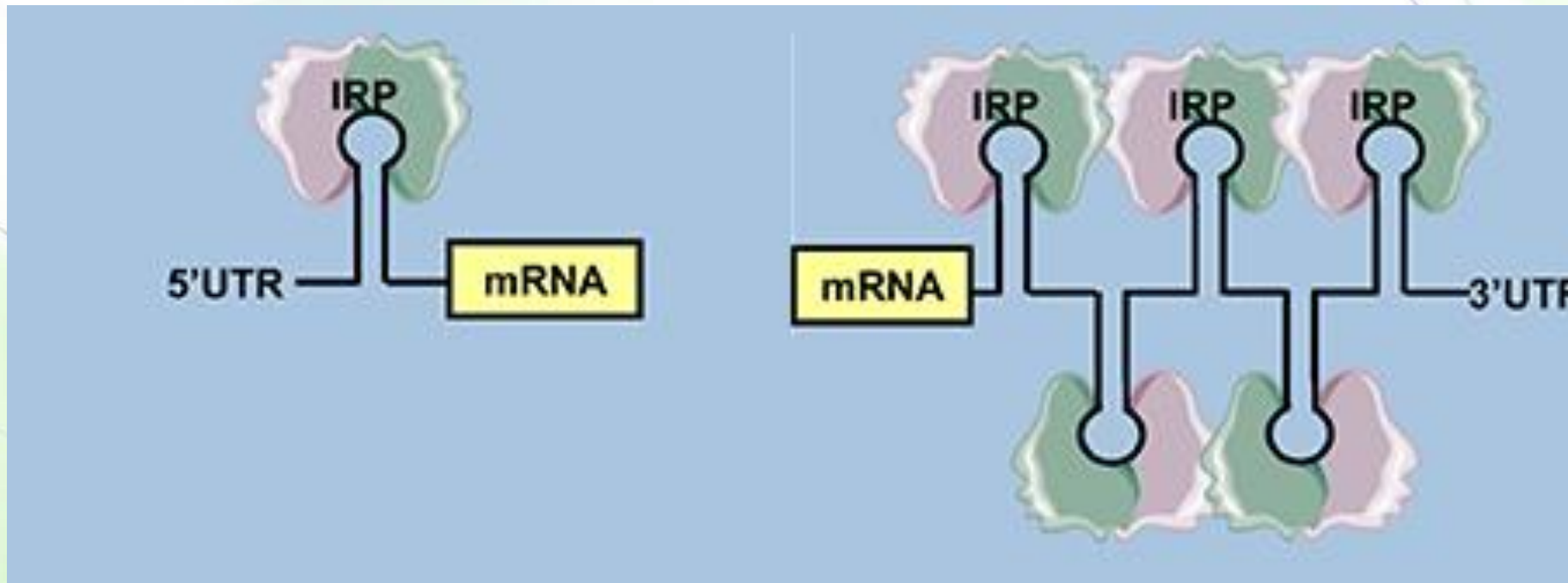
- In human iron-regulatory genes, there are genetic regions (of mRNAs, as well) called iron response elements (IREs).
- These regions also exist within the mRNAs of ferritin and transferrin receptor but at different sides.



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.

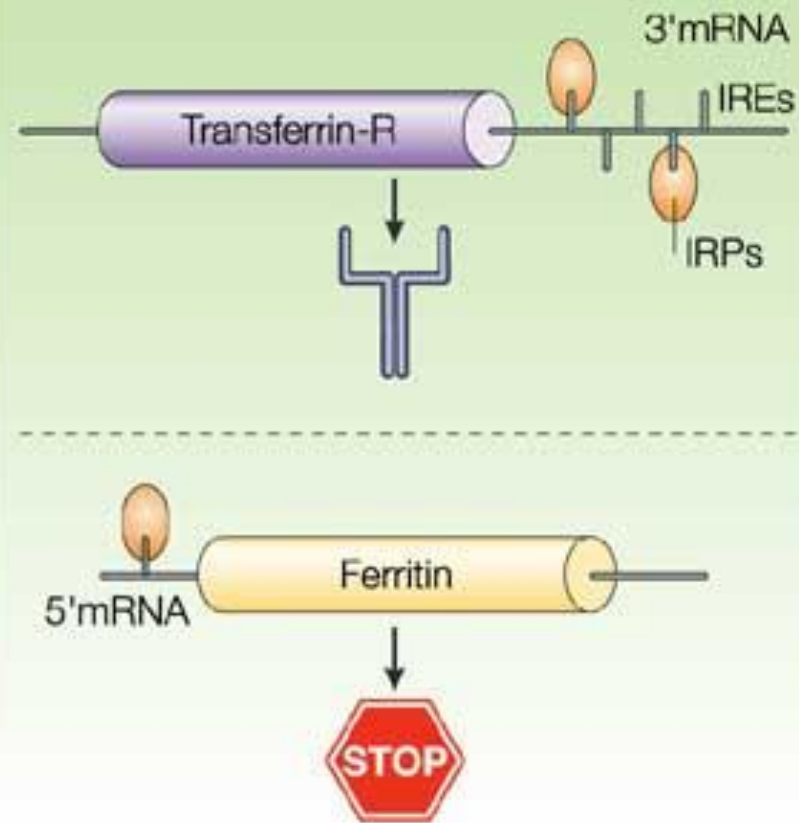


Effect on expression

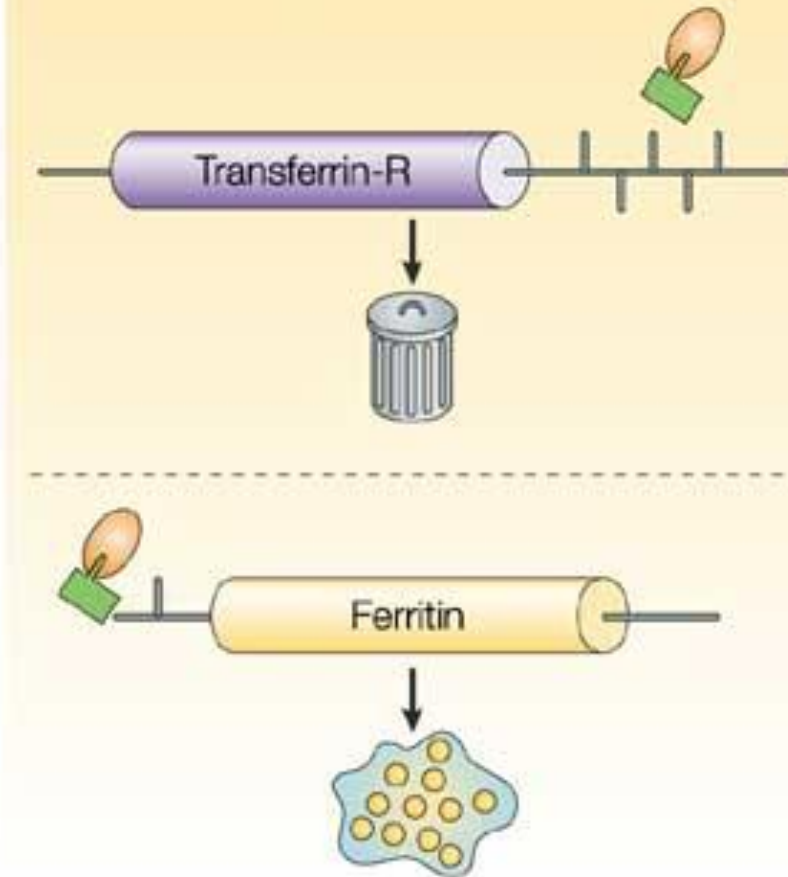


- When iron is abundant (high) in the cells, it binds to IRP, disabling the binding of IRP to the mRNAs of transferrin receptor and ferritin.
 - Transferrin receptor: mRNA is destabilized and is degraded, lowering protein level, and, hence, iron uptake.
 - Ferritin: Translation is activated and storage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

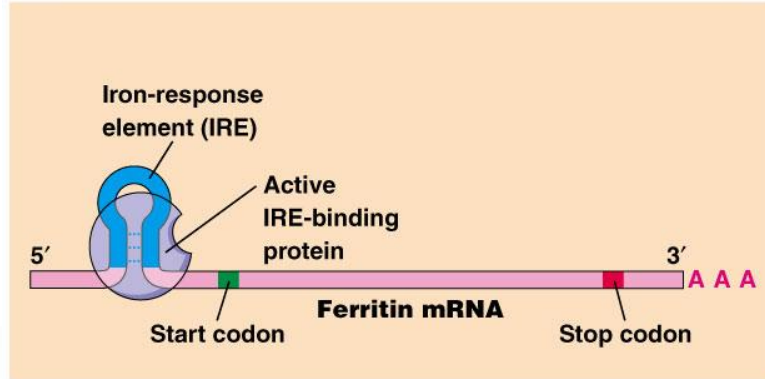


b Iron overload



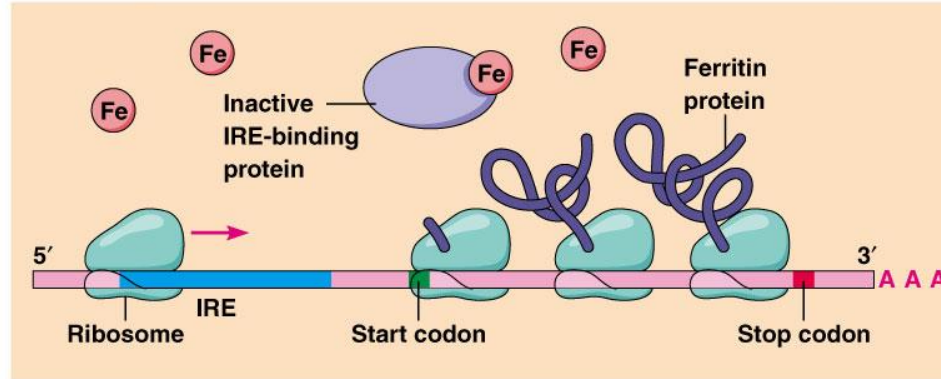
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(a) Low iron concentration. IRE-binding protein binds to IRE, so translation of ferritin mRNA is inhibited.

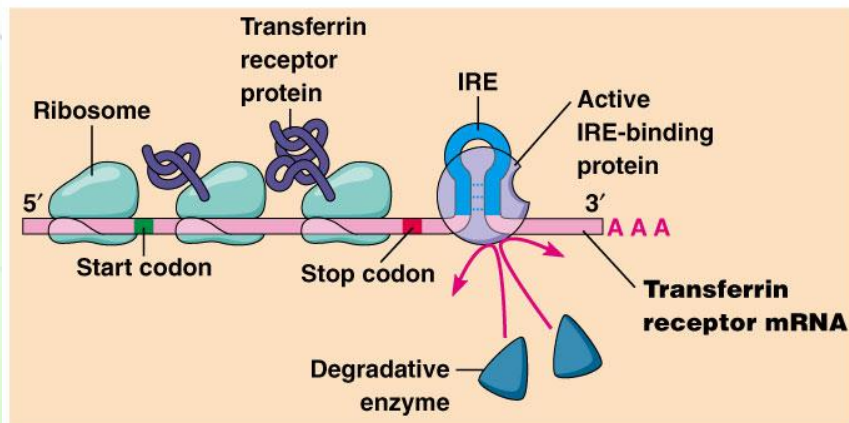


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(b) High iron concentration. IRE-binding protein cannot bind to IRE, so translation of ferritin mRNA proceeds.



(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.



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

