UNIT OVERVIEW: IRON HOMEOSTASIS, TOXICITY AND THE TREATMENT OF ANEMIA

Iron homeostasis (uptake, Tf, Ferritin, TfR)
Causes of iron deficiency (anemias)
Diagnosis of iron deficiency
Iron supplementation (drug interactions)
Iron overload (toxicity and mechanisms)

(Devlin, Chapter. 21)

Iron Homeostasis, reserves:

Total body Iron content: men 49, women 38 mg/kg
Serum Iron (Men: 75-150 ug/dL, women 60-140 ug/dL)

Major body reserves for iron are:
Blood (32 mg), Bone marrow (4 mg), liver (13 mg)

Breakdown of body iron:
28/26 mg/Kg hemoglobin
4/3 mg/Kg myoglobin
2/2 mg/Kg cytochromes, Fe/S enzymes
13/5 mg/Kg ferritin/transferrin

Thus ~60% of body iron is bound up in Hb.
Excretion of iron is typically very low (1-2 mg).

Iron Homeostasis, requirements:

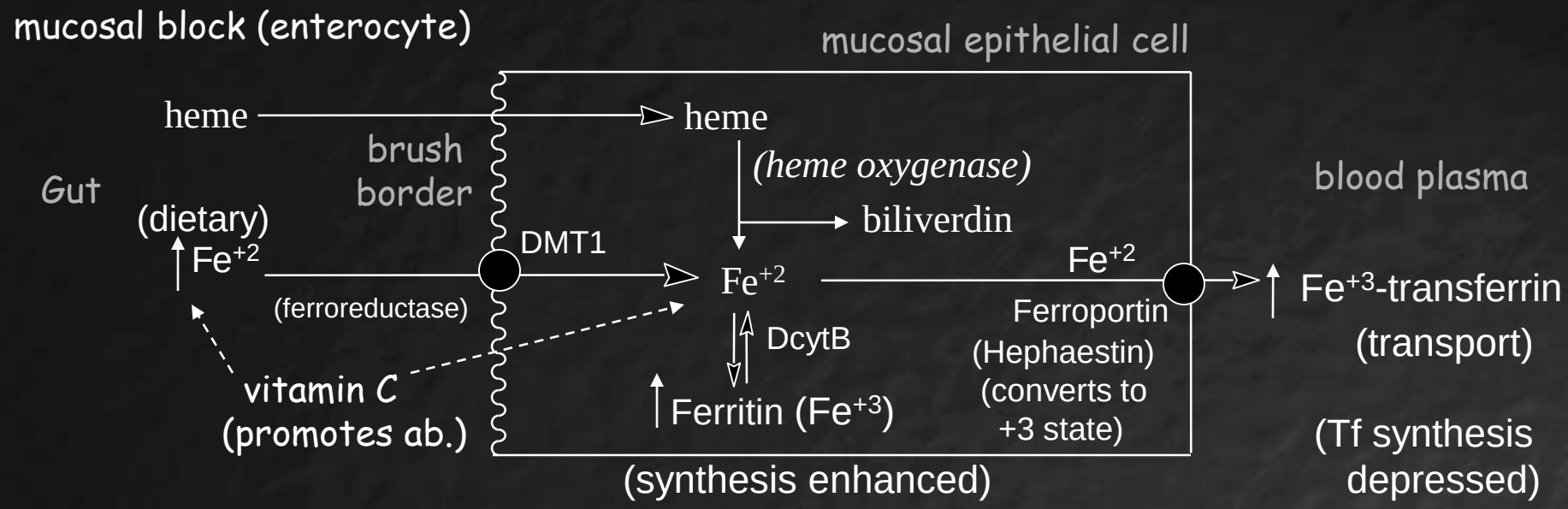
Daily iron requirements (normal):
1 mg/day men/postmenopausal women
2 mg/day menstruating women required
4-6 mg/day pregnant women

Reclaimed iron:
Catabolized red blood cells free up ~25 mg iron/day.

Free iron is toxic (Fenton reaction can produce OH*).

Thus iron needs to be bound to proteins to prevent cellular damage (Heintz bodies). Thus iron is bound to transferrin and ultimately ferritin.

Iron Homeostasis, absorption:



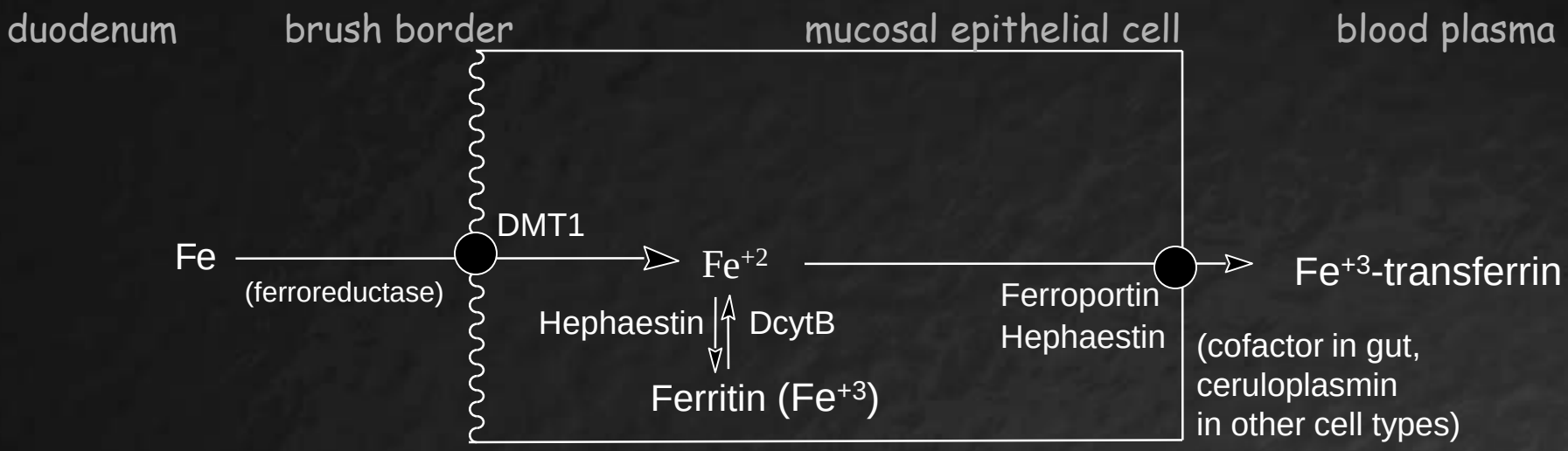
1. Iron / heme are released from food stuffs by proteolytic digestion in the gut. These are chelated by compounds that keep them soluble and available for absorption.

2. Mucosal uptake into intestinal epithelial occurs predominantly in the small intestine (proximal duodenum). Only about 10% of dietary iron is absorbed.

3. Iron enters the cell as either inorganic iron or heme. Heme is degraded in these absorptive cells by heme oxygenase, to release inorganic iron.

4. Iron is transported to tissues as inorganic iron bound to transferrin.

Iron Homeostasis, absorption:



Hepcidin:

- 25 aa peptide hormone produced in liver which regulates iron transport.

- An acute phase protein which acts by inhibiting ferroportin preventing the transport of iron out of the cell. This trapped iron is ultimately removed when cells are sloughed from the digestive tract.

Iron Homeostasis, absorption:

Conditions which increase iron absorption:

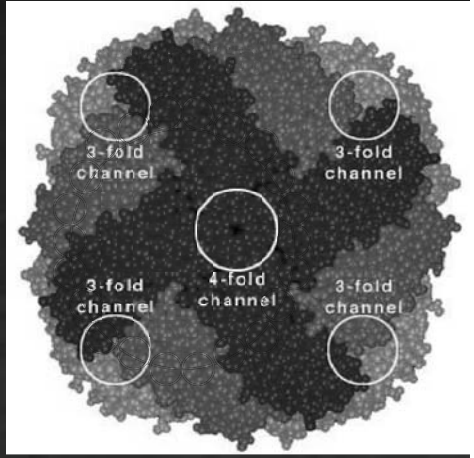
Low dietary iron
Low body iron stores *
Increased red cell production *
Low hemoglobin *
Low blood oxygen content *
* Result in decreased hepcidin production

Conditions which decreased iron absorption:

Systemic inflammation leading to increased hepcidin production

Iron proteins, ferritin:

- MW 450,000, primary role in iron storage (liver)
- 24 x 175 amino acids (18.5 kDa), holds 4500 $Fe(OH)_3$
- synthesized and secreted by liver
- protects the body from toxic effects of iron
-60% glycosylated (secreted form)



Normally ~20% iron loaded - there is therefore normally a low level of ferritin present in human plasma. Under conditions of iron overload, ferritin levels rise markedly. Iron released from ferritin by reduction and chelation. Ferritin internalized via ferritin receptor.

Iron incorporated into ferritin as $Fe^{2+} \longrightarrow Fe^{3+}$ferritin
(due to H chain ferroxidase activity)

Alcoholism: decreased transferrin-bound iron uptake. Increased ferritin receptors and increased hepatocyte iron overload.

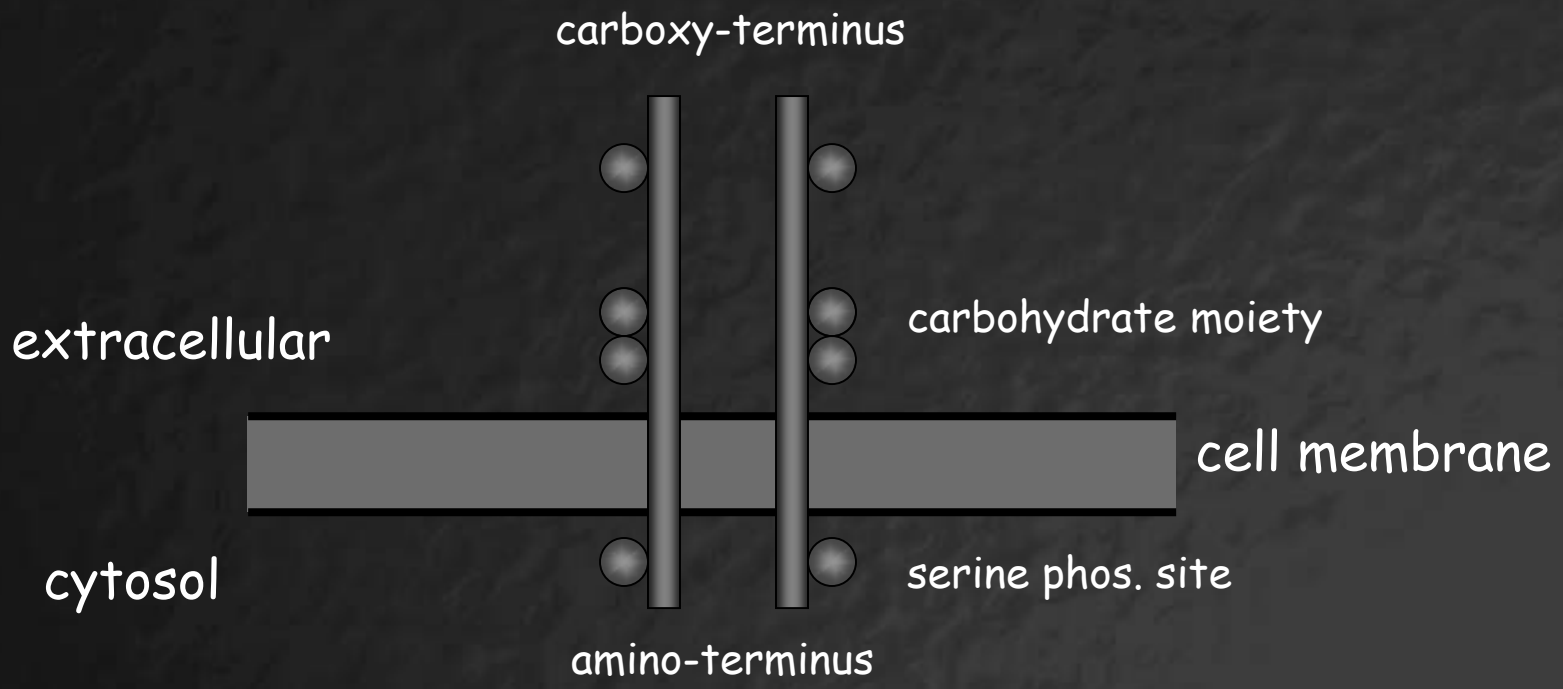
Iron proteins, transferrin:

- MW 76,000
- 679 amino acids (2 moles Fe^{+3}/mole Tf)
- glycoprotein synthesized and secreted by liver
- a plasma beta1-globulin
- 6% carbohydrate
- responsible for transporting ~25 mg Fe^{+3}/day (95% of iron present in blood plasma from catabolized RBC's.
- Transfers ~22 mg Fe/day to Hb. Typical saturation is 20-24% (33% overload)

-transferrin is taken up by hepatocytes, bone marrow, placenta via a transferrin receptor

Iron proteins, transferrin receptor:

Schematic diagram of transferrin receptor. Transferrin which is internalized at coated pits. The short N-terminal tails are critical for internalization.

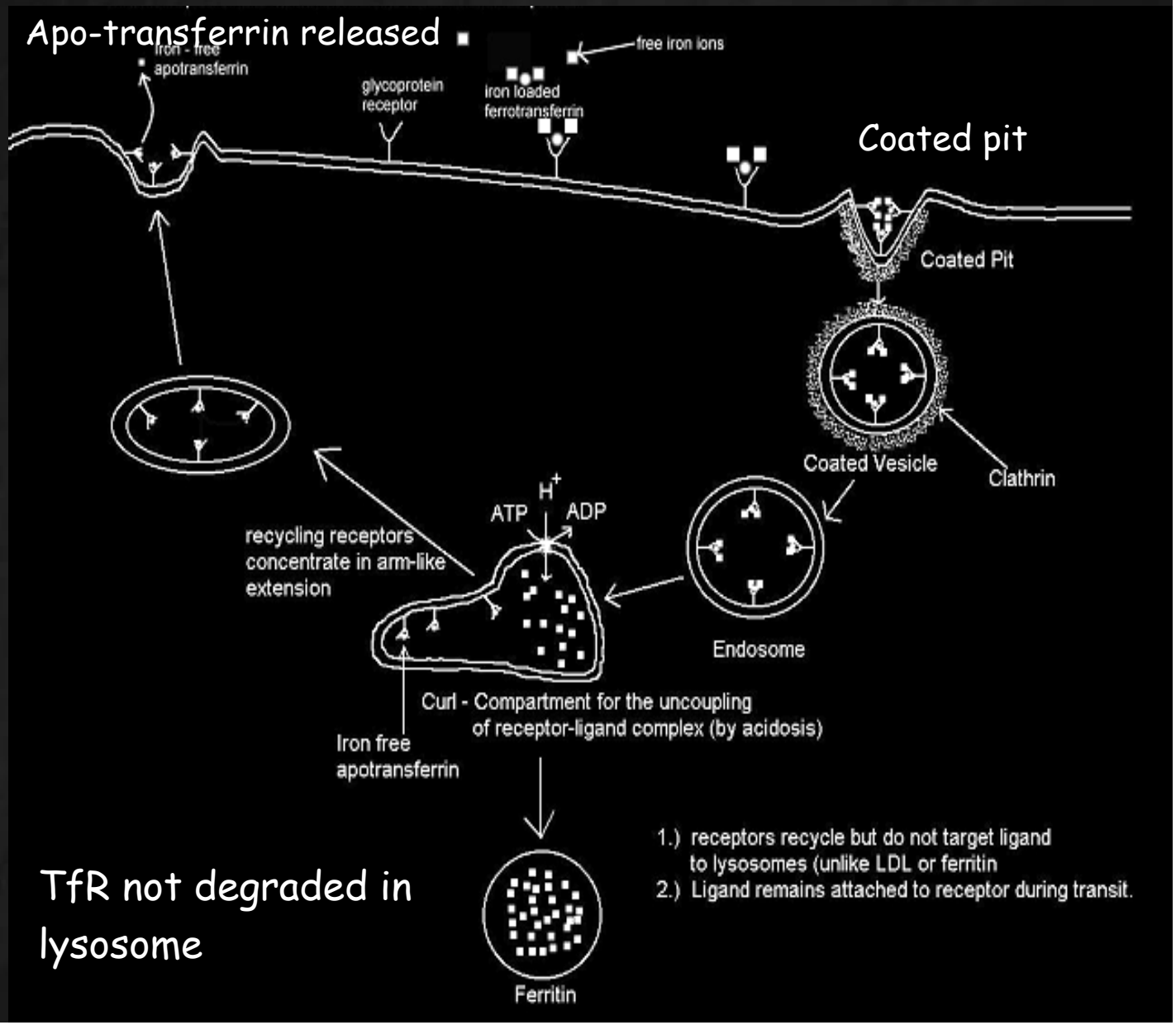


Stryer Fig. 35-34

Regulation of transferrin receptor:

When cellular Fe is low, TfR mRNA is stabilized and increased receptors synthesis occurs.

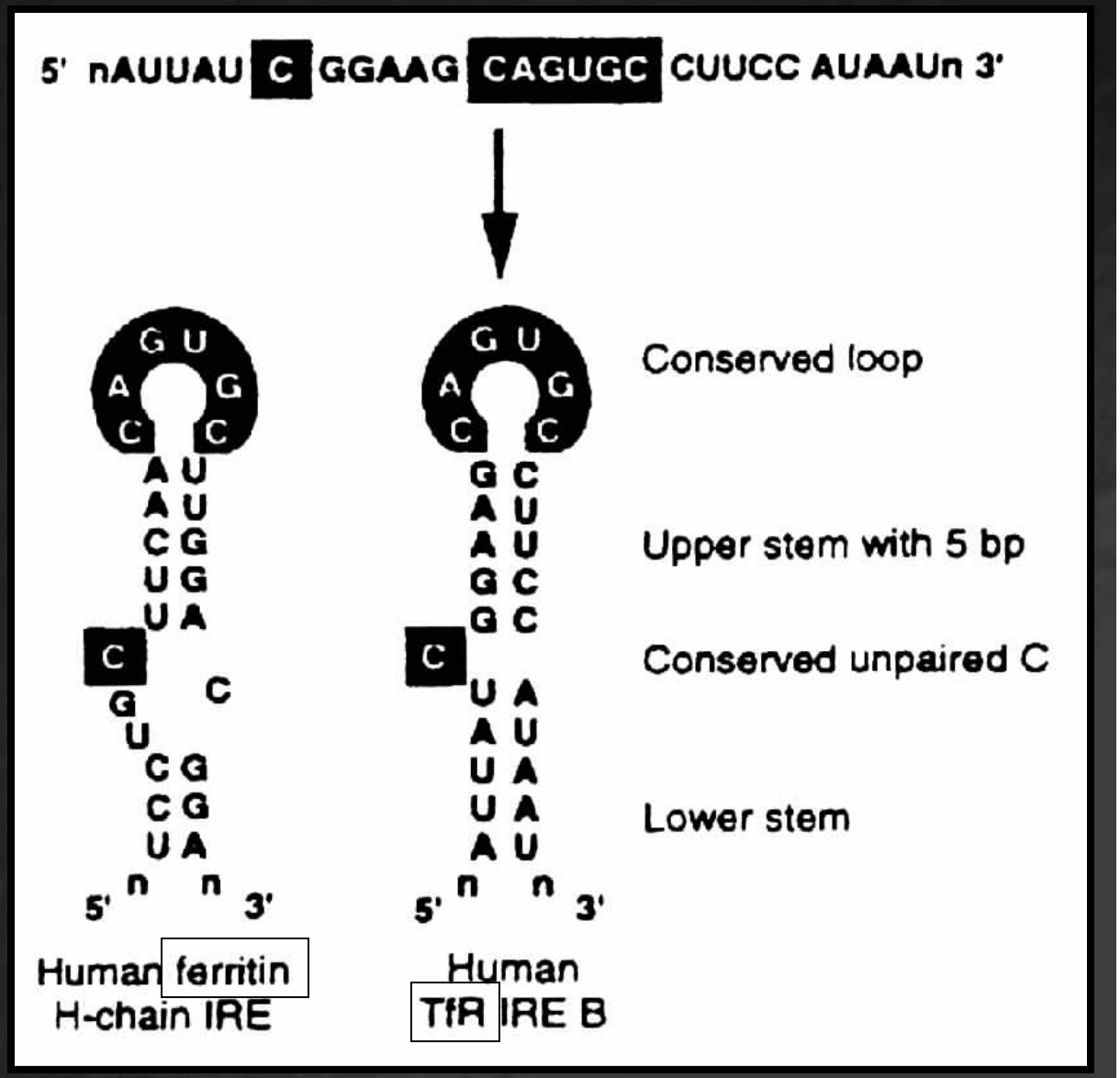
In contrast, when cellular iron levels are high, TfR mRNA is de-stabilized, reducing receptor levels.



TfR not degraded in lysosome

Iron-responsive elements:

Within the mRNAs of ferritin, the transferrin receptor, and aconitase, nucleotide sequences have been identified which defines a binding site for iron responsive proteins. Binding of these alters the translational efficiency of these mRNA's in opposite ways. In this manner, levels of cellular iron stores and iron transporters can be coordinated.

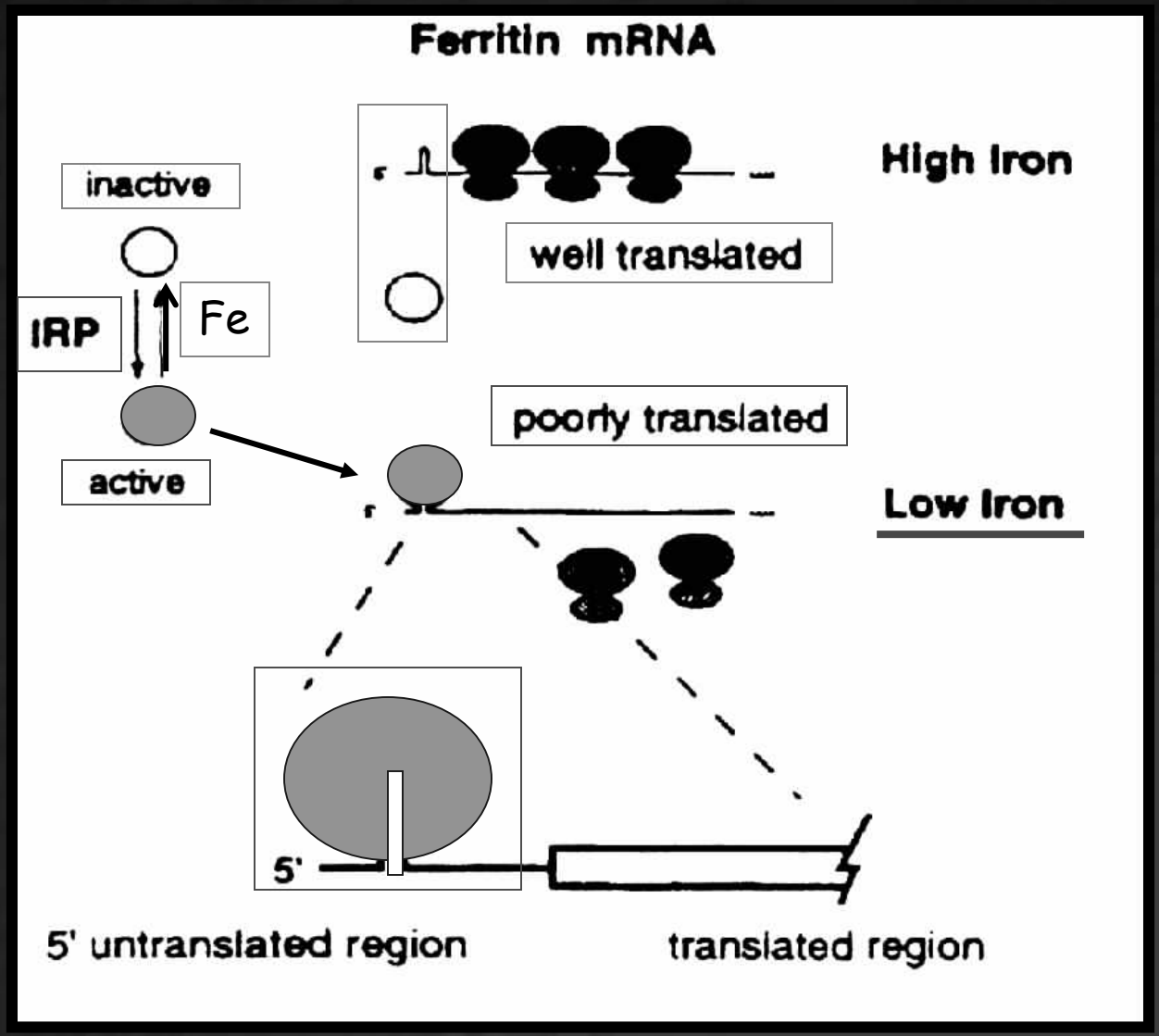


Nut. Rev. 56, S11-S19 (1998)

Regulation of ferritin:

Under conditions of low cellular iron, stimulates binding of IRP (iron responsive protein) to ferritin mRNA, preventing its translation.

Elemental iron stimulates ferritin synthesis by causing the release of IRP from IRE, inducing its translation.



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Regulation of ferritin:

Ferritin protein synthesis is increased under elevated levels of iron. Transcription is not increased - so this represents a change in translational efficiency of the mRNA pool.

This occurs as a result of iron acting on IRE-BP's (iron responsive element binding proteins) or IRP's to alter their conformation, which results in a reduction in the affinity for IRP's for the IRE.

When this happens, IRP's are released from the 5' IRE of ferritin mRNA, thereby enhancing ferritin mRNA translation.

Conversely, low levels of iron promote the binding of IRP complexes, thereby inhibiting ferritin mRNA translation.

Regulation of transferrin receptor:

In the case of the transferrin receptor this regulatory mechanism operates in reverse, high levels of available iron reduce rates of protein synthesis.

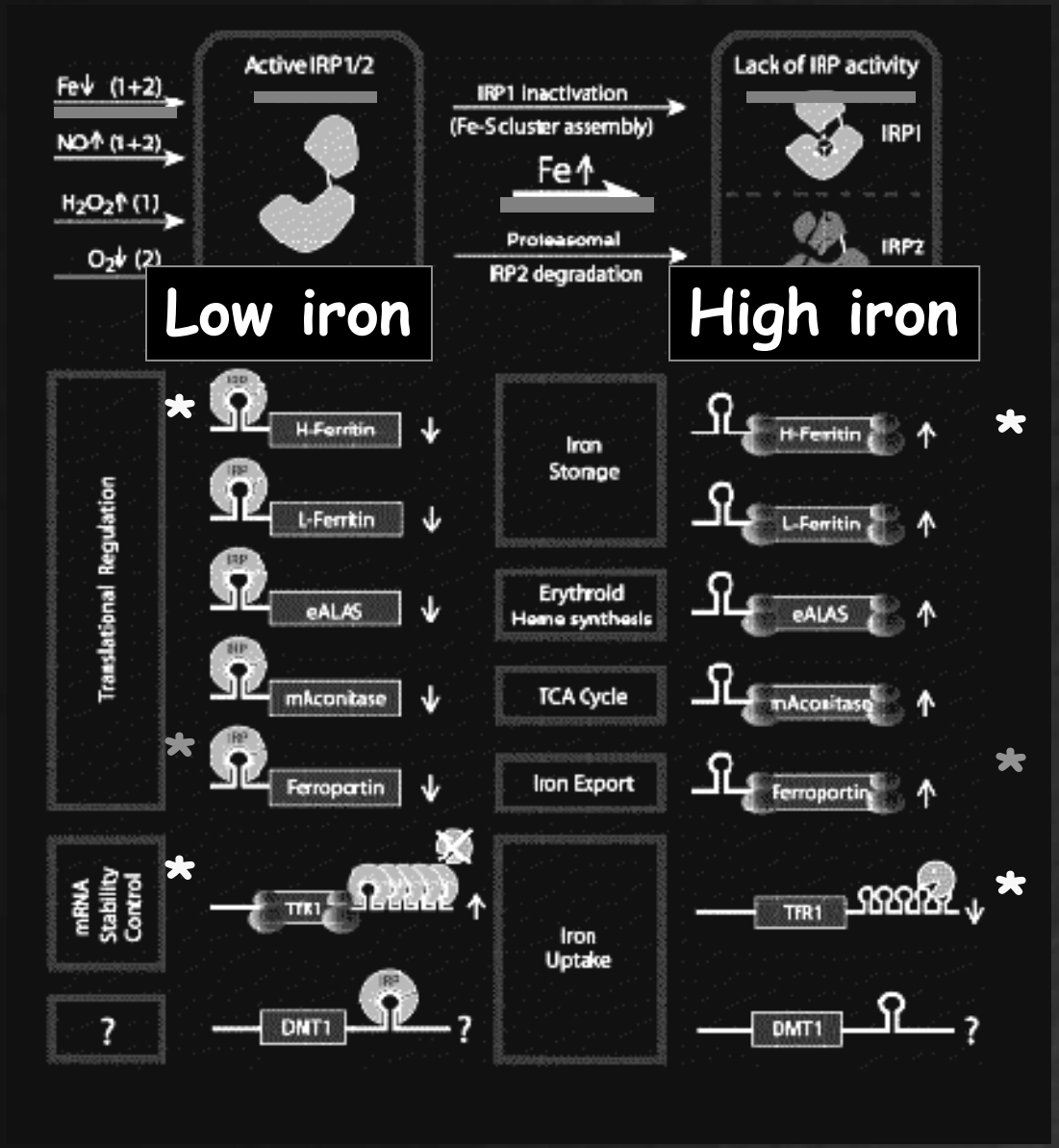
For the TfR, IRE's (iron responsive elements) are located at the 3' end of the gene outside the coding region. In the presence of insufficient iron, IRP's also bind to TfR mRNA. However in this context binding of IRP's DOES NOT alter translational efficiency.

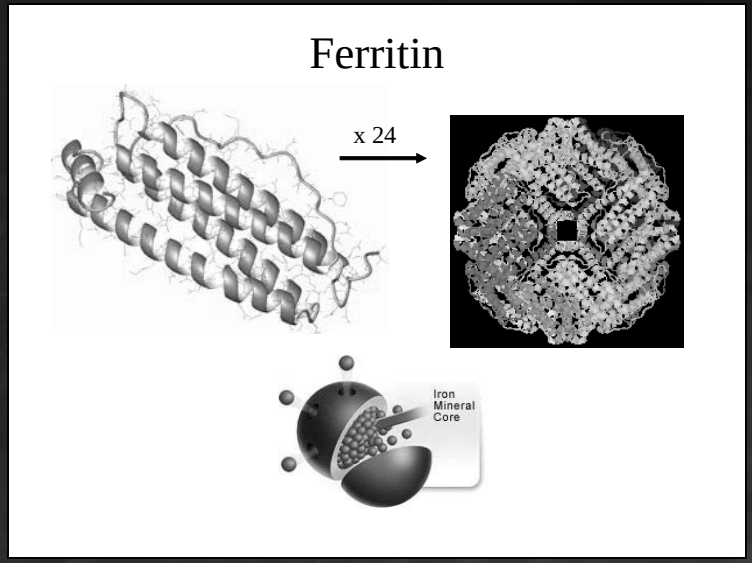
Thus under conditions of low iron TfR mRNA can still be efficiently translated.

Under conditions of high available iron, IRP binding to the TfR IRE is reduced. Because of the 3' location, loss of IRP binding enhances TfR mRNA degradation (lowering aggregate levels of translation).

The net effect is to keep free iron levels to a minimum

Regulation of iron:





Causes of Iron Deficiency:

- Direct blood loss (Acute / Chronic)

- Excessive RBC destruction (extrinsic – dietary)
  (B12, folic acid, iron – uptake or utilization)

- Excessive RBC destruction (extrinsic – env./drug induced)

- Excessive RBC destruction (intrinsic – genetic, Thal., HbS)

- Bone marrow abnormalities (hypochromic/microcytic)

Causes of Iron Deficiency, direct anemia:

Acute / Chronic anemia:

Tissue iron requirements greatly increase during: growth, menstruation, pregnancy, blood donations, pathological bleeding (peptic ulcer, hemorrhoids, bleeding gums, intestinal diseases, hookworm infestation, chronic hemodialysis).

Low tissue iron stores → Fewer RBC & paler/smaller → hypochromic, microcytic, i.e., less hemoglobin content

Causes of iron deficiency, dietary:

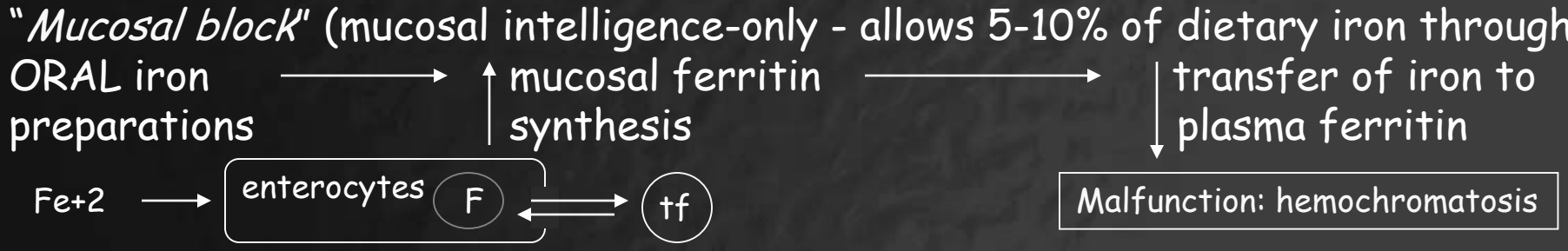
A. 56% of women in developing countries have serum ferritin <8ug/l and hemoglobin levels < 110 g/l. This can result in preterm birth, impaired mental and motor development among children.

B. Phytates (form 2% of cereals, nuts, legumes), Polyphenols in tea, vegetables, Phytic acid in soybeans. These agents inhibit iron absorption. Tannates in tea, coffee, veg. also decrease absorption.

C. Low meat intake (heme Fe - myoglobin or hemoglobin).

D. Lack of ascorbic acid in diet, ascorbic acid (Vit C) promotes non-heme Fe absorption by reducing Fe^{3+} to Fe^{2+} (particularly with gastric acid).

"*Mucosal block*" (mucosal intelligence-only - allows 5-10% of dietary iron through)



Diagnosis of iron deficiency:

Commonly used diagnostic criteria:

- ↓ hemoglobin level
- ↓ serum iron (normal 105 ug/dl) (low specificity)
- ↓↓ serum ferritin (decreased storage), often seen in infection or chronic inflammation (high specificity)
- ↑ elevated serum transferrin (low saturation)
- ↑ transferrin receptor - reflects tissue iron needs (not ↓ in infection or inflammation)
- ↑↑ protoporphyrin levels in RBC

Diagnosis of iron deficiency:

Can be difficult to distinguish dietary iron deficiency from anemia ! However the following can be informative:

Ferritin: low with pure iron deficiency but increased with acute phase response

Iron: low in both conditions

Transferrin: high in pure iron deficiency but decreased with acute phase response

Iron supplementation, dietary:

Fe fortification of wheat flour:
Canadian wheat flour is enriched with thiamine (Vit B1), riboflavin (Vit B2), niacin (Vit B3) and iron and folic acid. Cost: fortification of wheat flour with folic acid and iron costs approximately $1.50 (US) per metric ton.

Fe fortified formula:
*Not for breast fed babies <6 months (reserve stores sufficient) - may cause hemolysis in Vit E deficient premature infants

Fe from foodstuffs:
Red meats (10%), and green leafy vegetables (1-5%, note phytate and polypheynol content however), fish (not milk)

* Iron supplementation not recommended for non-anemic pregnant women - clinical trials have failed to demonstrate improved clinical outcomes for the mother or newborn. Supplementation can be harmful to individuals with hereditary hemochromatosis or hemosiderosis, ↑ risk of cancer /ischemic heart disease in those with modestly increase plasma iron levels.

Iron supplementation:

Lactoferrin, whey proteins in milk or cheese can complex to non-heme iron, increasing iron uptake. Vitamin C also enhance Fe uptake.

Parenteral iron preparations:
Used for patients who do not absorb oral preparations, as in inflammatory bowel disease, peptic ulcers etc, develop side effects, or are otherwise non-compliant. Note however the potential danger of anaphylaxis (identify patients before treatment) and iron overload.

Iron dextran (polymatose) NEW
Iron dextran – OLDER, intravenous or deep intramuscular
(withdrawn due to high incidence of anaphylaxis)
Iron sorbitol - OLDER intramuscular only
(pain, myalgia, abscess formation)

Iron supplementation, oral drugs:

For iron deficient individuals, 200-300mg elemental iron/day given for several weeks. Elemental iron amounts are shown in equivalents/100mg iron salt. For pregnant women, UNIFEC tablets (200mg $FeSO_4$ + 0.25mg folic acid) are given daily to women in 2/3 trimester.

Equivalent effective doses:

Ferrous sulfate 20 mg (standard)
Ferrous gluconate 12 mg
Ferrous succinate 35 mg
Ferrous fumarate 32 mg

Adverse effects

Nausea
Upper abdominal pain
Constipation/diarrhea
(take after meal)

However, iron from these sources can complex with a number of drugs, thereby reducing availability. These include: TETRACYCLIINE, PENICILLAMINE, QUINOLINE, LEVODOPA, METHYLDOPA, LEVOTHYROXINE, ETIDRONATE.

Iron overload:

Causes of iron overload:

- Hereditary hemochromatosis (mutation of HFE gene on chromosome 6). HFE codes for a major histocompatibility complex protein which modulates the transferrin receptor.
  0.5% of US population - homozygous, ~5% are heterozygous
- Induced hemochromatosis, 100,000 per year – ex. transfusion dependent anemia (thalassemia major and sickle cell)
- Others causes: alcoholic cirrhosis, oral iron therapy, African Bantus, iron cook pot

NOTE: Iron poisoning is a significant cause of lethality in children (>200ug elemental iron). Victims exhibit black stools, lethargy, severe acidosis, convulsions, coma, circulatory collapse, bloody diarrhea, hepatic and renal failure, hypotension.

Effects of iron poisoning / therapies:

Effects:

Unbound serum iron arises due to saturation of transferrin stores

Excessive free serum iron can accumulate in liver (Kupffer cells) - bone marrow, myocardium, pancreas due to no active excretion mechanism for iron.

Consequences:

Hepatomegaly (hepatoma), skin pigmentation, diabetes mellitus (major cause of death), hypogonadism, cardiac dysfunction (heart failure), liver cirrhosis. Haemachromatosis is a genetic example of this.

Anti-iron therapies:

Phlebotomy (acute) or desferoxamine chelation therapy (for refractory anemia) + ascorbate

Iron toxicity, mechanisms:

Toxic mechanisms:

Haber-Weiss reaction: $Fe^{3+} + O_2^{\bullet -} \longrightarrow Fe^{2+} + O_2$

(Fenton) $Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\bullet}OH$

Net reaction: $O_2^{\bullet -} + H_2O_2 \xrightarrow{[Fe^{3+} \quad Fe^{2+}]} O_2 + OH^- + {}^{\bullet}OH$

↓

Attack DNA, RNA, proteins, lipids

Primary hemochromatosis (>20g iron - <1g normally):

- Genetic disorder characterized by excessive storage of iron in tissues.
- In cases of hemochromatosis the mucosal block malfunctions.
- Cause appears to be excessive absorption of iron (mod. TfR).
- Leads to tissue damage. Organs most affected are liver, skin and pancreas.
- Patients with this disorder usually develop cirrhosis of the liver.
- May acquire pigmentation of the skin (bronzed disease) and diabetes.
- Disorder can be controlled by periodic withdrawal of phlebotomy.
- Disorder carries some evolutionary advantage as prevents anemia under condition of a low iron diet.
-~5% Caucasians carry a defective allele of the HFE gene, 1.5 million (USA) are homozygous for this defect.

(Trends in Biochemical Sciences. 24, 164-165 (1999)

Genetic causes of iron overload:

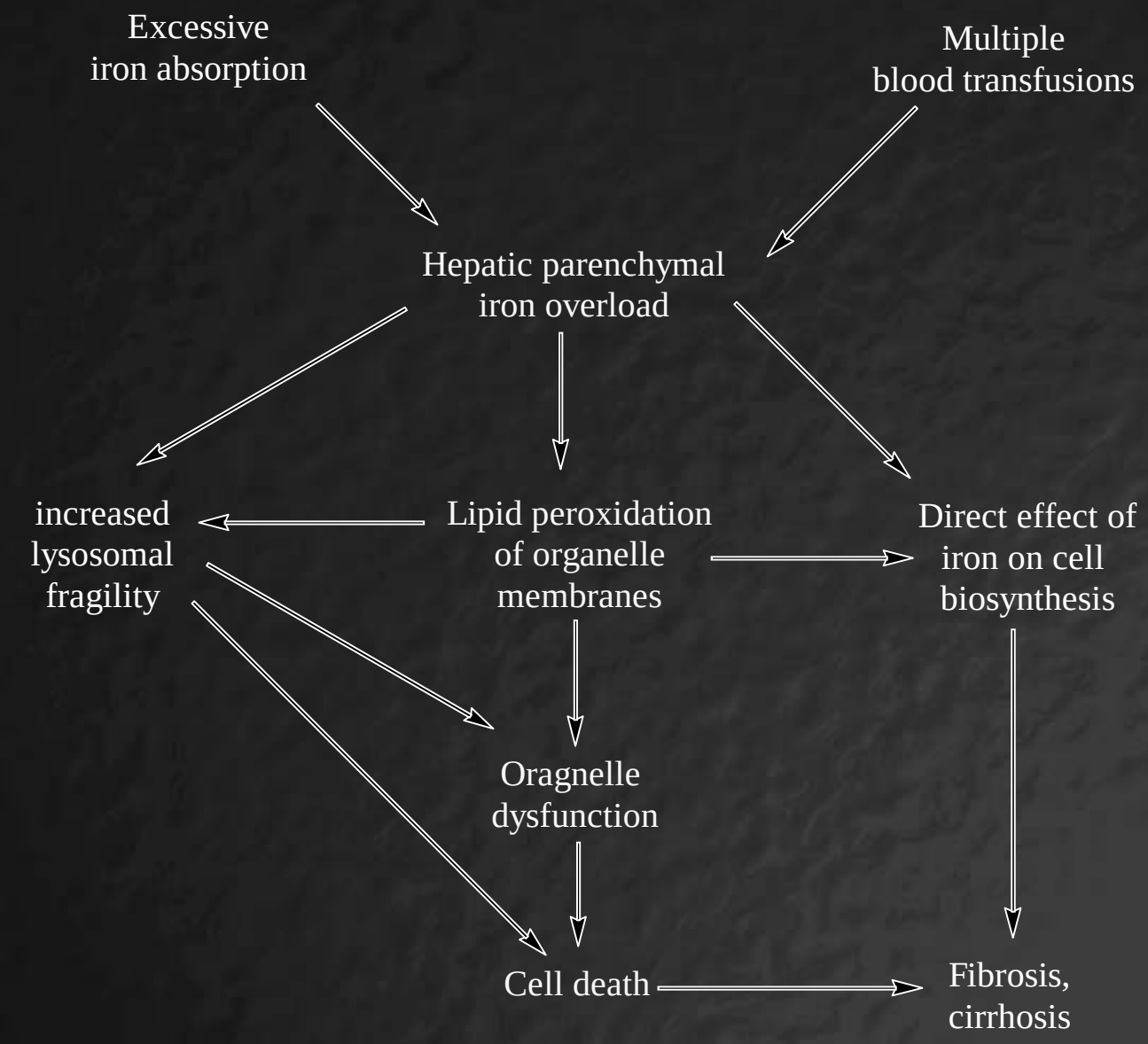
More than 95% of genetically caused IO cases are caused by a defect in HFE gene (C282Y-major, H63D-minor)

Other forms:
Low hepcidin activity
Ferroportin overactivity
These lead to increased gut absorption of iron

HFE-Related:
Type 1 – HFE (classic 6p21.3)

Non HFE Related:
Type 2a – Haemojuvelin (1q21)
Type 2b – Hepcidin (19q13)
Type 3 – Transferrin receptor 2 (7q22)
Type 4 – Ferroportin (2q32)

Liver injury during iron overload:



Fundamental diagnostic criteria: Iron values

Test	Total Iron	Transferrin	Transferrin saturation	Ferritin
Long term nutritional iron deficiency	Low	High	Low	Low
Hemochromatosis	High	Low	High	High
Chronic Illness (i.e. combating parasite)	Low	Low	Low	normal to high
Hemolytic Anemia (genetic or drug induced)	High	Low	High	High
Acute Iron Poisoning	High	Normal	High	Normal