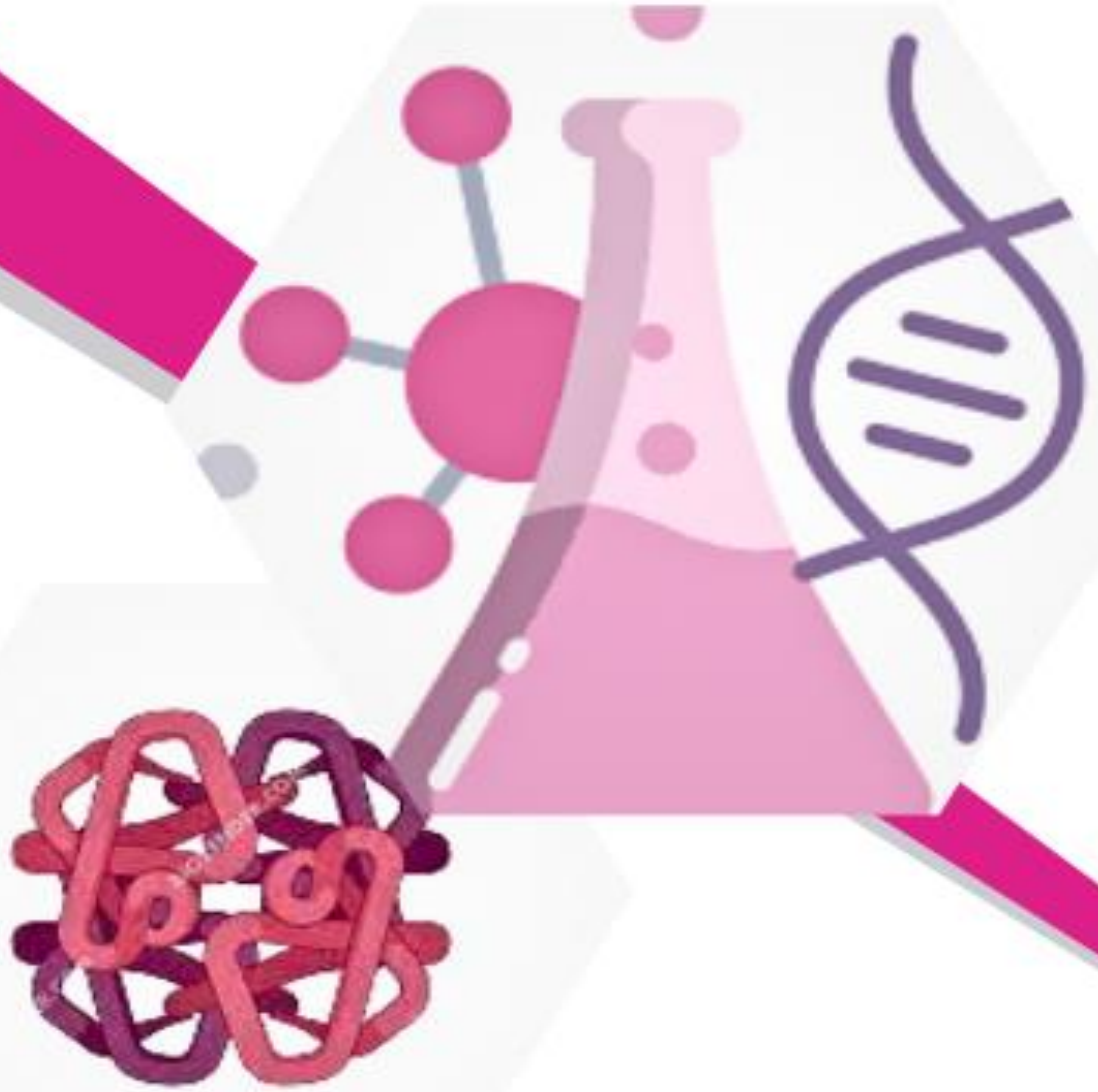
Doctor.021

no. 5

HLS BIOCHEMISTRY



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IMPORTANCE OF IRON

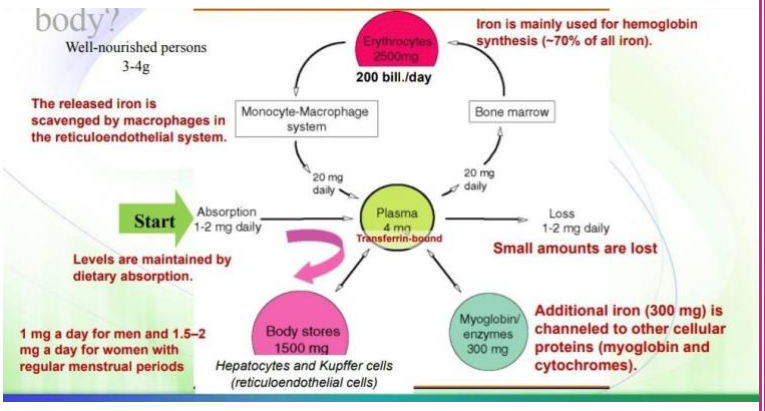
- **Within the body, iron exists in two oxidation states: ferrous (Fe2+) or, the highly insoluble** (that's why you can't see it through the body it will be bounded to proteins as ferritin and transferrin)**, ferric (Fe3+).**

Iron is really important, its involved in oxygenation , oxygen storge in muscles .

- **It is also the prosthetic group of several enzymes such as redox cytochromes and the P450 class of detoxifying cytochromes.**
- **Iron is important for metabolism and oxygen transport.**
- **Yet...**
- **Iron can be potentially toxic due its ability to form free radicals,** that's why you won't see it in the free state due to the toxicity of it **.**
- **Solution: iron is not free.**

WHAT IS THE LIFE CYCLE OF IRON IN THE BODY?

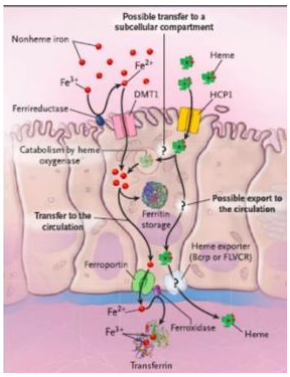
Explanation :



- **The amout of iron in well nourished persons is 3-4 g, it's a huge amount if we compare it to the daily absorbed amount (1-2mg) .**
- **After absorption very little amount will be founded in the plasma (4mg). So, where is the iron?**
- **Actually most of iron will go to the bone marrow for erythropoiesis , 70% of iron in our body is in the hemoglobin(around 2.5 g).**
- **After RBC death in the hemolysis process macrophages phagocytose them to preserve iron and to prevent toxicity as well.**
- **Beside the BM a huge amount of iron will be stored in certain places in our body mainly in hepatocytes and Kupffer cells ~1.5 g.**
- **Also , there is a good amount(~300mg) stored in muscles at myoglobin .**
- **Another amount of iron will be founded in certain metabolic enzymes as cytochromes (ex.P450)**
- **Notice that what we loose daily equils what we absorbed this means that iron is well preserved .**
- **Side note : females loose iron more than males due to the menstrual cycle.**

IRON ABSORPTION

STATE OF IRON



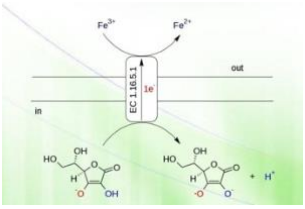
Under conditions of neutral or alkaline pH, iron is found in the ferric Fe3+ state and, at acidic pH, in the ferrous Fe2+ state.

In the stomach, iron will be in the ferrous state.

In the duodenum, iron is in the ferric state.

However, to be absorbed, dietary iron must be in its ferrous Fe2+ form.

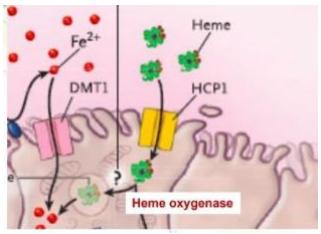
SITE OF ABSORPTION :



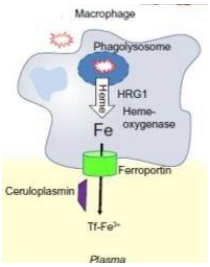
- **Ferrireductase enzyme on the enterocytes' brush border reduces Fe3+ to Fe2+ in a vitamin C-dependent reaction.** (vit c is so important for ferrireductase function)
- **Divalent metal transporter 1 (DMT1) transports iron into the cell.**
- **DMT-1 can transport other metal ions such as zinc, copper, cobalt, manganese, cadmium, and lead.**

If you ingest food full with heme (ex. red meat) , heme also can be absorbed by the HCP(Heme carrier protein) at the enterocytes surface .

HEME AS A SOURCE OF IRON



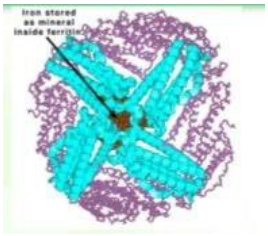
- **Iron can also be obtained from ingested heme.**
- **Heme is absorbed by a receptor called heme-carrier protein 1 (HCP- 1) and iron is released/**extracted **by heme oxygenase-1 (HO-1).**
- **In other cells such as macrophages** (they contain the same enzymes and transporters so they can ingest iron and heme)**, heme oxygenase also extracts iron from heme.**



- ***Note: Proton pump-inhibiting drugs such as omeprazole greatly reduce iron absorption*** (it's a serious problem for patients who take these drugs in large quantities).

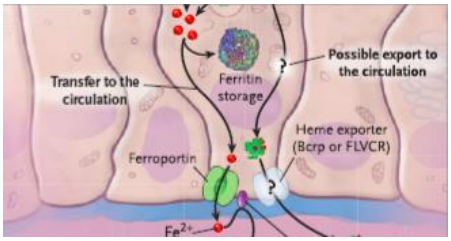
FATES OF IRON:

Inside the duodenal cell iron will back to the ferric state, after that iron ions have two fates :



Fate 1: storage

- **Cells can then store iron as ferritin.**
- **Each Ferritin complex can store about 4500 iron (Fe3+) ions.**
- **But, if cells are sloughed off from the tip of the villus into feces before absorption, iron is eliminated from the body.**



Notice that this is one of the ways of how we loose iron , it exists the body with dead duodenal cells.

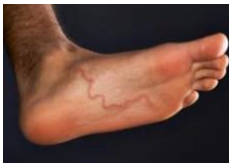
Fate 2: Transport

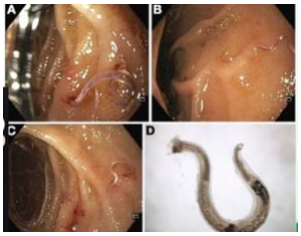
- **Iron is transported out via a basolateral transporter known as ferroportin** (its also founded in macrophages), **which is distributed throughout the body on all cells.**

INTESTINE -RELATED IRON METABOLISM DISORDERS

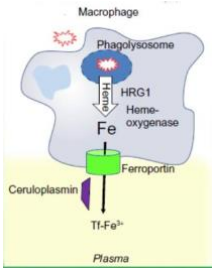
There are some diseases that affect iron amount in our body either by malabsorption or by loosing large quantities of intestinal cells (due to infection , inflammation or hemorrhage):

- **Iron malabsorption**
  - **Gastrectomy (total or partial)**
  - **Celiac disease (villous atrophy)**
  - **Crohn's disease**
  - **Helicobacter pylori**
- **Intestinal hemorrhage (gastrointestinal-mediated iron loss)**
  - **Gastric cancer , iron will be released into the plasma.**
  - **Ulcers**
  - **Inflammatory bowl disease**
  - **Hookworm infection , this worm stays in different places in our body including the intestine .**

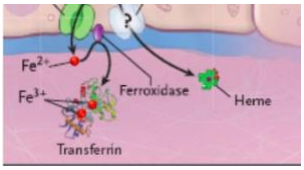




FERROXIDASE AND TRANSFERRIN

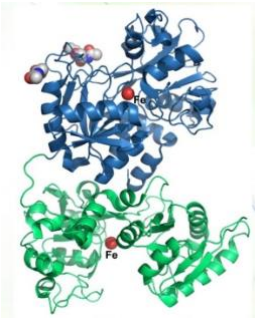


- **Once iron leaves the intestinal cells, an iron oxidase, known as hephaestin or ferroxidase, converts iron from the ferrous state to the ferric state** (so it can bound to transferrin)**.**
- **Nonintestinal cells use the plasma protein ceruloplasmin to oxidize iron.**
- **Iron is rapidly bound to transferrin, an iron-binding protein of the blood that delivers iron to liver cells and from liver cells for storage to other tissues via receptor-mediated endocytosis.**



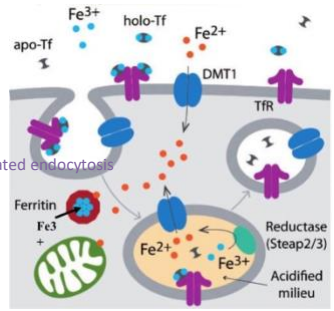
PROPERTIES OF TRANSFERRIN

- **Apotransferrin can bind several metals, but ferric, not ferrous, iron has the highest affinity forming ferrotransferrin.**
- **Transferrin contains two sites that bind ferric irons:**
- **iron-binding sites of transferrin are normally only about 1/3 saturated with iron.** This leaves a lot of binding sites in transferrin through our bodies (An impressive defensive mechanism).
- **When iron exceeds normal levels, non- transferrin-bound iron (NTBI) appears**(iron is bounded to proteins other than transferrin)**.**



RECEPTOR-MEDIATED ENDOCYTOSIS

- **Ferrotransferrin** (or holo transferrin)**binds to a transferrin receptor (TfR)**"this receptor was founded in many cell types bcs they need iron in many processes " **on the surface of cells triggering endocytosis into early endosomes (pH of 6.0).**
- **Early endosomes are transformed into late endosomes (pH of 5.0)**"iron will be released inside the vesicle due to the low pH" **where Fe3+ atoms dissociate, get reduced**(bcs of low pH) **into Fe2+ by the ferrireductase STEAP3, and are transported into the cytosol via DMT1.** When iron reach the cytosol it can be used by proteins(enzymes) or stored**.**
- **STEAP3 depends on vitamin C.**



Receptor mediated endocytosis

As what happened in the intestine.

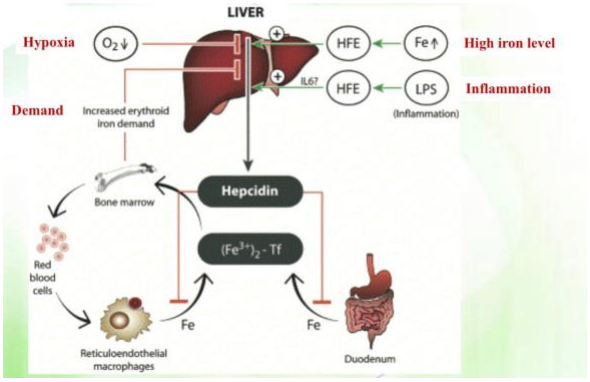
- **The apotransferrin-transferrin receptor complex is recycled back to the surface, apotransferrin**(means without iron) **dissociates, and the receptor binds another transferrin.**
- **Affinity of TfR to iron: diferric Tf (Fe2Tf) >monoferric Tf (Fe1Tf) >apo-Tf**

REGULATION OF PROTEIN FUNCTION

We will discuss certain proteins that have role in transport , readuction , oxygenation, absorption, and storage of iron.

HEPCIDIN (IRON SENSOR)

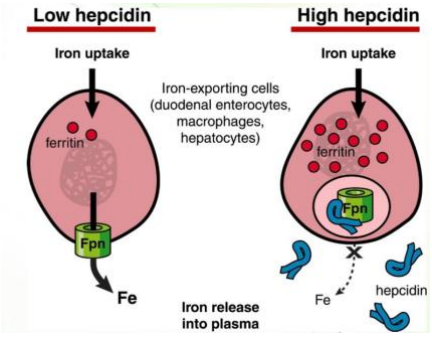
- **Hepcidin is a peptide hormone (25 amino acids) secreted by the liver and it reduces iron levels.** (iron increase , hepcidin increase علاقة طردية)
- **When iron level increases and in cases of inflammation, hepcidin secretion increases.**
- **When iron levels are low, there is high iron demand, or hypoxia, its release is suppressed.**



To sum up, hepcidin decreases iron absorption , and arrest it inside the intestinal cells and macrophages ,on the other hand ferritin increases . If the iron body level decreases the opposite will happen.

HOW DOES HEPCIDIN REDUCE IRON LEVELS IN THE BODY?

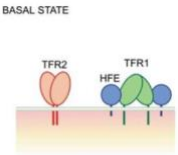
- **Hepcidin binds to the basolateral iron transporter ferroportin** (its founded in macrophages and enterocytes) **inducing ferroportin internalization and degradation**(by lysozymes)**.**
- **This results in higher iron storage, means less iron leaves the cell.**
- **Iron is eliminated in sloughed off intestinal cells**(they will die quickly due to the toxicity of the accumulated iron inside them)**.**



- **Iron is not released from macrophages.**
  - **Hepcidin also inhibits the presentation of the iron transporters (e.g. DMT1) in intestinal membranes decreasing iron absorption.**

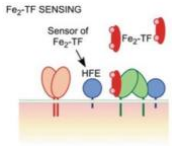
REGULATION OF HEPCIDIN

These are certain mechanisms that show you how iron absorption is regulated by numerous ,different , and highly regulated signaling pathways this indicates that iron is so important for our cells but its also toxic .

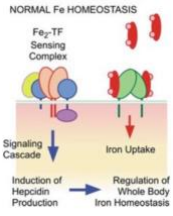


1)

a) Apo-TFR1 complexes with HFE during low or basal serum iron conditions.

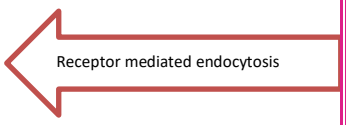


TFR1: For transferrin binding and iron entry into the cell
TFR2: it's a sensor , that stimulates hepcidin production according the iron amounts that enters the cell



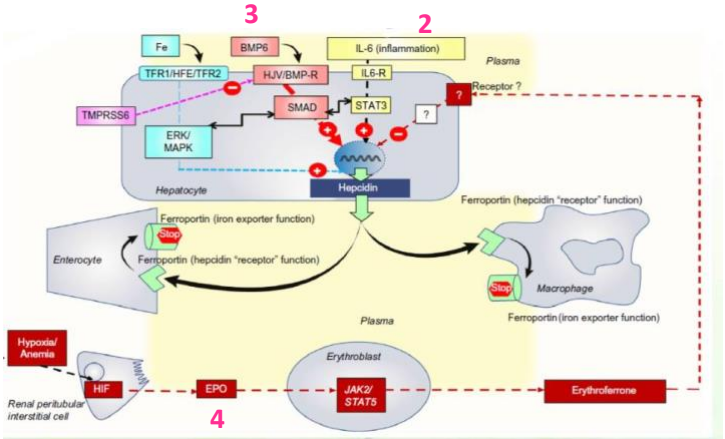
b) Holo-Tf (Fe2-Tf) binds TFR1 releasing HFE.

c) HFE binds TFR2 and induces an intracellular signal that stimulates hepcidin production (to balance inbody iron and the iron that will enter the cells).



2) Inflammatory cytokine, IL6, induces the expression of hepcidin /how? By binding to its own receptor (IL6-R)

Why? Because iron is important for many types of bacteria



3) Release of bone morphogenic protein 6 (BMP6) is induced by intracellular iron (induction occurs when the intracellular iron increases)**, which binds to its receptor (BMPR).**

BMPR is bound to hemojuvelin (HJV) protein stimulating the synthesis of hepcidin.

4)The expression of hepcidin is negatively regulated by anemia and hypoxia, which induce the synthesis of EPO (erythropoietin) by the kidney.

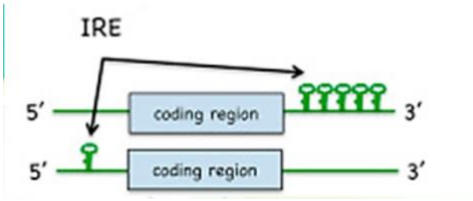
EPO (by binding to its own receptor) **stimulates the synthesis of erythroferrone , which inhibits the synthesis of hepcidin**(by binding to its own receptor on hepatocytes).

POST-TRANSCRIPTIONAL REGULATION OF EXPRESSION

IRON RESPONSE ELEMENT AND IRON REGULATORY PROTEIN

Now, lets dig deep into molecular biology!

* m-RNA will be produced and will be eventually translated into the different molecules that are responsible for the metabolism, transport and absorption of iron. This mRNA has different elements and sequences, our main focus is the Iron Regulatory Element (IRE), this element is found particularly at the Untranslated Region (UTR) of the mRNA either at the 5' or the 3' end.



The translation for the specific molecules that we need for iron metabolism starts at the coding region.

This coding region will produce either TfR 1, ferritin, DMT1 or ALAS.

Remember that Ferritin and ALAS (enzyme used in heme synthesis) are needed when there are huge amounts of iron, and DMT 1 and TfR are needed when there are low amounts of iron and we need to get iron into the cells.

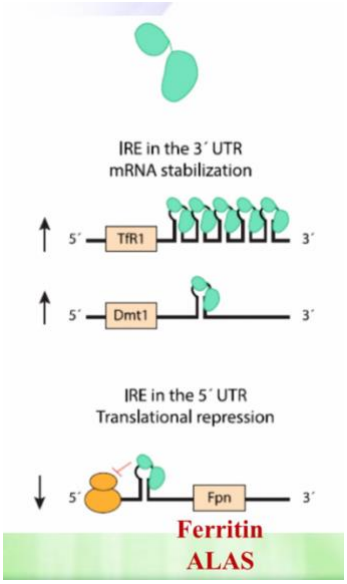
(So what we understand is that the start codon doesn't necessarily have to be at the 5' end nor the stop codon has to be at the 3' end).

Now, let's go back to our main discussion, the Iron Response regulatory element is the **binding site** for Iron Regulatory Protein IRP. Also, **the binding of the IRP is dependent on the amount of iron.** *Binding on the 3' end is stimulatory for translation and not binding on the 5' end is stimulatory and vice versa!*

Low amount of Iron:

Binding of IRP on the IRE found on the 3' UTR —> stabilizing mRNA —> increase in translation —> increase the amount of that product.

Notice that because the amount of iron is low, the IRP was able to bound to the IRE at the 3' stimulating the translation of TfR1 (transport of iron inside the cell) and DMT1(increase iron absorption), and that does make sense as we need these products to facilitate increasing the amount of iron.



Binding of IRP on the IRE found on the 5' UTR —> inhibit the translation of ALAS and Ferritin.

ALAS and ferritin are used for iron metabolism and storage, these two processes take place when there is a sufficient quantity of iron, which is not the case here.

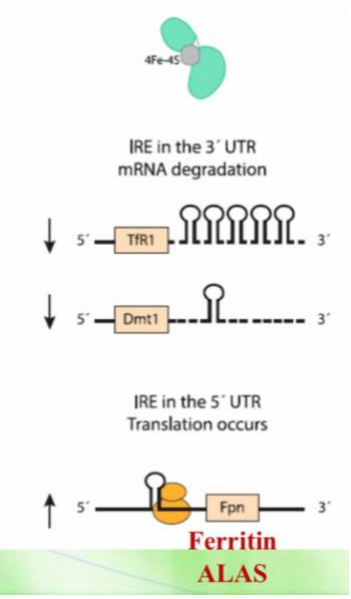
ALAS: Amino Levulinic. Acid Synthase

High levels of Iron: (upregulation for storage and inhibition of absorption)

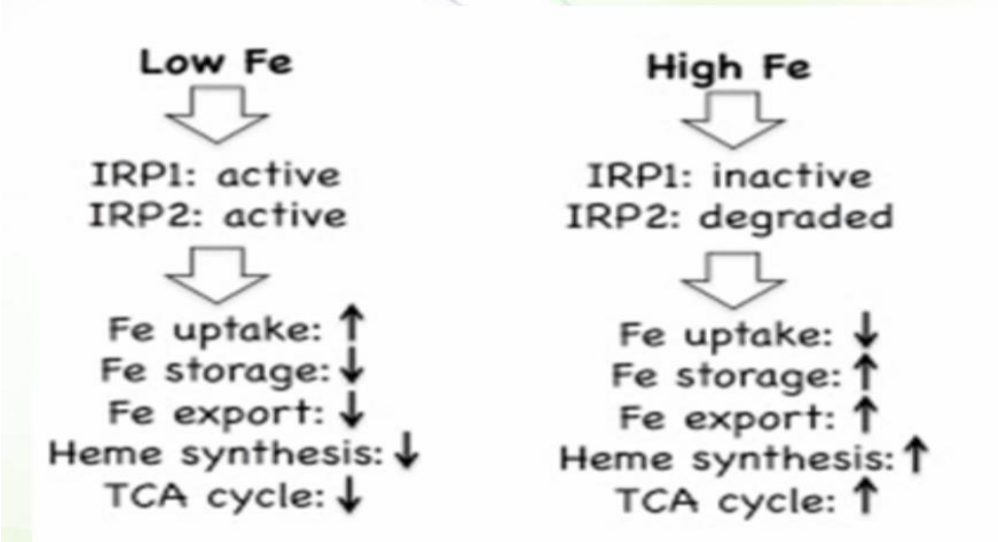
Iron will bind to the IRP and will remove it for the IREs.

Now that the IRP is removed from the 3’ end —> mRNA is unstable —> degradation of mRNA —> low amounts of TfR1 and DMT1 as there is no need to increase the amount of iron inside the cells, it is TOXIC!

Iron will also remove the IRP from the 5’ UTR —> the translation is resumed —> producing ferroportin, ferritin and ALAS, so that we can use the great amounts of iron in storage and metabolism.



So, here is a summary!



IRON-RELATED DISEASES

- **Hereditary hemochromatosis (HH)** increase of iron in the body
  - **Iron-deficiency anemia** low amounts of iron in the body leading to anemia

* HEREDITARY HEMOCHROMATOSIS (HH)

It is a group of disorders in iron metabolism that is characterized by excess iron absorption, saturation of iron-binding proteins and deposition of hemosiderin in the tissues.

- **more commonly in males than in females (why?)** of course due to the menstrual cycle

- **The primary cause of hemochromatosis is the inheritance of an autosomal recessive** (mutation on both chromosomes) **allele designated as HFE (type I or primary HH) , but four other genes that regulate the hepcidin–ferroportin axis can also be involved.**

When large amounts of iron are deposited inside cells, that will cause denaturation of proteins, the denatured proteins aggregate together forming the unsoluble hemosiderin.

GROUPS/CLASSES OF HEREDITARY HEMOCHROMATOSIS

- **Type 1 (hemochromatosis protein, HFE-dependent)**

  Most common

- **Type 2A (HJV-dependent)** remember the hemojuvelin protein that binds to the BMPR
- **Type 2B (hepcidin-dependent)** related to hepcidin production and release
- **Type 3 (TfR2-dependent)**
- **Type 4 (ferroportin-dependent)** ferroportin that is responsible for releasing iron from intestinal cells and macrophages.

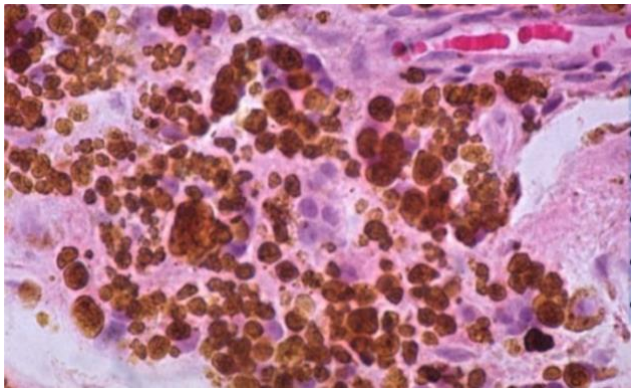
  Autosomal dominant disorder

HEMOSIDERIN

- **The normal total body iron stores may range from 2 to 6 gm, but persons with hemochromatosis have much greater stores exceeding 50 gm.**
- **If the capacity for storage of iron in ferritin is over-saturated, iron is stored as water- insoluble deposits known as hemosiderin, mainly in macrophages.**
- **Excess hemosiderin leads to cellular dysfunction and damage.**

"قَالَ كَمْ لَبِثْتُمْ فِي الْأَرْضِ عَدَدَ سِنِينَ (112) قَالُوا لَبِثْنَا يَوْمًا أَوْ بَعْضَ يَوْمٍ فَاسْأَلِ الْعَادِّينَ (113) قَالَ إِن لَّبِثْتُمْ إِلَّا قَلِيلًا ۖ لَّوْ أَنَّكُمْ كُنتُمْ تَعْلَمُونَ (114) أَفَحَسِبْتُمْ أَنَّمَا خَلَقْنَاكُمْ عَبَثًا وَأَنَّكُمْ إِلَيْنَا لَا تُرْجَعُونَ (115) فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۖ لَا إِلَٰهَ إِلَّا هُوَ رَبُّ الْعَرْشِ الْكَرِيمِ(116)" سورة المؤمنون

- **Affected organs and conditions**
  - **Liver (hepatic fibrosis)**
  - **Pancreas (diabetes mellitus)**
  - **Joints (arthropathy)**
  - **Skin (pigmentation)**
  - **Heart (cardiomyopathy)**
  - **Gonadotrophin-secreting cells (hypogonadotrophic hypogonadism)**

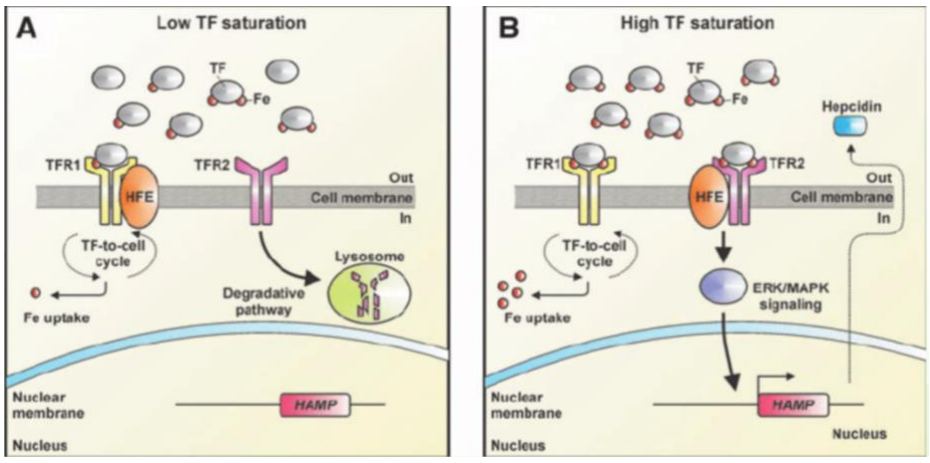


High amounts of iron that exceeds the binding capacity of ferritin so the proteins inside cells will be damaged and denatured exposing the hydrophobic regions causing clustering and aggregation forming hemosiderin.

Hemosiderin accumulation inside cells can further damage these cells and tissues.

REGULATION OF TRANSFERRIN RECEPTOR

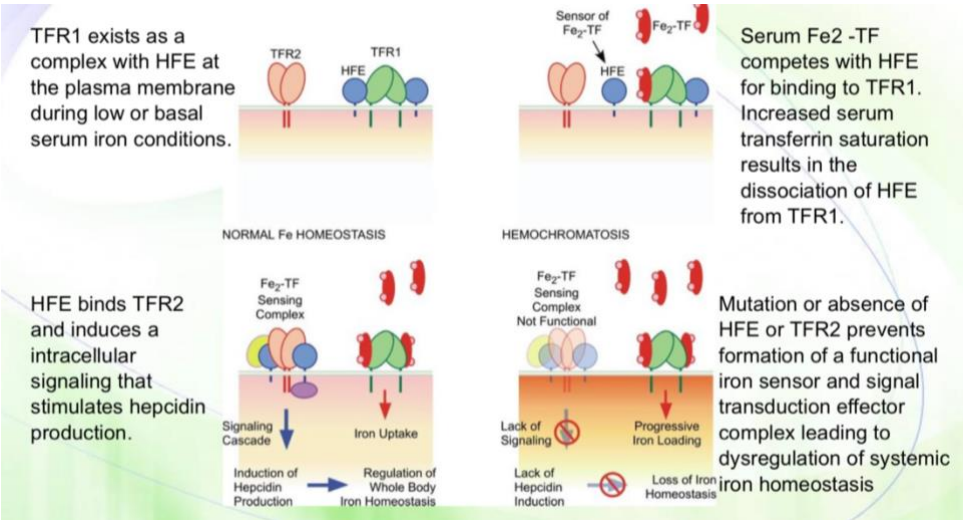
- **HFE is a major histocompatibility complex (MHC) class-1 gene.**
- **Normal HFE complexes with TfR1 reducing iron transfer into cells.**
- **Mutated HFE has a reduced presence on membrane and/or lack of interaction with Tfr1, leading to the loss of inhibition of transferrin receptor, and, therefore, increased iron uptake and storage.**



We previously mentioned the TfR1 and that is bound to HFE protein that dissociates from TfR1 as the holoprotein (transferrin + Fe) binds to TfR1, and that will cause the receptor-mediated endocytosis of TfR1.

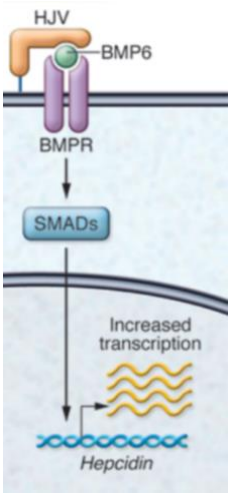
HFE binds to TfR2 (sensor), triggering it to send a signal to the inside of the cell to balance out the amounts of iron, and that will stimulate the production of **hepcidin!**

MECHANISM OF ACTION



JUVENILE HEMOCHROMATOSIS (teenagers and young)

- **Type 2A hereditary hemochromatosis**
- **AKA HFE2 (HJV)-dependent hereditary hemochromatosis**
- **Mutations in HJV gene, which encodes the protein "hemojuvelin", account for the majority of JH.**
- **Normal HJV upregulates expression of hepcidin.**
- **Type 2B is also juvenile hemochromatosis but is caused by mutations in hepcidin gene.**

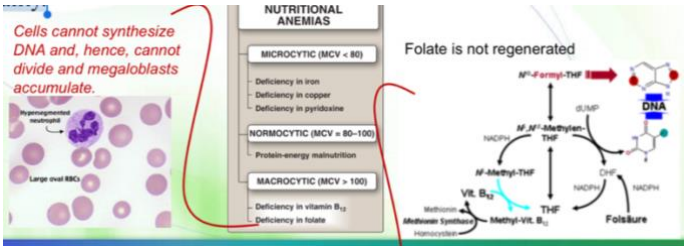


Notice that BMP6 (a protein that is released when there are high levels of iron) is associated with the BMPR, this receptor is dependent on the functional **hemojuvelin,** so when HJV is defective the receptor wont be functioning and the signal wont enter the cell. This will result in decreased transcription of hepcidin.

In type 2B, the signal is working in a good manner but the hepcidin that is produced is defective itself.

IRON-DEFICIENCY ANEMIA

- **Anemias are characterized by a deficiency in the number of mature erythrocytes in the circulation, lowering the oxygen-carrying capacity of the blood, causing tissue hypoxia, and clinical symptoms such as fatigue, weakness, increased cardiac output, as well as increased morbidity and mortality.**



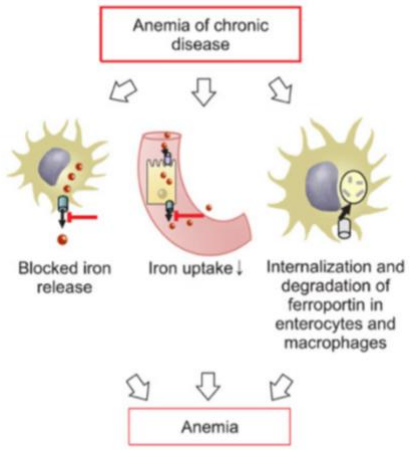
Deficiency in vitamin B12 or folate are common causes for anemia(IDA), in this case we will have macrocytic cells, megaloblasts, as they will not be able to divide.

Vitamin B12 and Folate are important for the production of nucleotides which is dependent on the presence of THF(tetrahydro folate) and the renewal of THF depends on vitamin B12.

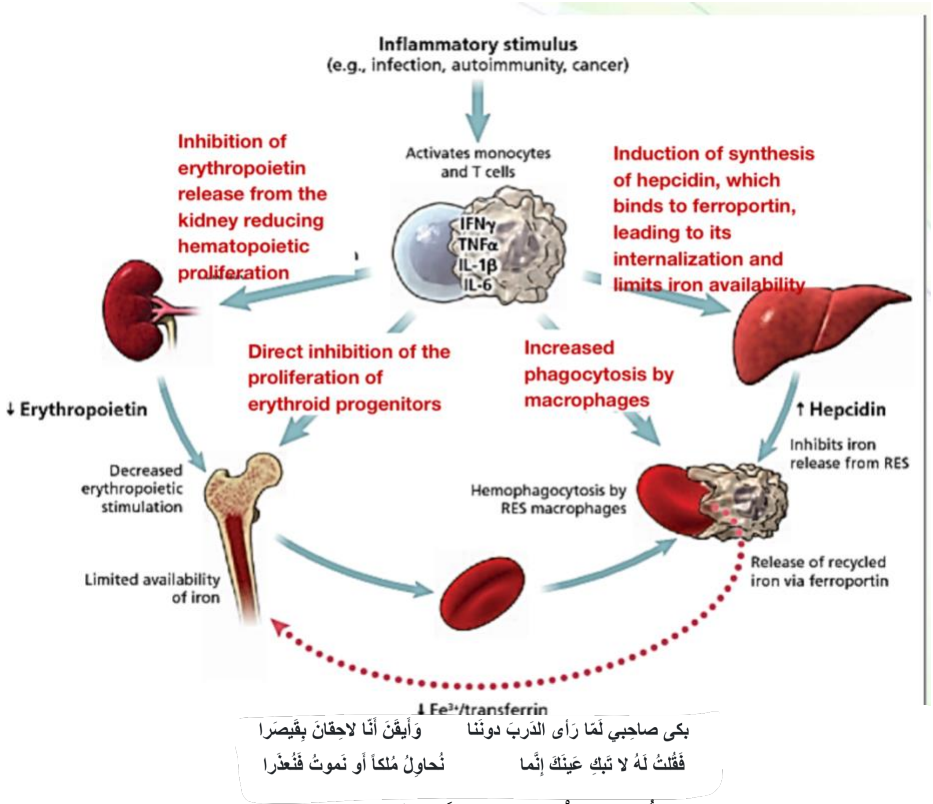
So, the deficiency in these vitamins will result in defective DNA synthesis.

ANEMIA OF CHRONIC DISEASE

- **Causes: chronic kidney disease, chronic infections and chronic inflammatory diseases**
- **Inflammatory cytokines—> increased hepcidin production by hepatocytes —>downregulation of ferroportin expression in major iron- exporting cells such as macrophages, duodenal enterocytes, and hepatocytes—> decreased enteric iron absorption and, perhaps more importantly, to increased iron retention within splenic macrophages and hepatocytes..**



ADDITIONAL MOLECULAR CONSEQUENCES OF CHRONIC INFLAMMATION



بكى صاحِبي لَمّا رَأى الدَربَ دونَنا وَأيقَنَ أنّا لاحِقانَ بِقَيصَرا

فَقُلتُ لَهُ لا تَبكِ عَينَكَ إنَّما نُحاوِلُ مُلكاً أو نَموتُ فَنُعذَرا

وَقُل رَّبِّ اغْفِرْ وَارْحَمْ وَأَنتَ خَيْرُ الرَّاحِمِينَ

دعواتكم لنا و لأخواننا في غزة و فلسطين و للمؤمنين أجمعين

Page 12 , juvenile hemochromatosis , type 2B instead of type 2A