Metabolism of iron

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Edited by Dr. Motamed Qadan
Hematopoietic-lymphatic system

Resources

- This lecture
- Yiannikourides and Latunde-Dada. A Short Review of Iron Metabolism and Pathophysiology of Iron Disorders. Medicines 2019, 6, 85. https://www.mdpi.com/2305-6320/6/3/85
- Lippincott's Biochemistry, 7th edition

- The Medical Biochemistry page, Iron and Copper Metabolism https://themedicalbiochemistrypage.org/iron-and-copper-homeostasis/
- Fleming and Ponka, Iron Overload in Human Disease, N Engl J Med 2012;366:348-59, https://www.nejm.org/doi/full/10.1056/nejmra1004967
- Brissot and Loréal, Iron metabolism and related genetic diseases: A cleared land, keeping mysteries, Journal of Hepatology 2016 vol. 64 j 505–515, https://www.sciencedirect.com/science/article/pii/S0168827815007424?via%3Dihub

Importance of iron

- Within the body, iron exists in two oxidation states: ferrous (Fe^{2+}), or the ferric (Fe^{3+}) which binds to anions, water and peroxides forming insoluble compounds.
- It is also present in 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 when reacting with H_2O_2

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$$

  - Solution: iron is not free.

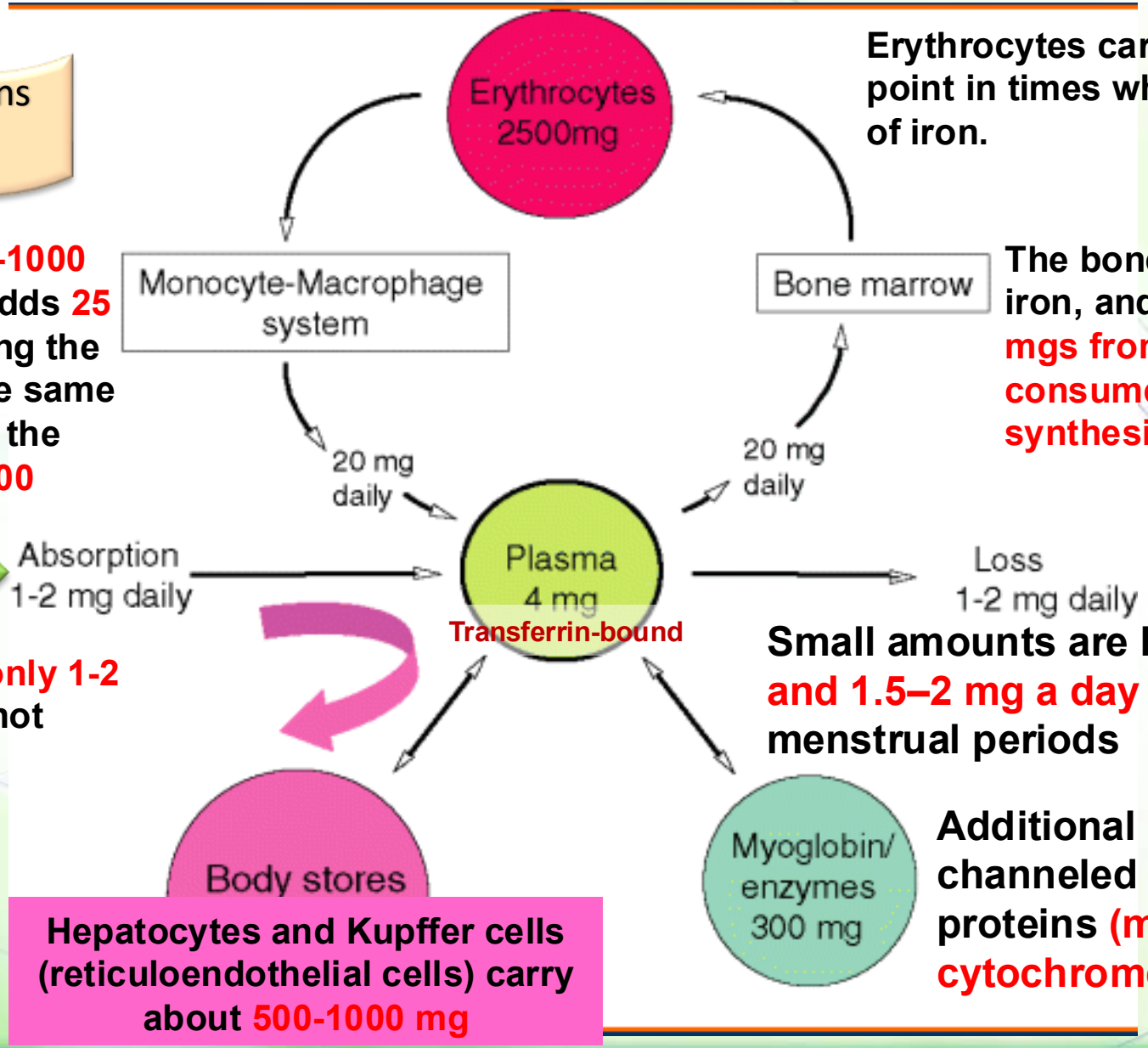
What is life cycle of iron in the body?

Well-nourished persons
3-4g

At any point in time macrophages carry 200-1000 mg of iron, of which it adds 25 mg daily from scavenging the RBCs and it releases the same amount (25 mg daily) to the bone marrow to make 200 Billion RBCs daily.

Start

18-20 mgs are consumed but only 1-2 mgs are absorbed, the rest is not absorbed.



Erythrocytes carry 2500 mg at any point in times which is about 70% of iron.

The bone marrow carries 150 mg of iron, and gets a daily amount of 25 mgs from macrophages which are consumed (the 25 mgs) to synthesize new RBCs.

Small amounts are lost 1 mg a day for men and 1.5–2 mg a day for women with regular menstrual periods

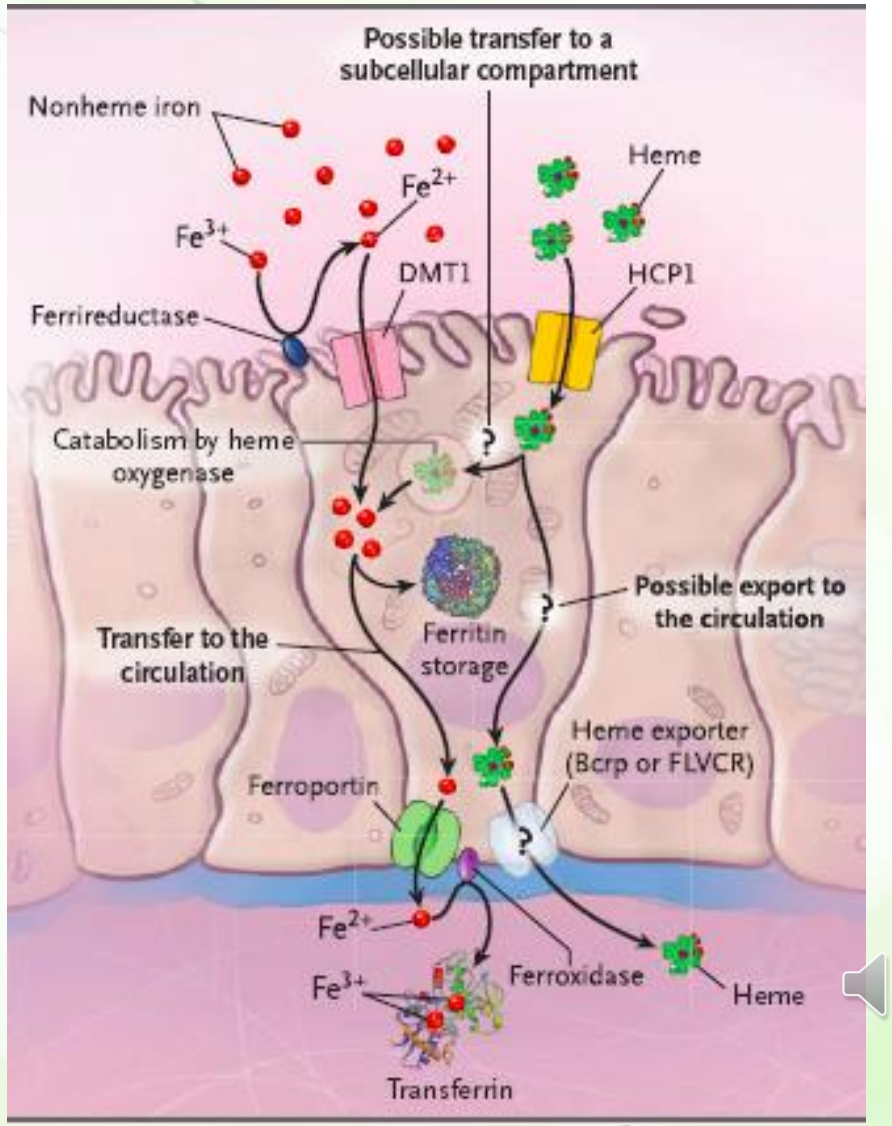
Additional iron (300 mg) is channeled to other cellular proteins (myoglobin and cytochromes).

Hepatocytes and Kupffer cells (reticuloendothelial cells) carry about 500-1000 mg

Iron absorption

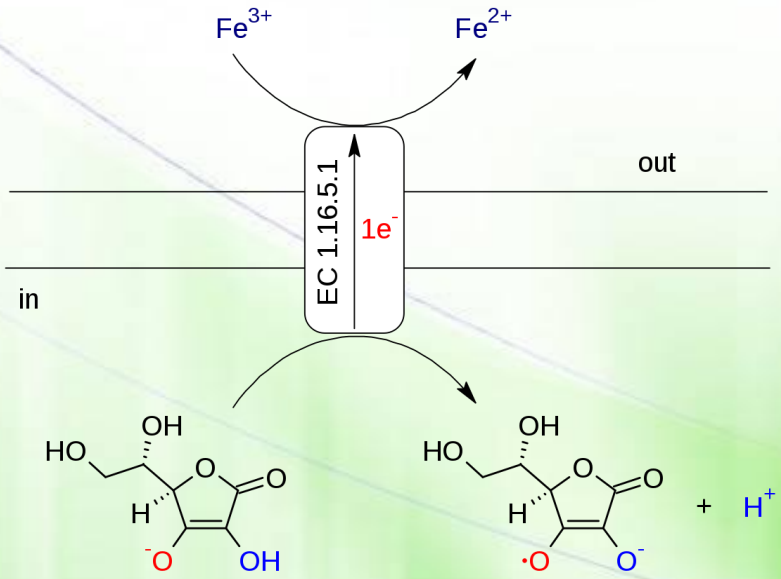
State of iron

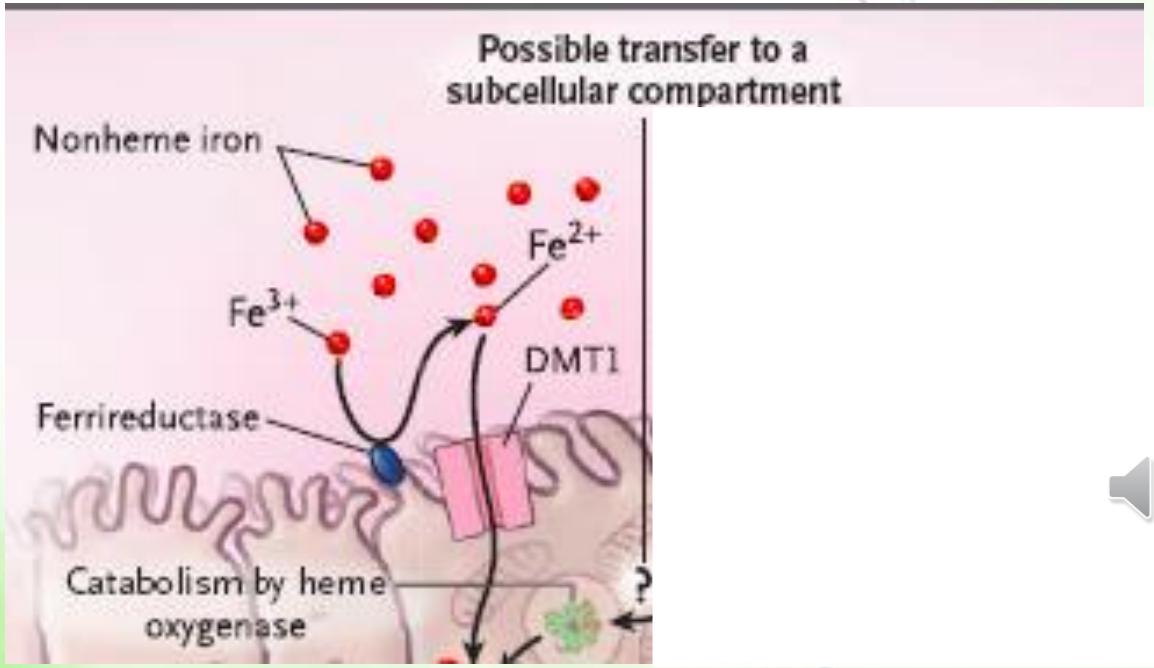
- Under conditions of neutral or alkaline pH, iron is found in the ferric Fe^{3+} state and, at acidic pH, in the ferrous Fe^{2+} state.
  - In the stomach, iron will be in the ferrous state.
  - In the duodenum, iron is in the ferric state.
- However, to be absorbed, dietary iron must be in its ferrous Fe^{2+} form.
- Plant based iron is in the Fe+3 state while animal based is in the Fe+2 state.
- Although plant iron is converted to Fe+2 in the stomach, it is still not as efficiently absorbed.



Site of absorption

- Ferrireductase enzyme on the enterocytes' brush border reduces Fe^{3+} to Fe^{2+} in a vitamin C-dependent reaction, where Vitamin C supplies the electron.
- 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.

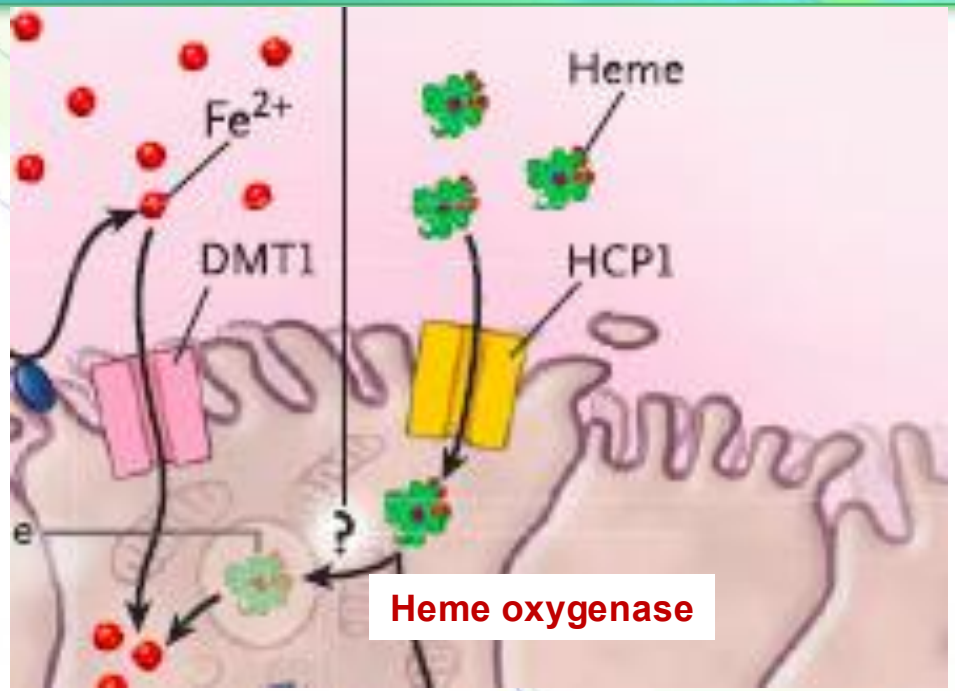


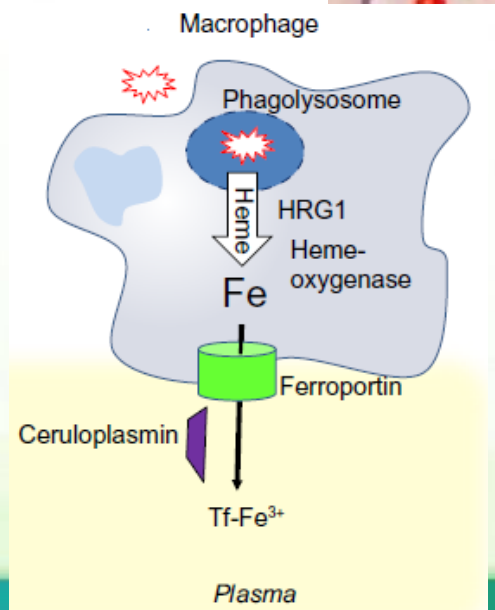


Heme as a source of iron

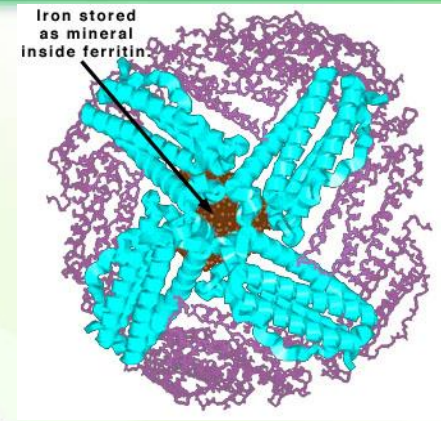
- Iron can also be obtained from ingested heme.
- Heme is absorbed by a receptor called heme-carrier protein 1 (HCP-1) and iron is released by heme oxygenase-1 (HO-1).
- In other cells such as macrophages, heme oxygenase also extracts iron from heme.

Proton pump-inhibiting drugs such as omeprazole greatly reduce iron absorption.



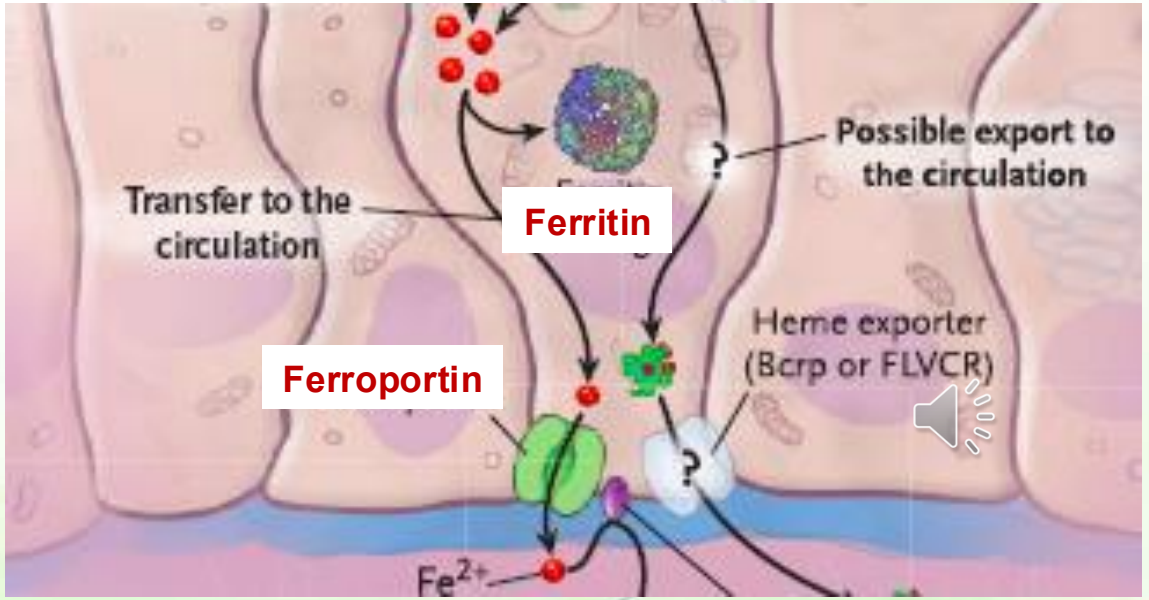


Fates of iron



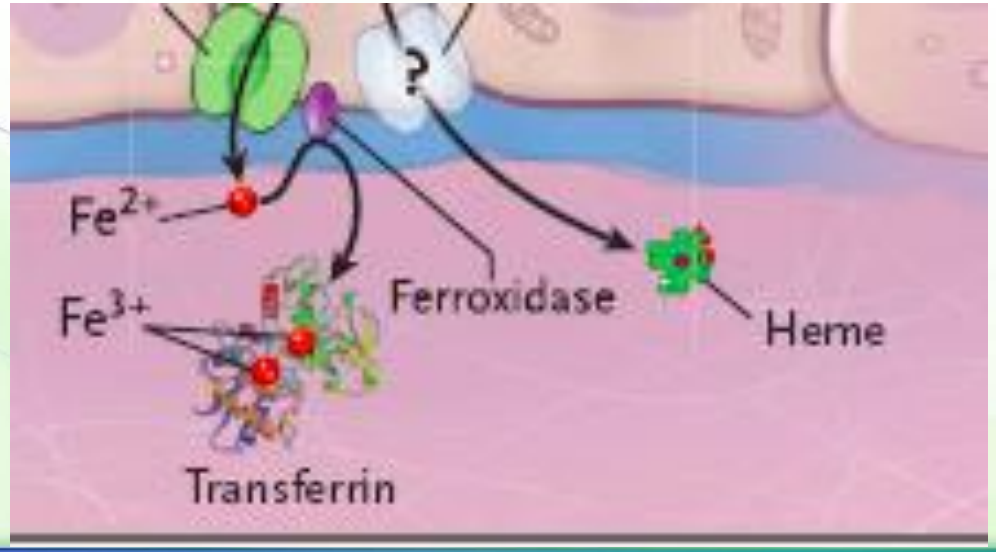
Fate 1: storage

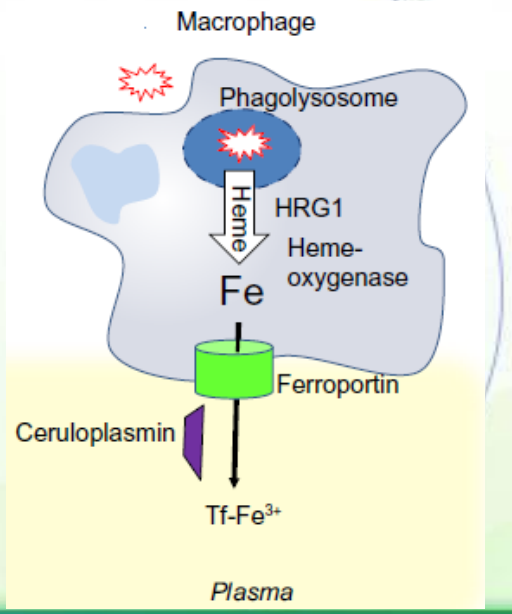
- Cells can store iron as ferritin.
  - Each Ferritin complex can store about 4500 iron ions. Ferritin is present in all storage locations including liver cells, macrophages..
- But, if cells are sloughed off from the tip of the villus into feces before absorption, iron is eliminated from the body.
- Fate 2: Transport
- Iron is transported out via a basolateral transporter known as ferroportin, which is distributed throughout the body on all cells.



Ferroxidase and transferrin

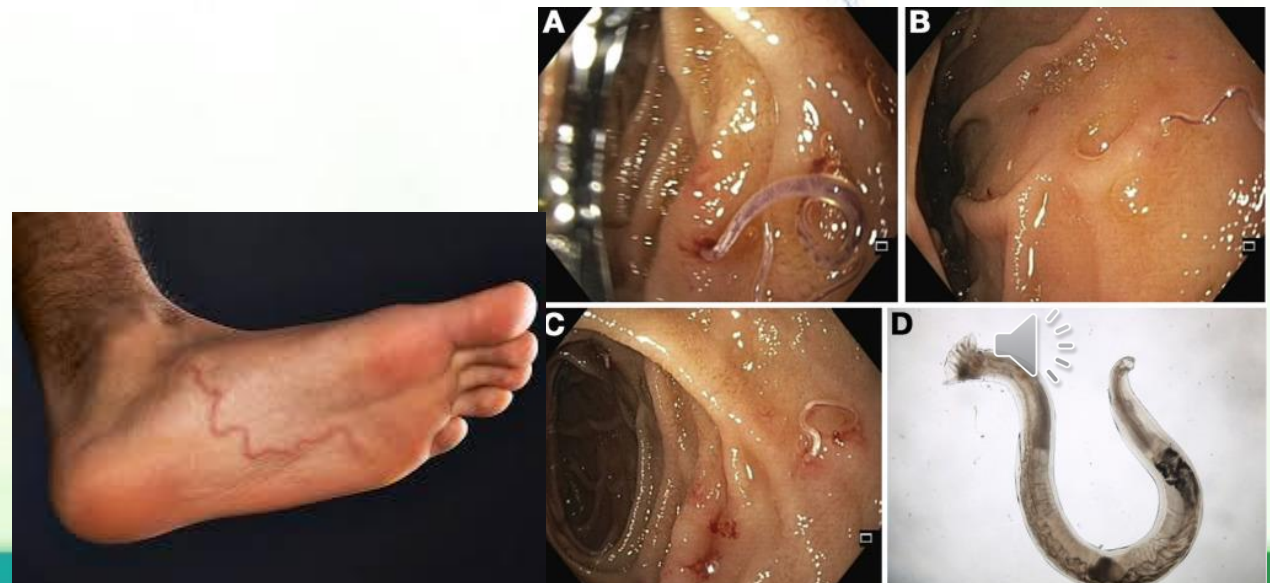
- Once iron leaves the intestinal cells, an iron oxidase, known as hephaestin or ferroxidase, converts iron from the ferrous state to the ferric state. If stored as Fe+2 it would be active and may participate in non desirable reactions, thus it is stored as Fe+3.
  - Nonintestinal cells use the plasma 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.





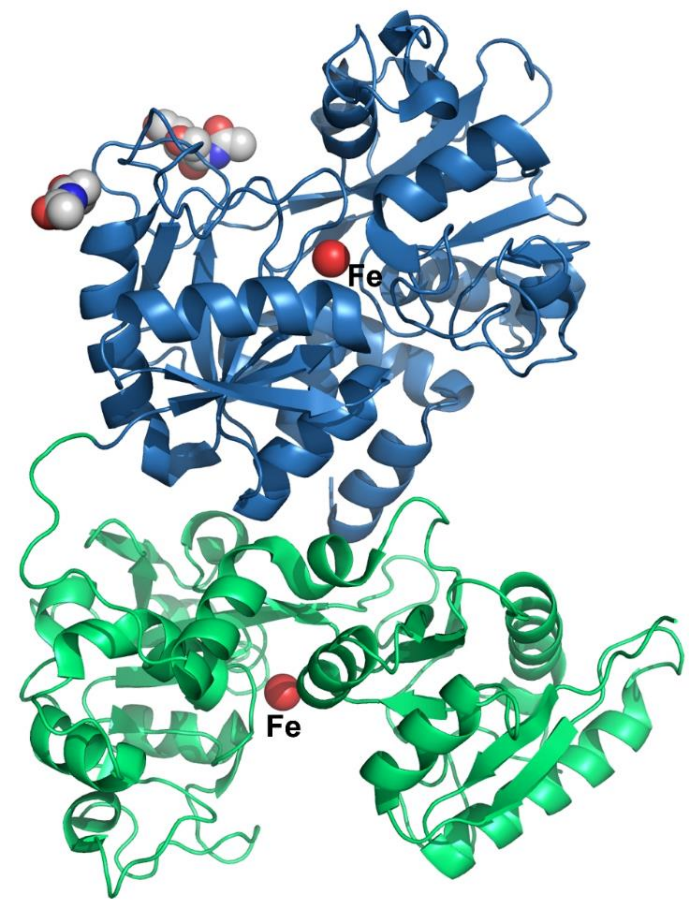
Intestine-related iron metabolism disorders

- Iron malabsorption
  - Gastrectomy (total or partial)
  - Celiac disease (villous atrophy)
  - Crohn’s disease
  - Helicobacter pylori
- Intestinal hemorrhage (gastrointestinal-mediated iron loss)
  - Gastric cancer
  - Ulcers
  - Inflammatory bowl disease
  - Hookworm infection



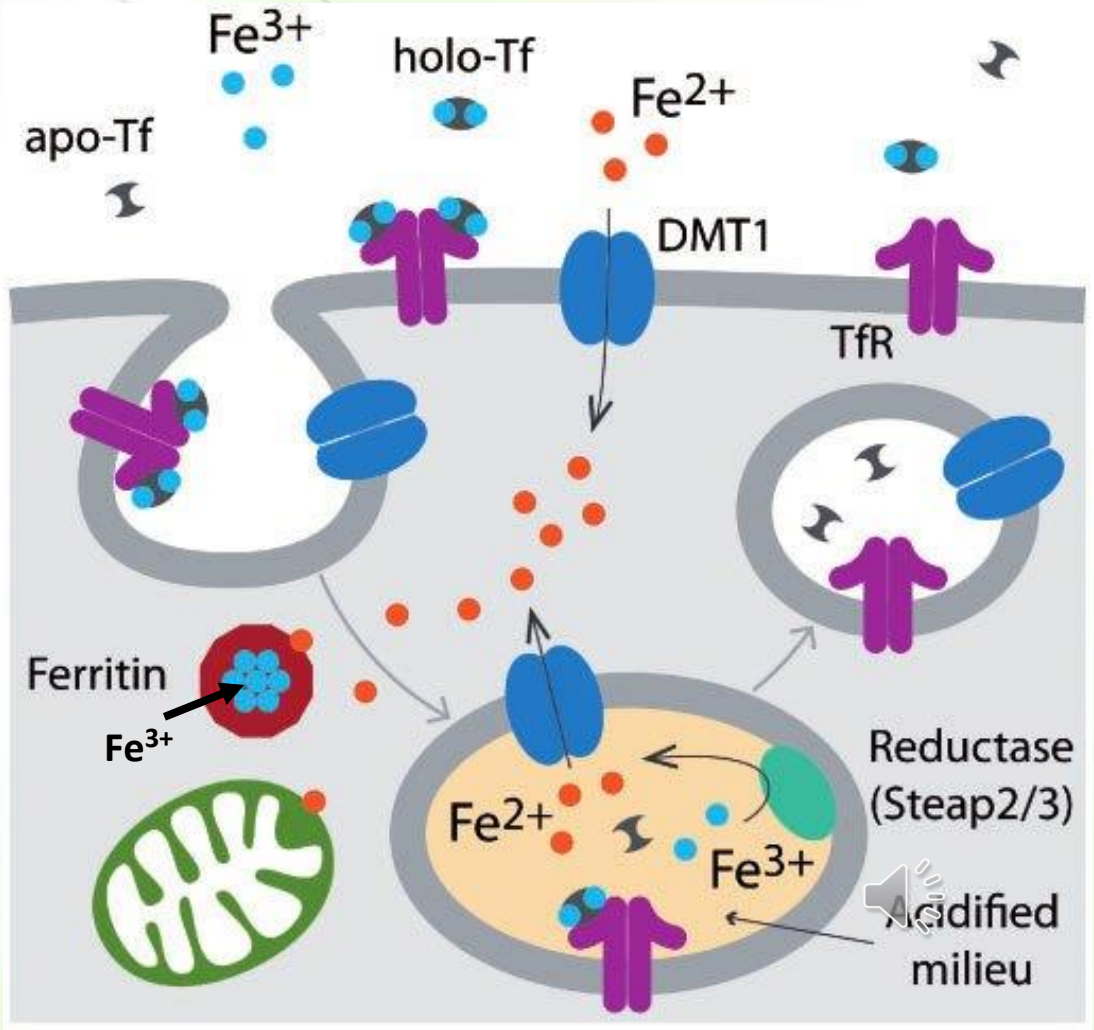
Properties of transferrin

- 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.*
- When iron exceeds normal levels, non-transferrin-bound iron (NTBI) appears.



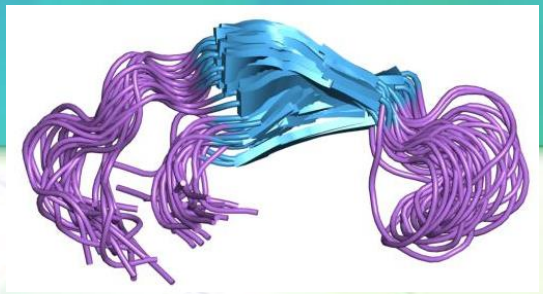
Receptor-mediated endocytosis

- Ferrotransferrin binds to a transferrin receptor (TfR) 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) where Fe^{3+} atoms dissociate, get reduced into Fe^{2+} by the ferrireductase STEAP3, and are transported into the cytosol via DMT1.
  - STEAP3 depends on vitamin C.
- The apotransferrin-transferrin receptor complex is recycled back to the surface, apotransferrin dissociates, and the receptor binds another transferrin.
- Affinity of TfR to iron: diferric Tf (Fe2Tf) >monoferric Tf (Fe1Tf) >apo-Tf

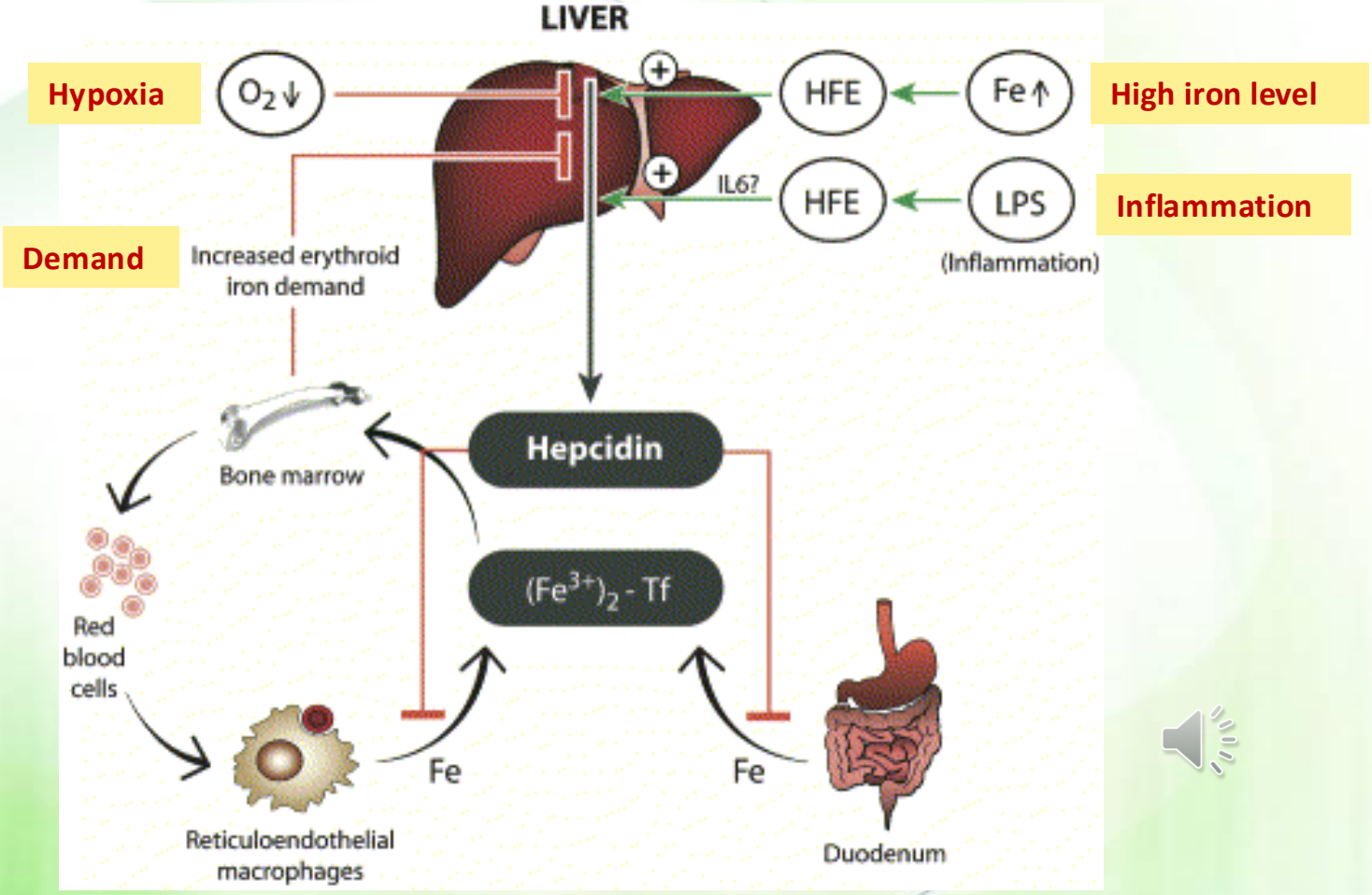


Regulation of iron in the body

Hepcidin (iron sensor)

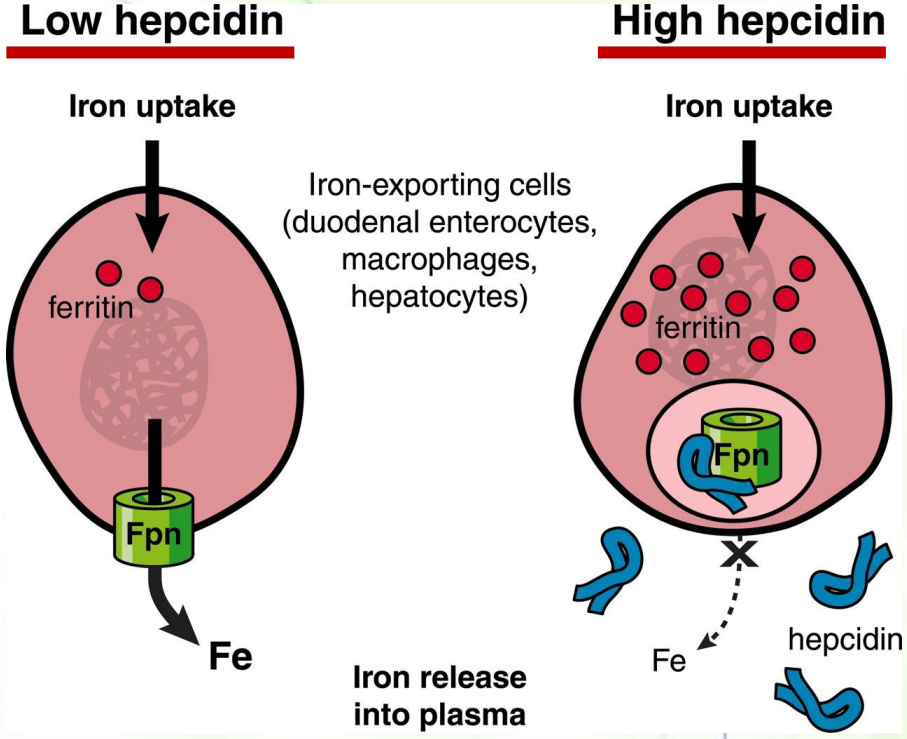


- Hepcidin is a peptide hormone (25 amino acids) secreted by the liver and it **reduces** iron levels.
- When iron level increases and in cases of inflammation, hepcidin secretion increases.
- When iron levels are low, there is high iron demand, or hypoxia, its release is suppressed.



How does hepcidin reduce iron levels in the body?

- Hepcidin binds to the basolateral iron transporter ferroportin inducing ferroportin internalization and degradation.
  - This results in higher iron storage.
    - Iron is eliminated in sloughed off intestinal cells.
    - Iron is not released from macrophages.
- Hepcidin also inhibits the presentation of the iron transporters (e.g. DMT1) in intestinal membranes decreasing iron absorption.

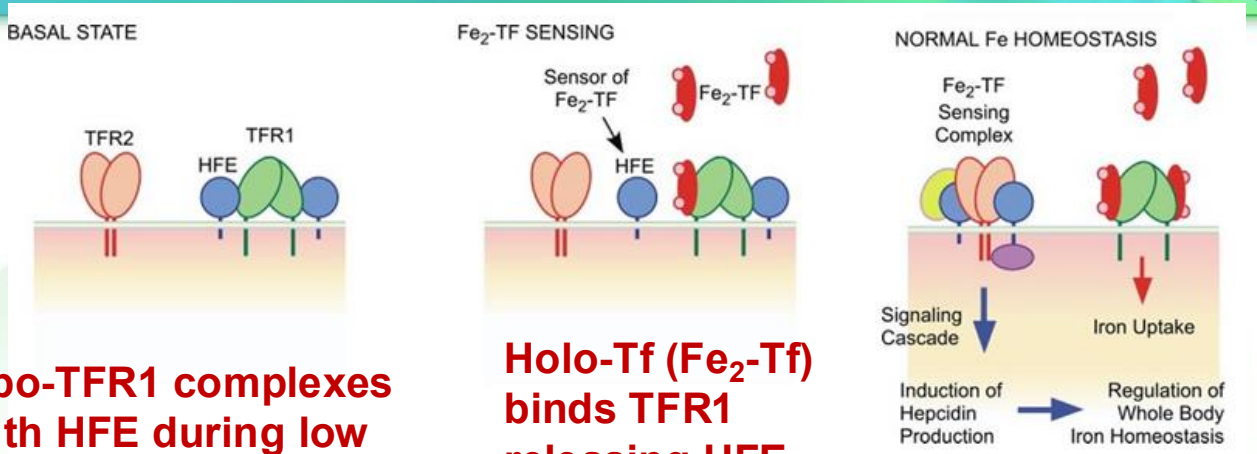


What happens when iron increases

What happens when iron increases

- Release of hepcidin through

1



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

Holo-Tf (Fe_2-Tf) binds TFR1 releasing HFE.

HFE binds to TFR2 sending a signaling cascade to increase hepcidin production

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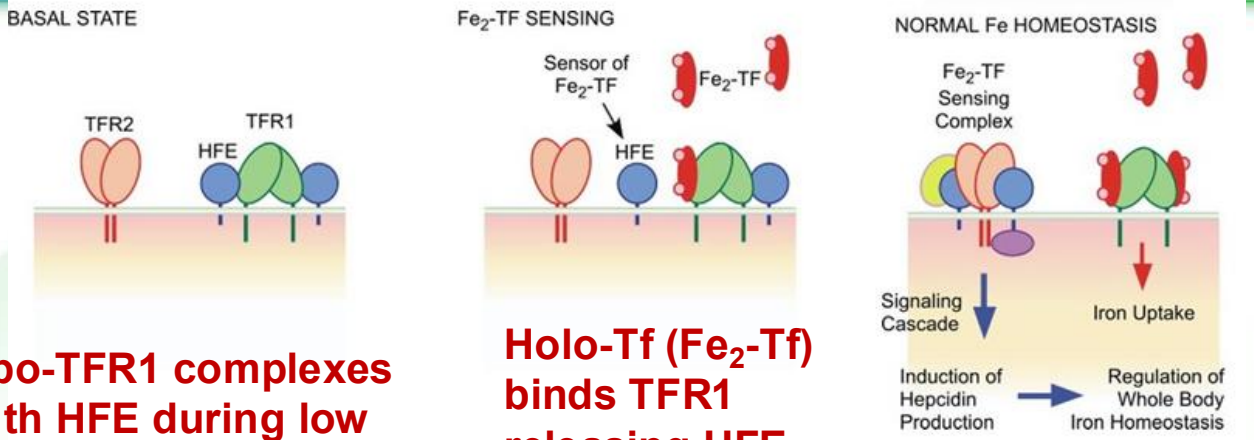

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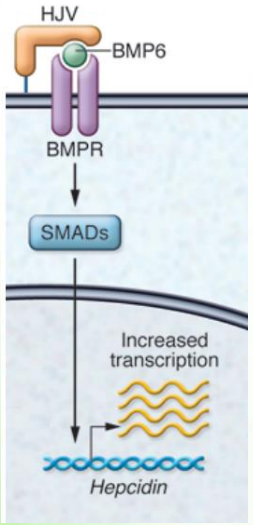

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High intracellular iron binds to bone morphogenic protein 6 (BMP6), which binds to its receptor (BMPR). BMPR is associated with Hemojuvelin (HJV), thus upon binding to BMP6, both proteins start a cascade of a signaling pathway inside the cell to upregulate hepcidin production.

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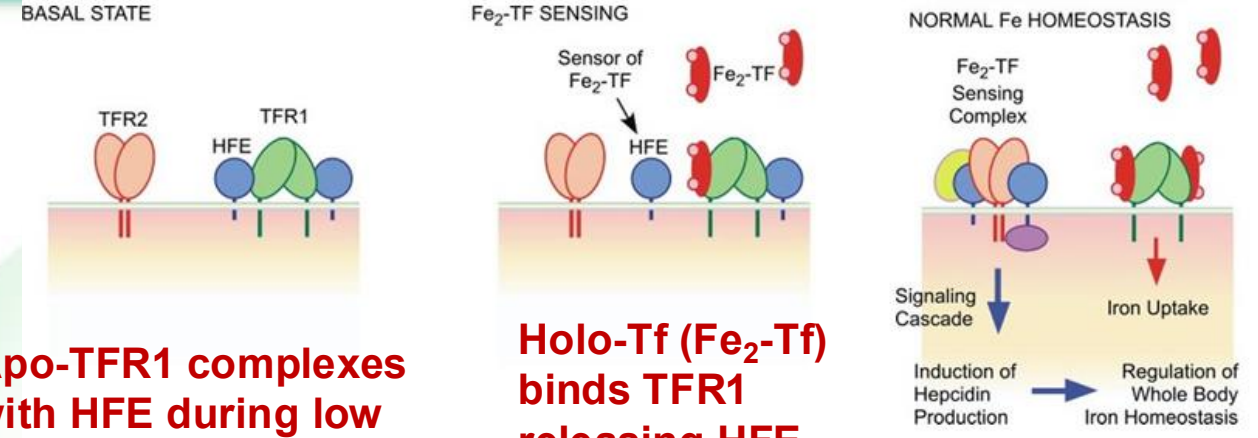

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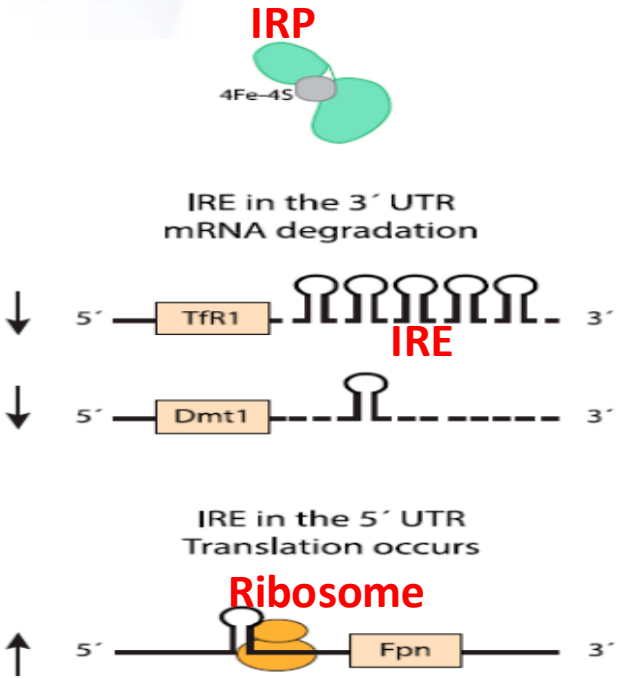

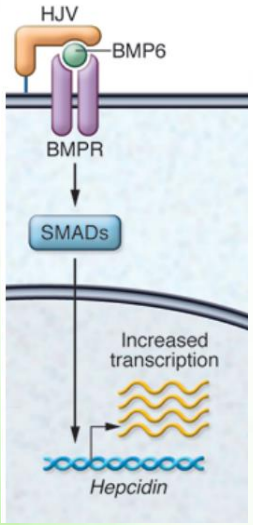

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At the transcriptional mRNA level:

A- In the 3 UTR of TfR1 and Dmt1, the binding of iron to IRP removes it from the IRE in both the 3 end and the 5 end. This causes TfR1 and DMT1 mRNA to become unstable and downregulated which reduces iron uptake.

B- In the 5 UTR region of Fpn the IRP is removed allowing the Ferroportin to be expressed probably to allow iron circulation. In case of too much iron, hepcidin downregulates ferroportin to prevent overload of iron in other tissues.

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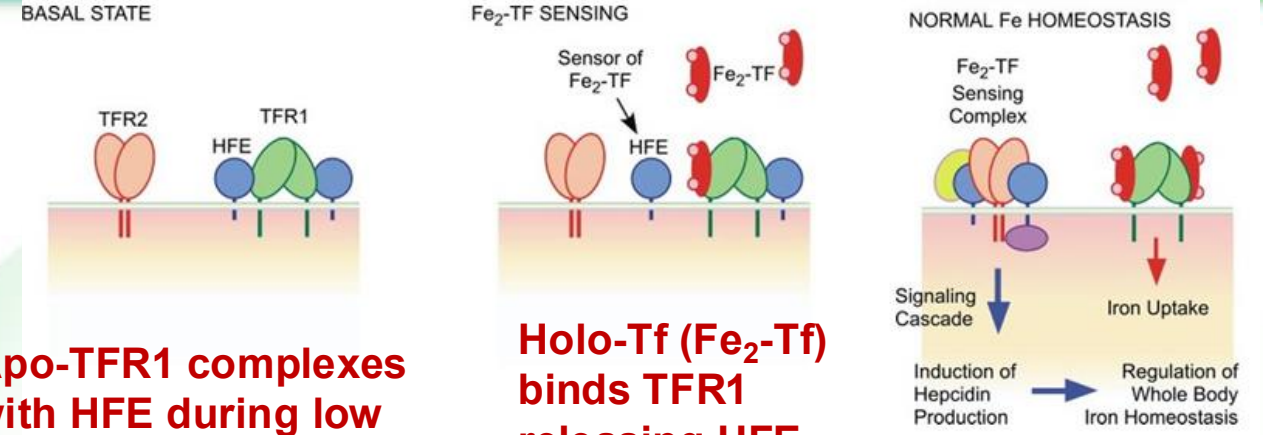

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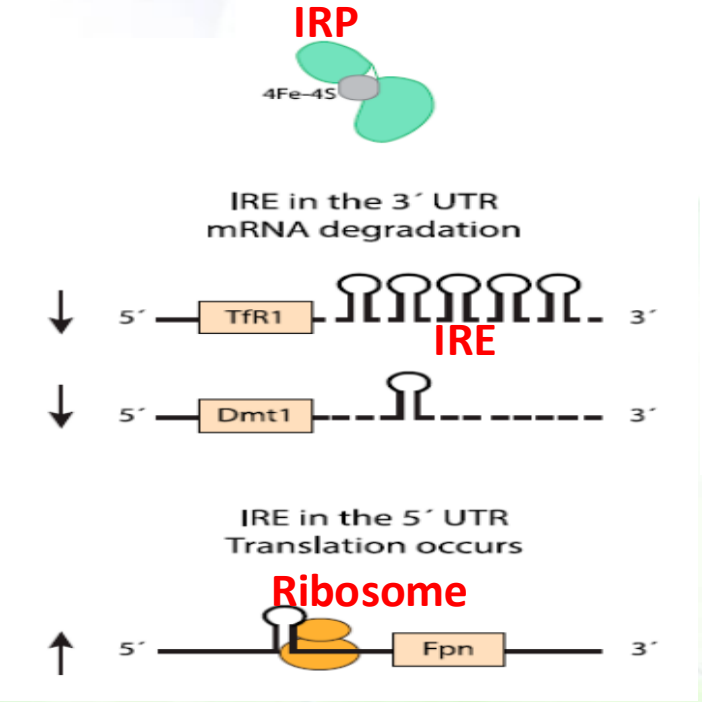
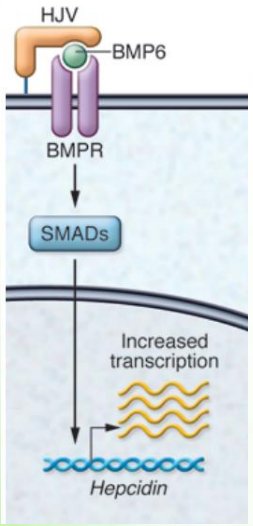


High Fe
IRP1: inactive
IRP2: degraded
Fe uptake: ↓
Fe storage: ↑
Fe export: ↑
Heme synthesis: ↑
TCA cycle: ↑



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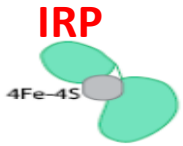
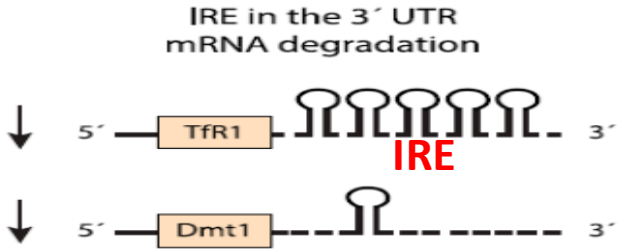
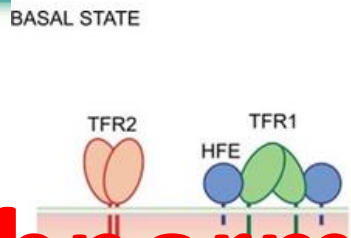
What happens when iron increases

Release of hepcidin through

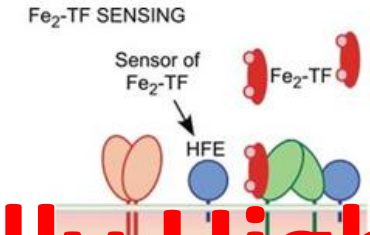
Normally High (1) Abnormally High







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



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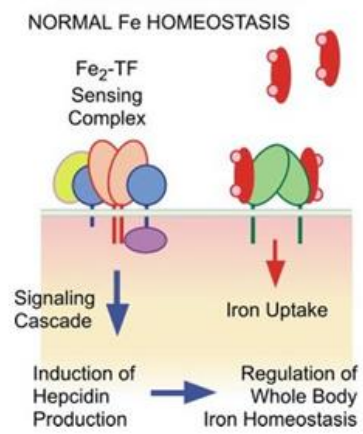
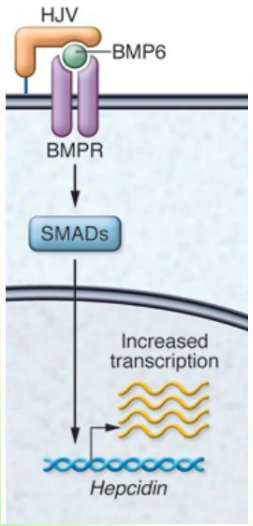


HFE binds to TFR2 sending a signaling cascade to increase hepcidin production



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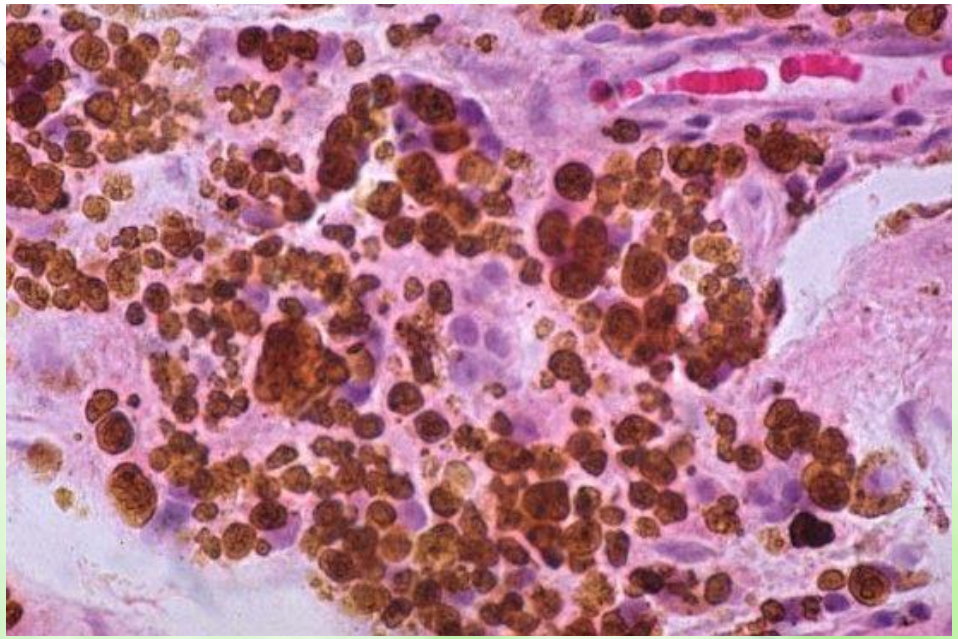
Hemosiderin

- 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.



Affected organs and conditions

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

Disease of high iron overload Hereditary hemochromatosis

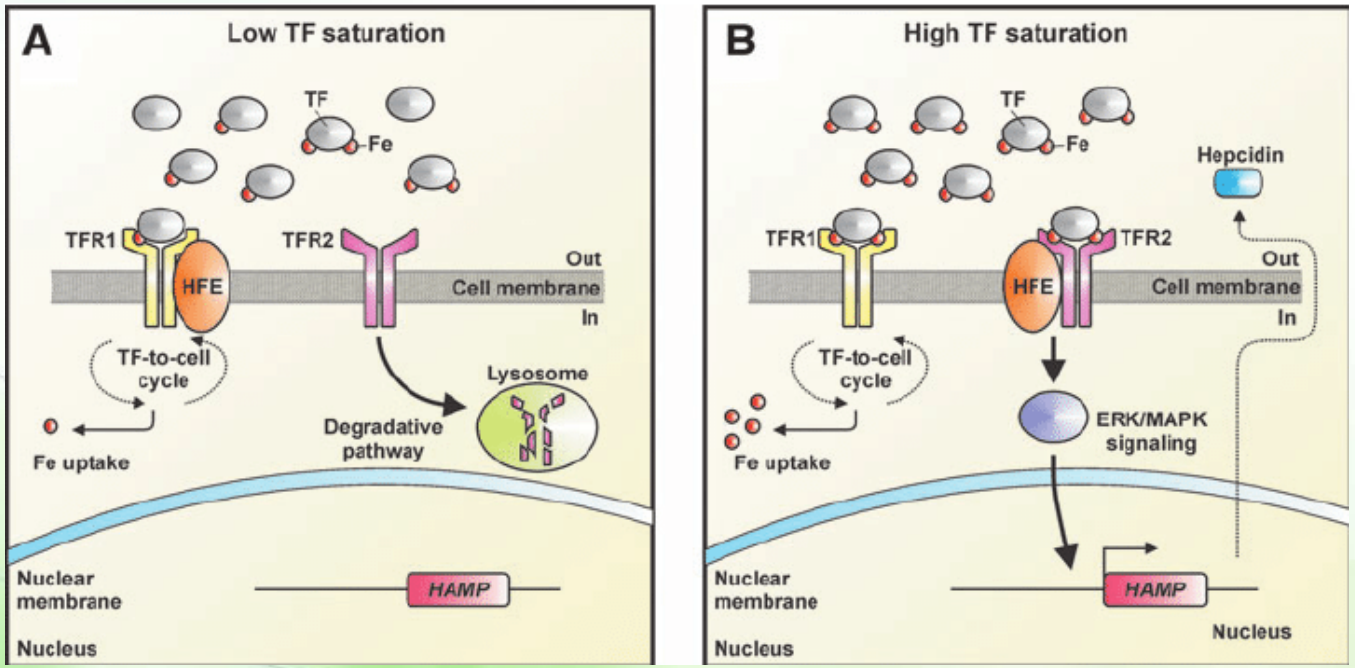
- It is a group of disorders in iron metabolism that is characterized by excess iron absorption, saturation of iron-binding proteins and deposition of hemosiderin in the tissues.
- The primary cause of hemochromatosis is the inheritance of an autosomal recessive allele designated as HFE (type I or primary HH) , but four other genes that regulate the hepcidin–ferroportin axis can also be involved.

Groups/classes of hereditary hemochromatosis

- Type 1 (hemochromatosis protein, HFE-dependent)
  - Most common
- Type 2A (HJV-dependent)
- Type 2B (hepcidin-dependent)
- Type 3 (TfR2-dependent)
- Type 4 (ferroportin-dependent)
  - Autosomal dominant disorder

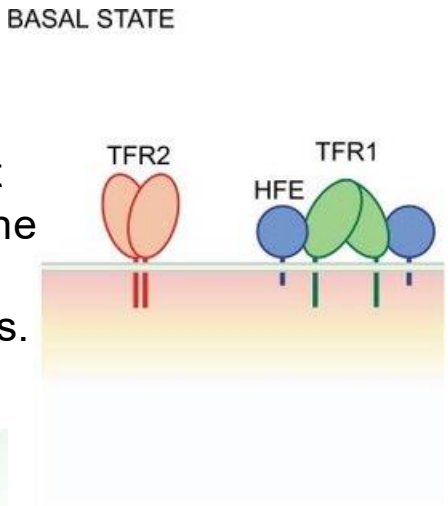
Regulation of transferrin receptor

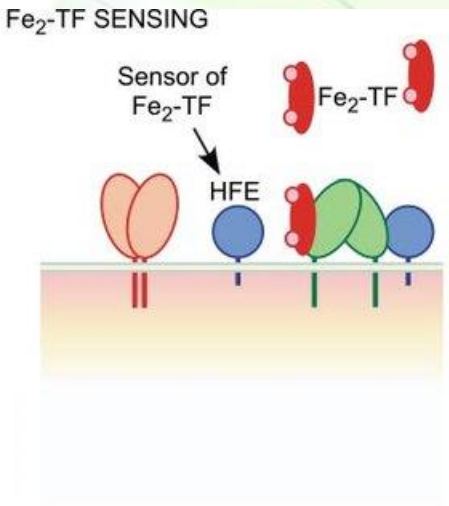
- HFE is a major histocompatibility complex (MHC) class-1 gene.
- Normal HFE complexes with TfR1 reducing iron transfer into cells.
- Mutated HFE has a reduced presence on membrane and/or lack of interaction with Tfr1, leading to the loss of inhibition of transferrin receptor, and, therefore, increased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



Mechanism of action

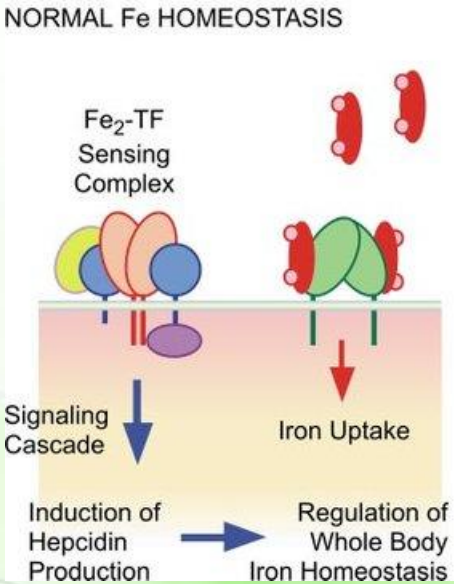
TFR1 exists as a complex with HFE at the plasma membrane during low or basal serum iron conditions.

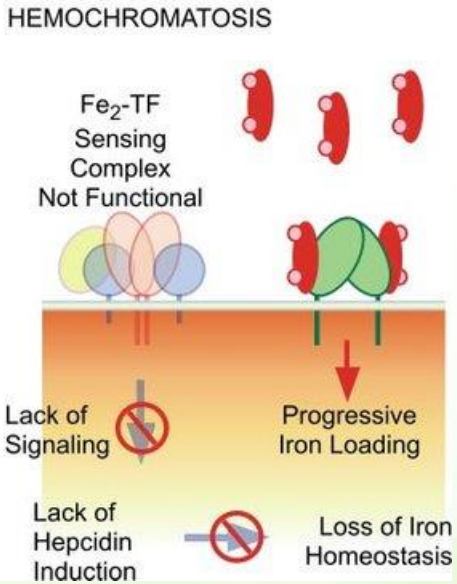




Serum Fe2 -TF competes with HFE for binding to TFR1. Increased serum transferrin saturation results in the dissociation of HFE from TFR1.

HFE binds TFR2 and induces a intracellular signaling that stimulates hepcidin production.



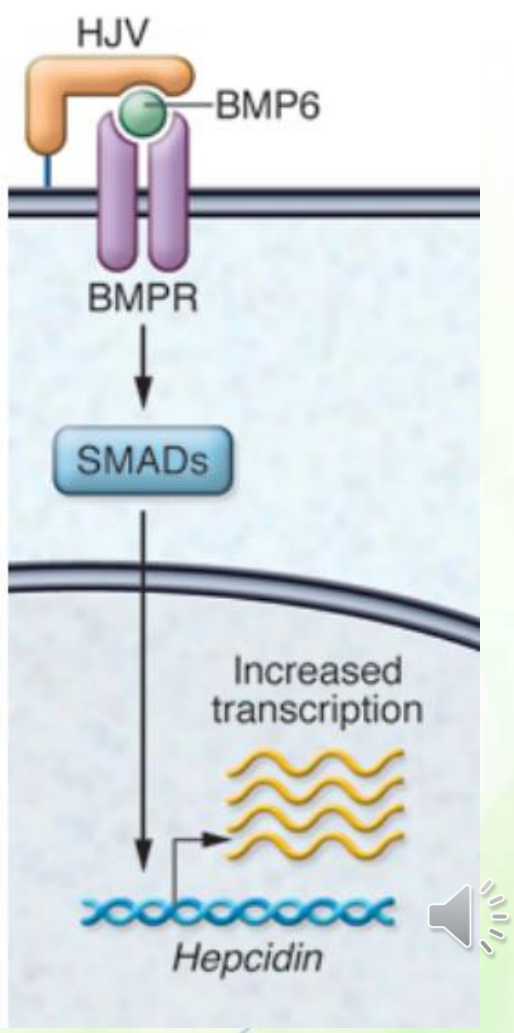


Mutation or absence of HFE or TFR2 prevents formation of a functional iron sensor and signal transduction effector complex leading to dysregulation of systemic iron homeostasis

Juvenile hemochromatosis

- 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.



What happens in case of low iron

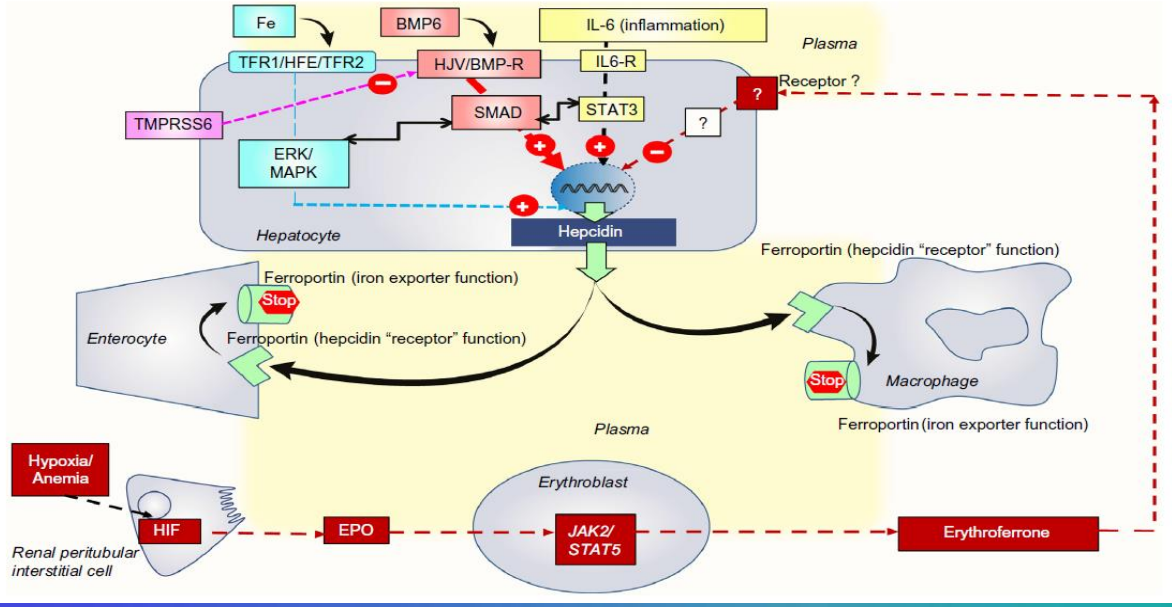
- 1. The expression of EPO (erythropoietin) by the kidney. EPO stimulates the synthesis of erythroferrone which is a protein produced by growing RBCs, thus inhibiting the synthesis of hepcidin.

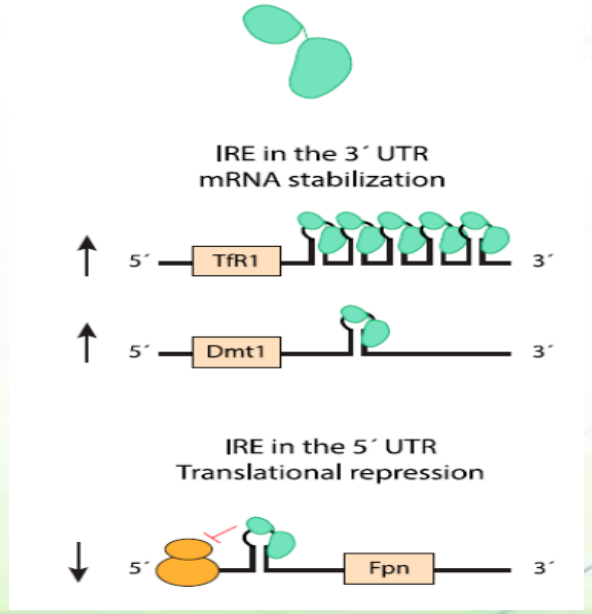
- 2. At transcriptional level there is a sequence in the DNA called Iron response element (IRE) that binds to iron response protein (IRP). The binding of this protein to IRE at the 3 UTR causes stabilization of the mRNA allowing it to be translated. Thus:
  A- Upregulation of TfR1 to increase iron absorption since it functions in engulfing TF-Fe+2 complex.
- B- Upregulation of DMT1 which functions in absorbing iron directly
- C- Downregulation of Ferroportin production to prevent loss of iron. The binding of IRP to the 5 UTR causes downregulation of the protein as it prevents its translation.

Low Fe

IRP1: active
IRP2: active

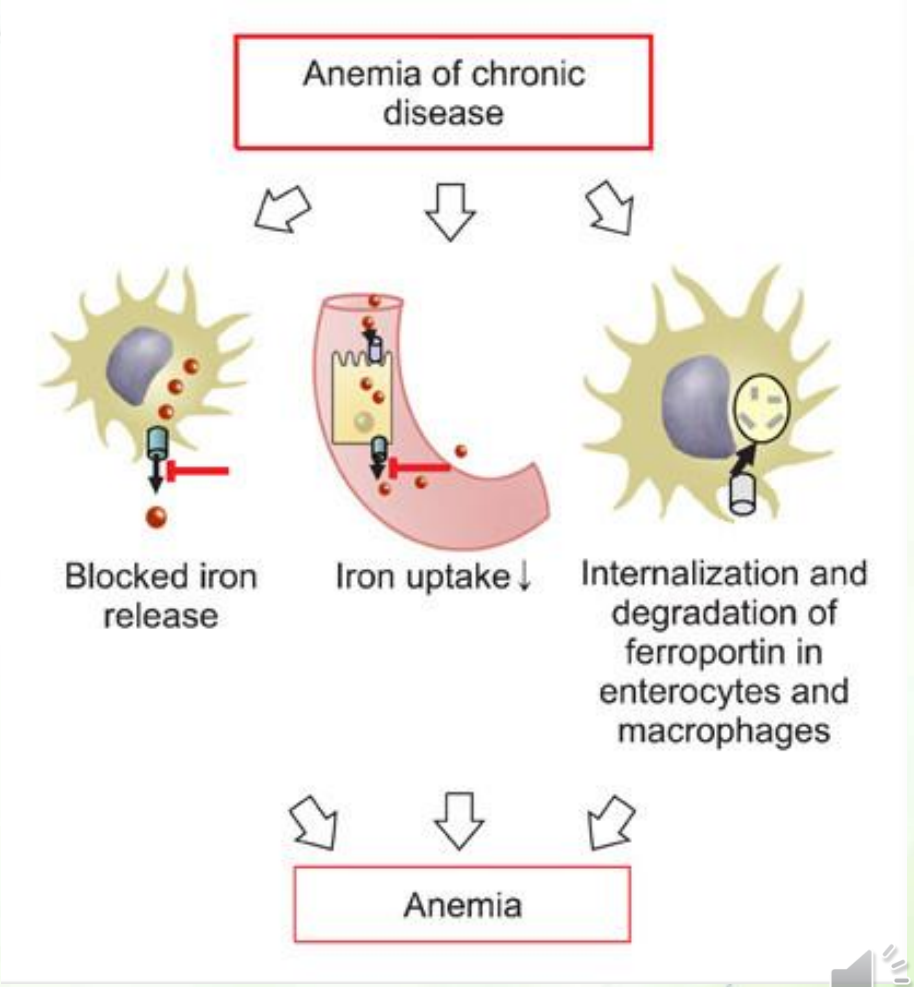
Fe uptake: ↑
Fe storage: ↓
Fe export: ↓
Heme synthesis: ↓
TCA cycle: ↓





What happens in case of chronic inflammation

- Inflammation causes the release **Inflammatory cytokines, including IL6, which iduces the expression of hepcidin.**
- 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 → Anemia

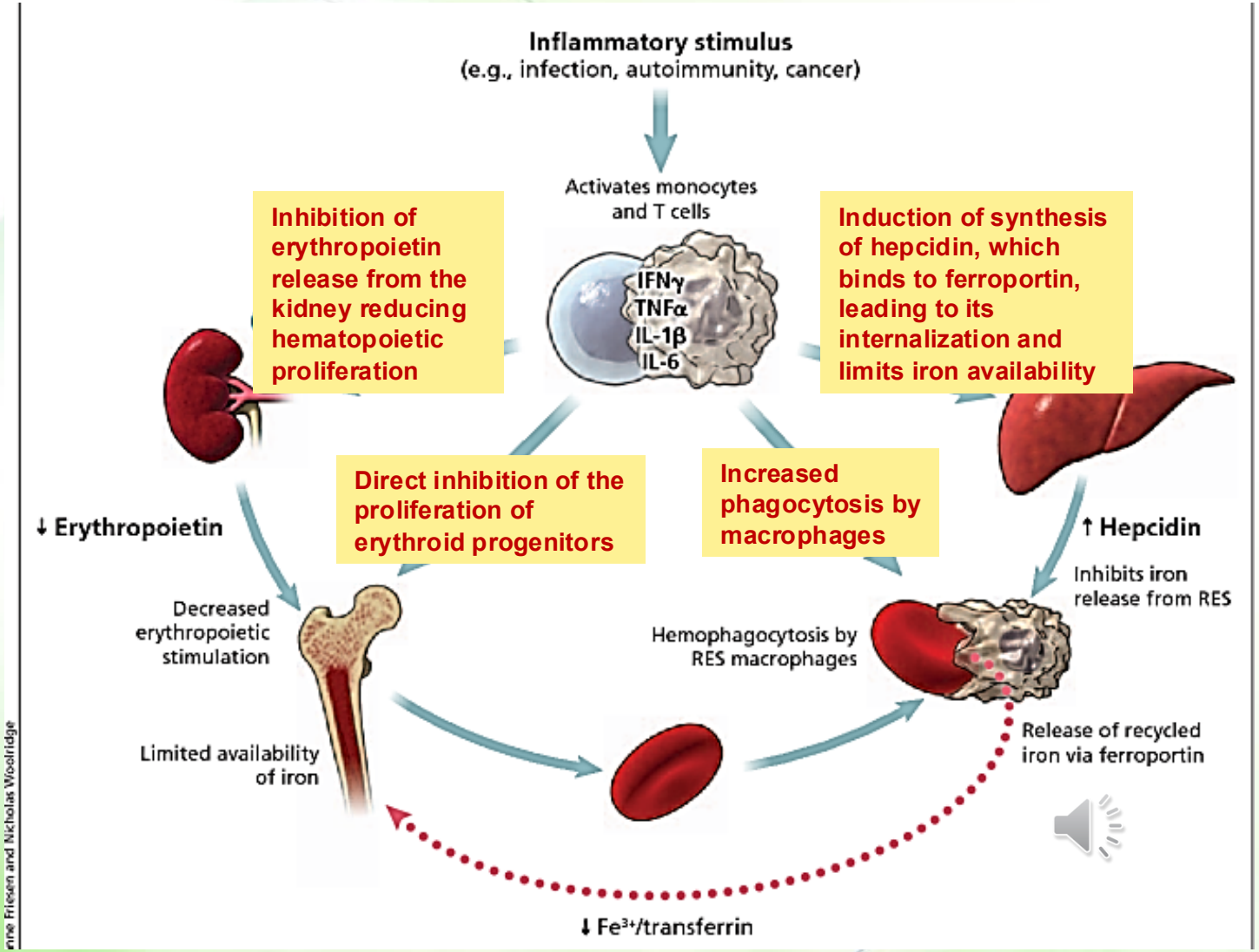


Additional molecular consequences of chronic inflammation

Besides IL6 release which causes the release of hepcidin leading to anemia, inflammation also:

1- decreases erythropoietin release from kidneys, which causes a decline of proliferation of erythroid progenitors.

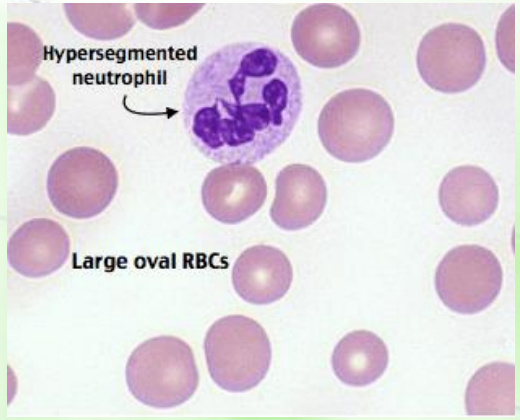
2- Increases phagocytosis of RBCs and reduces the release of the iron from the phagocytosed cells as Ferroportin is reduced.



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.

Cells cannot synthesize DNA and, hence, cannot divide and megaloblasts accumulate.



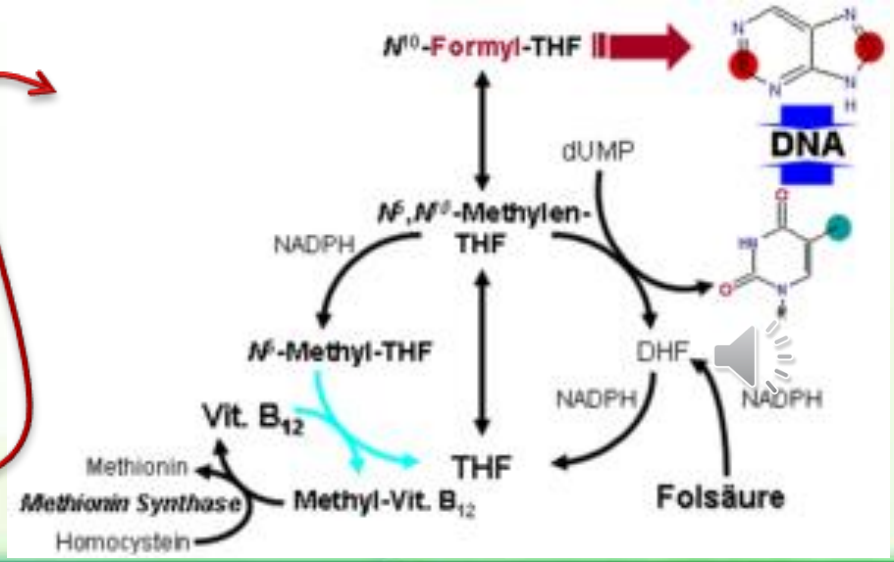
NUTRITIONAL ANEMIAS

MICROCYTIC (MCV < 80)
- Deficiency in iron
- Deficiency in copper
- Deficiency in pyridoxine

NORMOCYTIC (MCV = 80–100)
- Protein-energy malnutrition

MACROCYTIC (MCV > 100)
- Deficiency in vitamin B_{12}
- Deficiency in folate

Folate is not regenerated



Other issues associated with chronic inflammation

- Inflammatory cytokines → increased hepcidin production by hepatocytes → downregulation of ferroportin expression in major iron-exporting cells such as macrophages, duodenal enterocytes, and hepatocytes → perhaps more importantly, increased iron retention within splenic macrophages and hepatocytes→ Hemosiderin.

Important final notes

- Hepcidin is produced by the liver to control all the iron in the blood while each cell works on its own to get rid of iron.
- Where do each of the steps that control the blood iron take place?
- The major cells that control iron in the body are liver cells, intestinal cells and macrophages.
- Most cells produce ferritin and ferroportin DMT1 and TfR1 and TfR2 is present in the liver, spleen, macrophages and intestinal cells. BMP6 and HJV are present on the hepatocytes.
- Transferrin vs ferritin
- Hypogonadotropic hypogonadism
- The acidity of the stomach increases the solubility of the iron in both the Fe+2 and Fe+3 otherwise the become insoluble and it provides the environment for the iron to become Fe+2
- Proton pump-inhibiting drugs such as omeprazole greatly reduce iron absorption. Also, the increase the production of hepcidin.
- The 4 types of genetic diseases that cause hemochromatosis
- Plant iron vs animal iron