



BIOCHEMISTRY SHEET

LEC 2

تبييض : عبادة العايد

تدقيق : عمران يونس

حوسبة: براءة احمد

Hemoglobin synthesis

ملحوظة : التبييض يشمل اللون الأزرق و الأخضر.

Hemoglobin synthesis :

Consist of 8 reactions , 4 of them are mitochondrial and 4 reactions are cytosolic (1 mitochondrial , followed by 4 cytosolic reactions , followed by 3 mitochondrial reactions) .So intermediates of hemoglobin synthesis reactions are going from and to mitochondria through the mitochondrial bilayer membrane .

Why don't you react to what happened in one place?

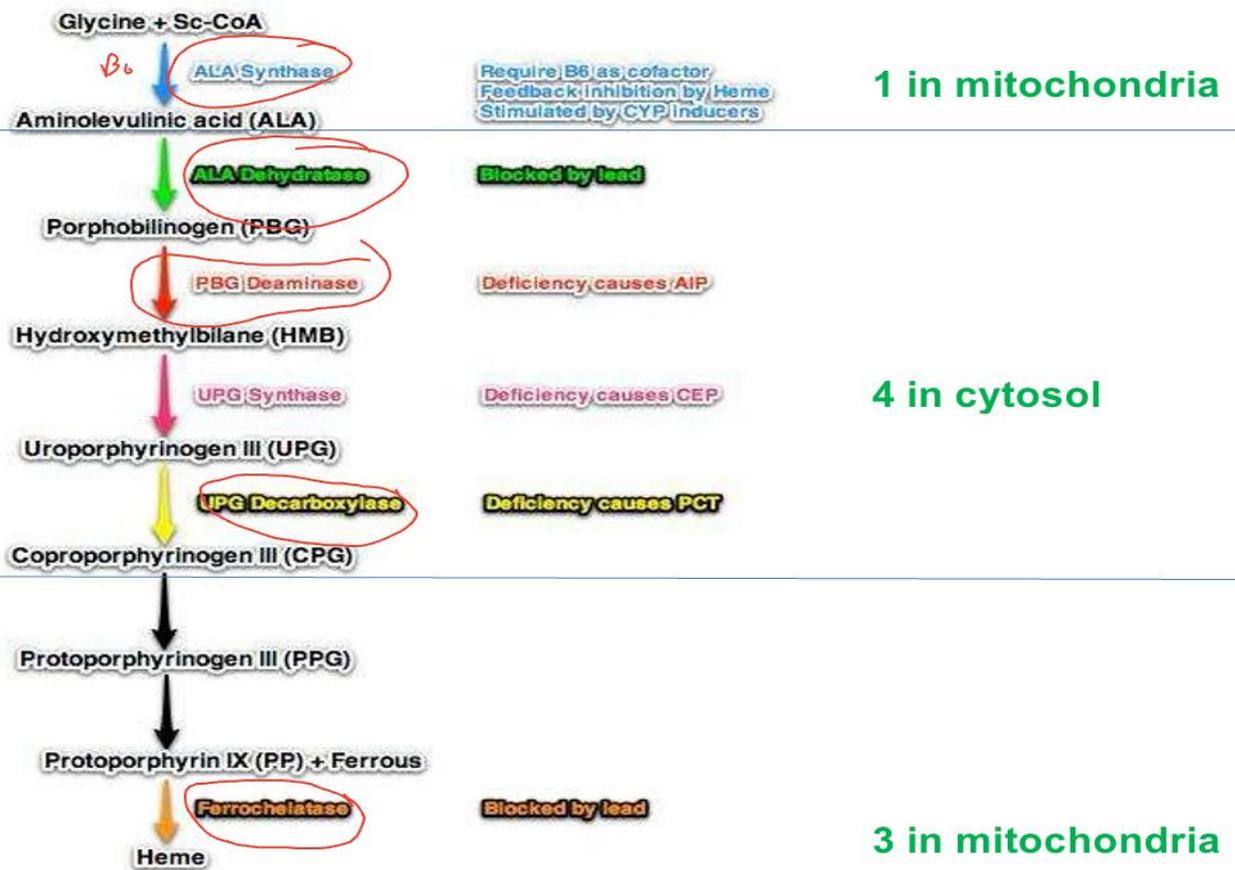
It's a way of enzyme activity regulation, called Compartmentation or Compartmentalization Which means that not all reactions happen at the same site inside the cell, so adding more regulatory factors. double layer mitochondrial membrane

*if all heme synthesis step are done in the cytoplasm NO control over production of Heme increase the production of globin more hemoglobin more erythrocytes (polycythemia)

1. Increase the blood viscosity
2. Slow blood flow
3. Thrombi -> stagnation of blood
4. Adding more load on the cardiac muscle & may be heart failure

The treatment of polycythemia are transfusion of blood (التبرع بالدم ونقله) Because if we want to treat it medically we have to inhibit the secretion of EPO .

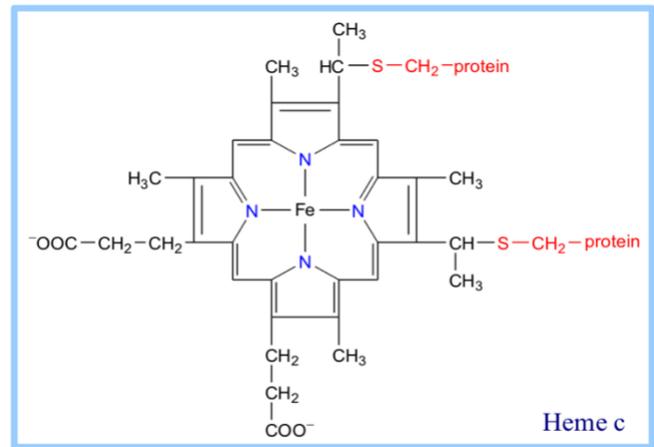
*All cells that have mitochondria are able to synthesize heme



Mnemonic for steps in heme synthesis

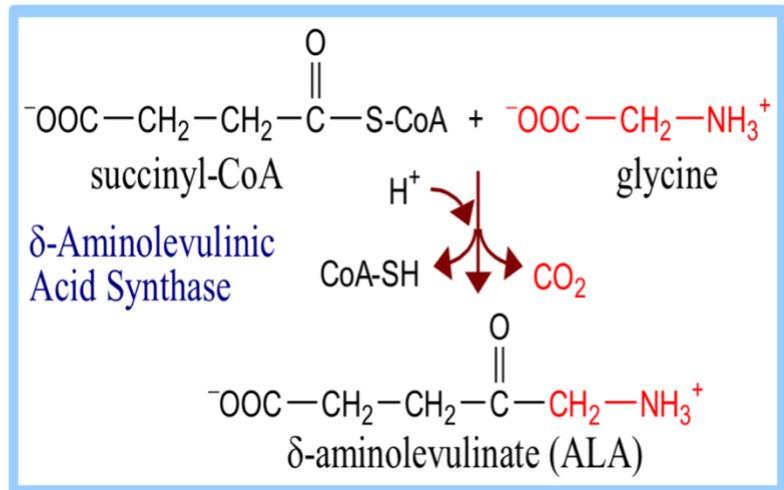
- S – SOME - Succinyl CoA
- G – GOOD - Glycine
- D – DOCTORS - Delta-Amino Levulinic Acid
- P – PALPATE - Porphobilinogen
- H – HEART - Hydroxymethylbelane
- U – UNDER - Uroporphobilinogen 3
- C – COVER - Coproporphyrinogen 3
- P- PRODUCES - Protoporphyrinogen
- P- PURE - Protoporphyrin
- H – HEME - Heme

- Heme is the prosthetic group of hemoglobin, myoglobin, & cytochromes.
- Heme is an asymmetric molecule.



Heme synthesis

- Heme synthesis begins with condensation of glycine & succinyl-CoA, with decarboxylation, to form δ -aminolevulinic acid (ALA).
- Pyridoxal phosphate (PLP) serves as coenzyme for δ -aminolevulinic acid synthase (ALA synthase), an enzyme related to transaminases.



The first reaction is condensation reaction Succinyl-CoA + glycine

=

Lead to decarboxylation (removing of CO₂) and produced the most important intermediate in this pathway (aminolevulinic acid) by the key regulatory enzyme (ALA synthase).

This enzyme (ALA synthase) works by aid of co-factor(pyridoxal phosphate) which is derivative of vit-B₆

Actually, vit-B₆ and its derivatives share in transaminase reactions. The ALA synthase enzyme belongs to transaminases, although it doesn't make transamination. As it uses the same cofactor which is used in all transaminase reactions .

- CoA~SH & the glycine carboxyl are lost following the condensation.
- ALA synthase is catalyzing the committed step of the heme synthesis pathway, & is usually rate-limiting for the overall pathway.
- Regulation occurs through control of gene expression.
- Heme functions as a feedback inhibitor, repressing the transcription of ALA synthase gene in most cells.
- A variant of ALA synthase expressed only in developing erythrocytes is regulated instead by availability of iron in the form of iron-sulfur clusters.

There are two forms of ALAS:

1-ALAS1 is considered a house-keeping gene and is expressed in all cells (located on chromosome 3).

House keeping gene :-

Working all the time at the constant rate

Doesn't require inducer or other stimulation factors , working all the time at constant rate .

Found in all cells which has mitochondria found on chromosome 3 which are found in all mitochondriae .

2-ALAS2 is an erythroid-specific form of the enzyme, expressed only in fetal liver and adult bone marrow (located on the X chromosome).

ALAS1 > controlled by feedback inhibition (end product inhibition) .

ALAS2> controlled by the content of iron .

- 1. Feedback inhibition = the enzyme is existing but its activity is inhibited by the end product**
- 2. Feedback regulation = the enzyme will not exist because here the end product is not acting on the activity of the enzyme , it acts on the production of the enzyme itself from the gene , so it is called (genetic level regulation)**

ALAS1	ALAS2
expressed in all cells	expressed only in fetal liver and adult bone marrow
Controlled by the heme (feedback inhibition) علاقه عكسية	controlled under the effect of availability of iron علاقة طردية
located on chromosome 3	located on the X chromosome

PBG synthase

(porphobilinogen synthase), also called ALA dehydratase, catalyzes condensation of two molecules of δ aminolevulinate to form the pyrrole ring of porphobilinogen (PBG).

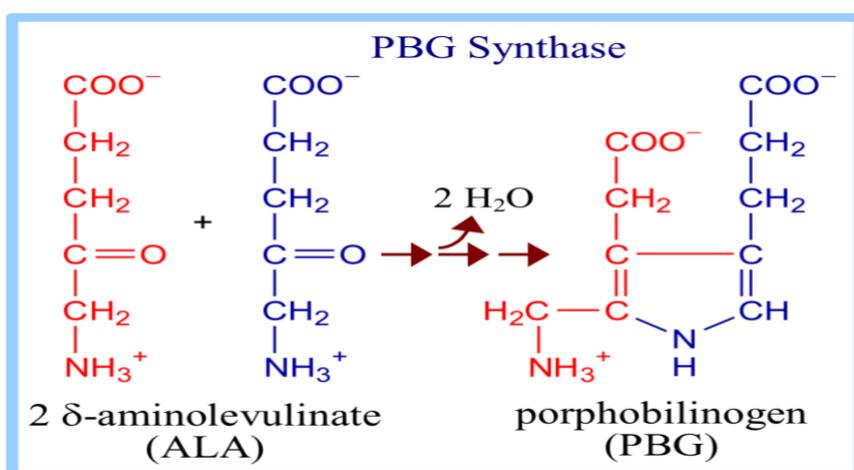
- Dehydratase = remove water molecule

- The first pyrrole ring produced are (porphobilinogen)

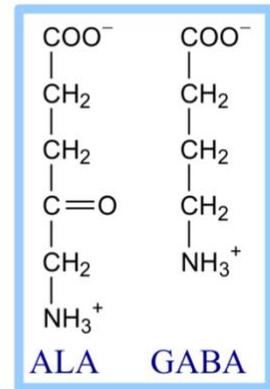
2. - the porphobilinogen which produced from reaction of PBG synthase enzyme (ALA dehydratase enzyme) , has different substitutions that bind to carbon atoms ... one with acetate and other with propionate , so the heme is an asymmetric molecule .

-The Zn^{++} in the active of site mammalian porphobilinogen synthase, acting as binding sites for ligands including cysteine S , it can also bind Pb^{++} (lead).

- Inhibition of porphobilinogen synthase by Pb^{++} results in elevated blood ALA, as impaired heme synthesis leads to de-
Repression of the transcription of ALA synthase gene.



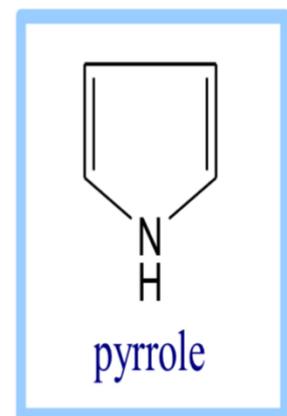
- High ALA is thought to cause some of the neurological effects of lead poisoning, although Pb^{++} also may directly affect the nervous system.
- ALA is toxic to the brain, perhaps due to:
 - 1- Similarity in the structures between ALA and GABA (γ - aminobutyric acid).
 - 2- ALA autoxidation generates reactive oxygen species (oxygen radicals).



If lead (Pb^{+2}) in high concentration binding to Zn^{+2} in the active site of ALA dehydratase enzyme , it will inhibit the activity of this enzyme so only one step are done in pathway , so no production of hemoglobin , and the ALA which produced from first step will be accumulated because ALA has similar structure of GABA (which is the main inhibitory neurotransmitter) , the accumulated ALA will bind to GABA receptors , this will lead to loss of balance between excitatory & inhibitory neurotransmitter functions , so convulsions occur .

**** number of inhibitory neurotransmitters is much less than number of excitatory neurotransmitters so if there is an analogue of GABA that bind to its receptor, this results in loss of function of GABA and loss of balance between neurotransmitters = increase excitatory functions ends up with convulsions**

- Porphobilinogen (PBG) is the first pathway intermediate that includes a pyrrole ring.
- The porphyrin ring is formed by condensation of 4 molecules of porphobilinogen.
- Porphobilinogen deaminase (hydroxymethylbilane synthase) catalyzes successive PBG condensations, initiated in each case by elimination of an amino group. it leads to the formation of the tetrapyrrole hydroxymethylbilane.



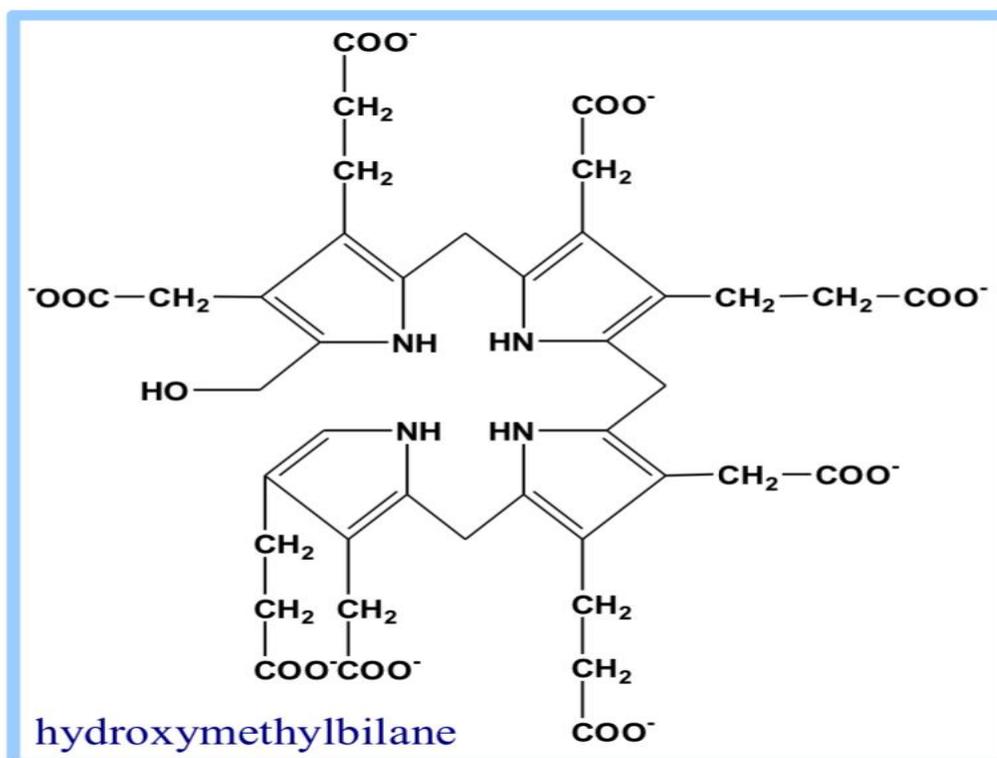
Hydroxymethylbilane has two fates:

Hydroxymethylbilane molecule :

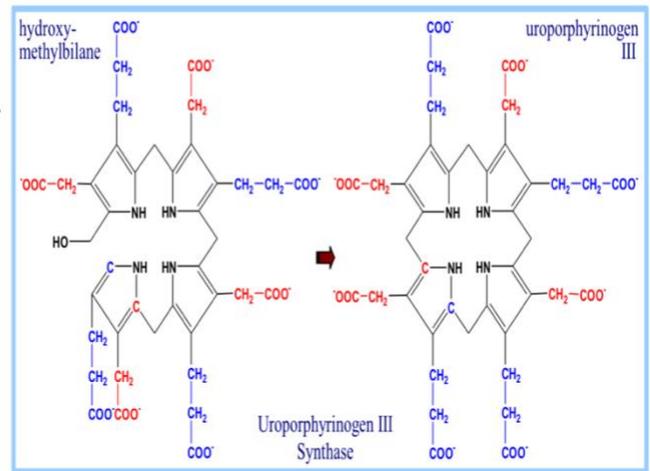
It is a molecule which is produced from reaction between 4 PBG molecules by deamination of its amino groups and linearization of it by hydroxymethylbilane synthase enzyme
So it is linear molecule contains 4 pyrrole rings .

1- The most important is regulated, enzymatic conversion to uroporphyrinogen III, the next intermediate on the path to heme which is mediated by a holoenzyme comprised of uroporphyrinogen synthase plus a protein known as uroporphyrinogen III cosynthase.

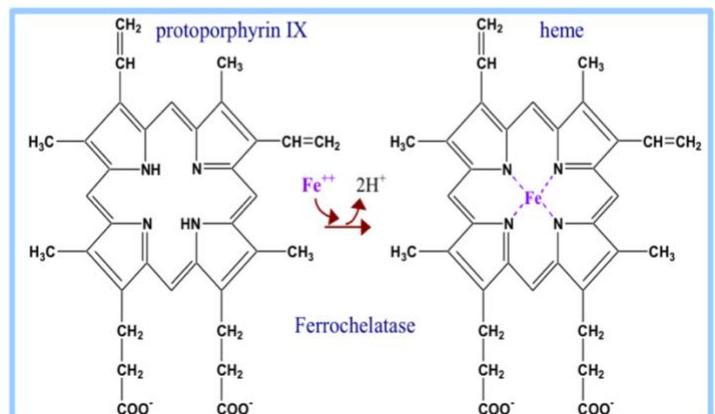
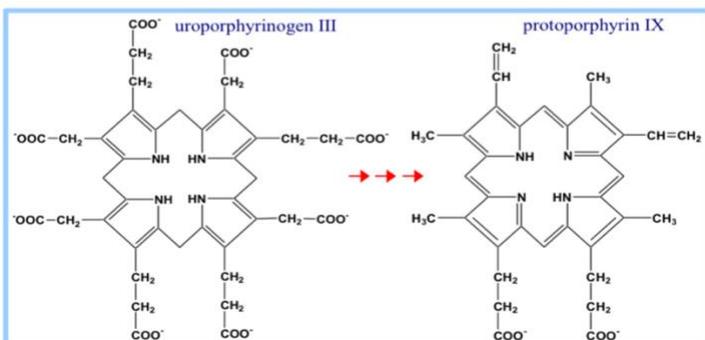
2- Hydroxymethylbilane can also non – enzymatically cyclize forming uroporphyrinogen I.



- Uroporphyrinogen III synthase converts the linear tetrapyrrole hydroxymethylbilane to the macrocyclic uroporphyrinogen III.
- Uroporphyrinogen III synthase catalyzes ring closure & flipping over of one pyrrole to yield an asymmetric tetrapyrrole.
- The distribution of acetyl & propionyl side chains, as flipping over of one pyrrole yields an asymmetric tetrapyrrole.



- Uroporphyrinogen III is the precursor for synthesis of vitamin B12, chlorophyll, and heme, in organisms that produce these compounds.
- Conversion of uroporphyrinogen III to protoporphyrin IX occurs in several steps.
 - All 4 acetyl side chains are decarboxylated to methyl groups (catalyzed by uroporphyrinogen decarboxylase)
 - Oxidative decarboxylation converts 2 of 4 propionyl side chains to vinyl groups (catalyzed by Coproporphyrinogen oxidase)
 - Oxidation adds double bonds (Protoporphyrinogen oxidase).
 - Fe^{++} is added to protoporphyrin IX via Ferrochelatase, a homodimeric enzyme containing 2 iron-sulfur clusters.
 - A conserved active site His, along with a chain of anionic residues, may conduct released protons away, as Fe^{++} binds from the other side of the porphyrin ring, to yield heme.



Hydroxymethylbilane is cyclized .. which mean ring number 1 will binding ring number 4 by an enzyme known as (Uroporphyrinogen synthase)

This enzyme has two states :

- Non - enzymatically ... it will bind 1 & 4 rings in the same substitution (acetate / propionate ---- acetate propionate ----- acetate propionate ----- acetate propionate)

This will form structure called (Uroporphyrinogen I)

- Enzymatically in the presence of special co- factors called (Uroporphyrinogen III co-synthase).....during binding of 1&4 rings , ring number 4 will turn around itself so give more assymetry in the structure of heme .. the binding will be as (acetate / propionate ----- acetate / propionate ----- acetate / propionate ----- *propionate / acetate*)

The difference in ring number 4 because of flipping over or rotation of this ring around itself .

**** only Uroporphyrinogen III will continue in heme synthesis .

Uroporphyrinogen I has no function , this because there are no enzyme can act upon it or react with it .

Uroporphyrinogen decarboxylase enzyme will go on last reaction inside the cytosol , which is decarboxylation reaction , by removing CO₂ from each acetate group so it converts to methyl group (acetate = CH₃-COO

.....
methyl = CH₃ **** so decarboxylated acetate = methyl)

The molecule produced are known as (Coproporphyrinogen) which has substitution as follow (methyl/ propionate ---- methyl/ propionate ---- methyl/ propionate ----- propionate methyl)

Coproporphyrinogen will go in the mitochondria to give the rest of reactions in hemoglobin synthesis .

It will go in oxidative decarboxylation reaction of two of propionate groups on ring 1 & 2 to convert it to vinyl groups by enzyme called (coproporphyrinogen oxidase) , so the substitution of the new molecule are as follow (methyl / vinyl ---- methyl / vinyl ----- methyl / propionate ----- propionate / methyl)

This structure is known as (protoporphyrinogen) which will go into oxidation by (protoporphyrinogen oxidase) to convert it into (protoporphyrin) , which has double bond because the bonds which link the pyrrole rings with each other are not methylene bonds , it is methylene so this enzyme forms double bonds in the methylene groups that link the 4 pyrrole rings together .

the final asymmetry of hemoglobin is attributed to :

1. Acetate propionate on each ring
2. More asymmetry on ring 4 by its rotation around itself .
3. Another asymmetry by decarboxylation of all acetate groups to convert it to methyl groups .
4. More asymmetry by oxidative decarboxylation of propionate groups on the 1&2 pyrrole

Q: Ferrous in the heme molecule has 6 bonds , although its valency are 4 not 6 , why ??

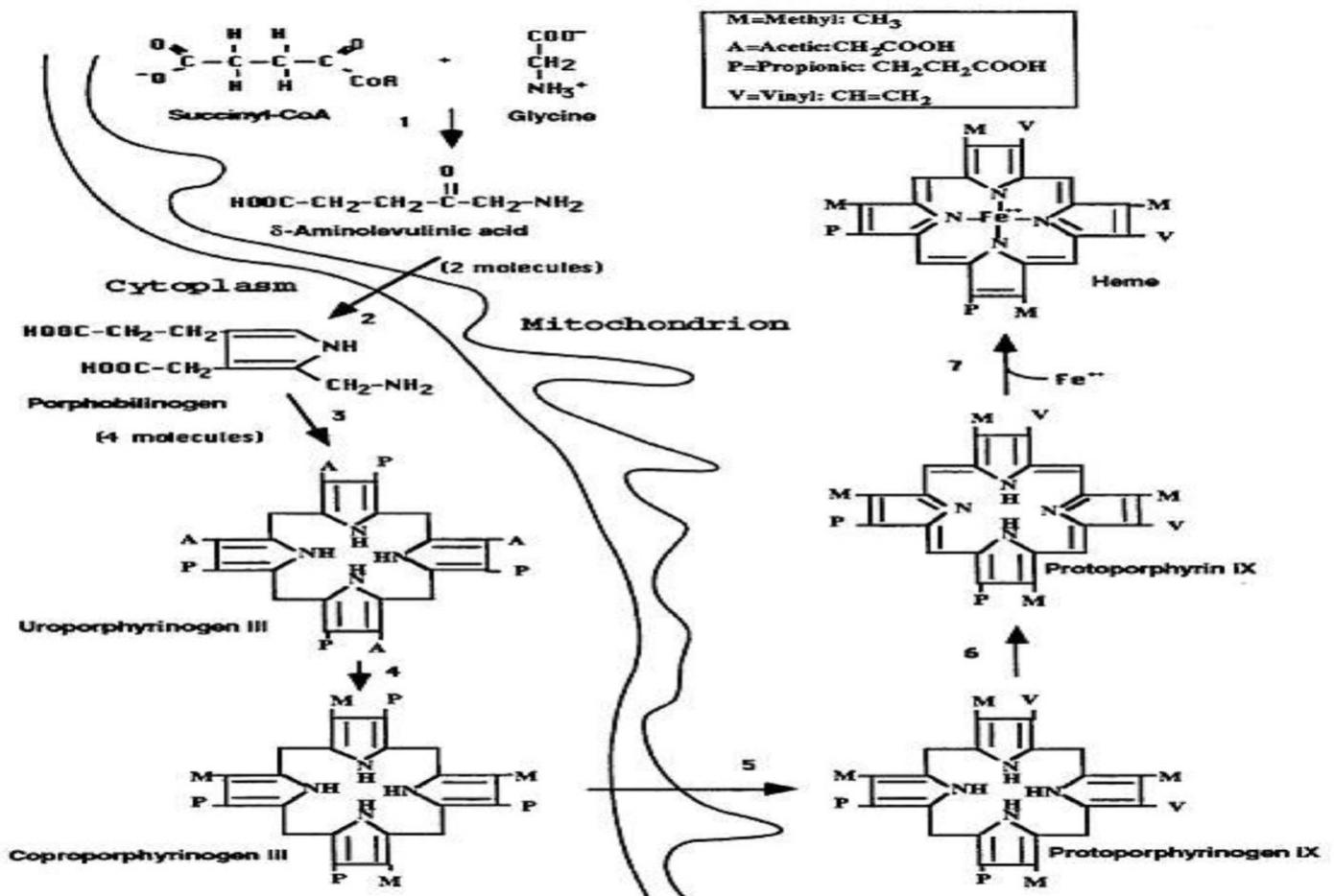
A: although there are a lot of defense mechanism to prevent oxidation of ferrous iron into ferric , but some times oxidation occur , so there are enzyme inside erythrocytes known as (methemoglobin reductase enzyme) which will convert hemoglobin to its active functional form after any change of any part of hemoglobin into methemoglobin .

Bonds of ferrous are :

4 bonds with the nitrogen of four pyrrole rings .

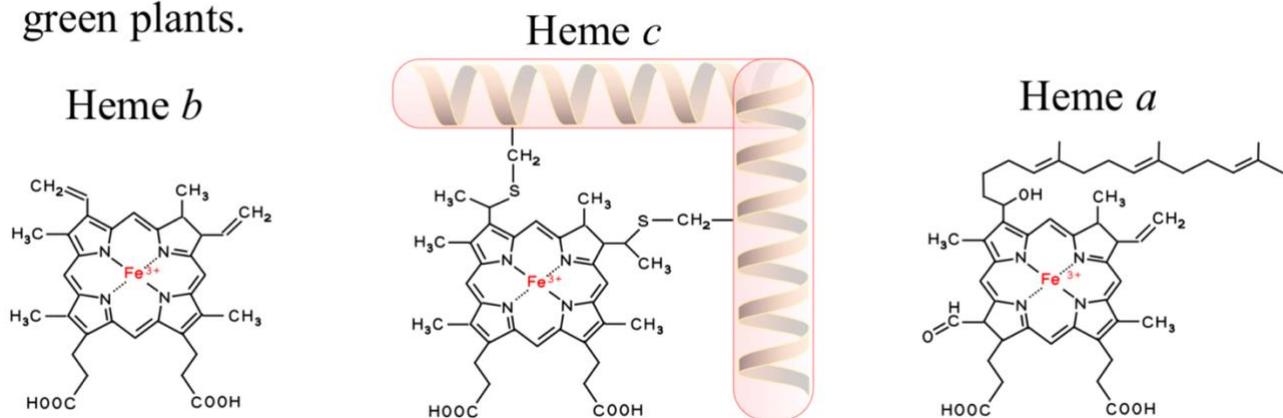
one with proximal histidine

one with O₂



Pathway of Heme Biosynthesis

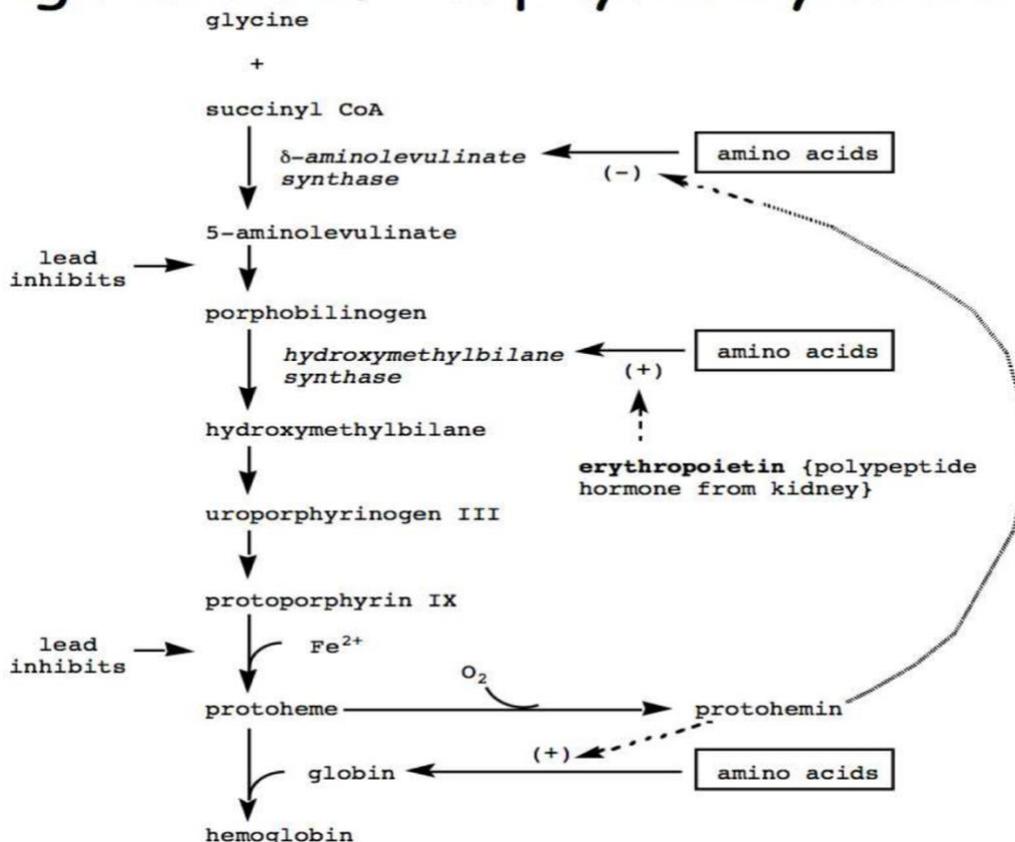
- In addition to the heme *b* found in hemoglobin, there are two different forms of heme found in cytochromes such as those involved in the process of oxidative phosphorylation.
- Cytochromes of the *c* type contain a modified iron protoporphyrin IX known as heme *c*.
- In heme *c* the 2 vinyl (C=C) side chains are covalently bonded to cysteine sulfhydryl residues of the apoprotein.
- Only cytochromes of the *c* type contain covalently bound heme.
- Heme *a* is also a modified iron protoporphyrin IX.
- Heme *a* is found in cytochromes of the *a* type and in the chlorophyll of green plants.



- Regulation of transcription or post-translational processing of enzymes of the heme synthesis pathways differs between erythrocyte forming cells & other tissues.
- In erythrocyte-forming cells there is steady production of pathway enzymes, limited only by iron availability.
- In other tissues expression of pathway enzymes is more variable & subject to feedback inhibition by heme.
- The rate-limiting step in hepatic heme biosynthesis occurs at the ALA synthase catalyzed step, which is the committed step in heme synthesis.
- The Fe^{3+} oxidation product of heme is termed hemin which acts as a feed-back inhibitor on ALA synthase.
- Hemin also inhibits transport of ALA synthase from the cytosol into the mitochondria as well as represses synthesis of the enzyme.

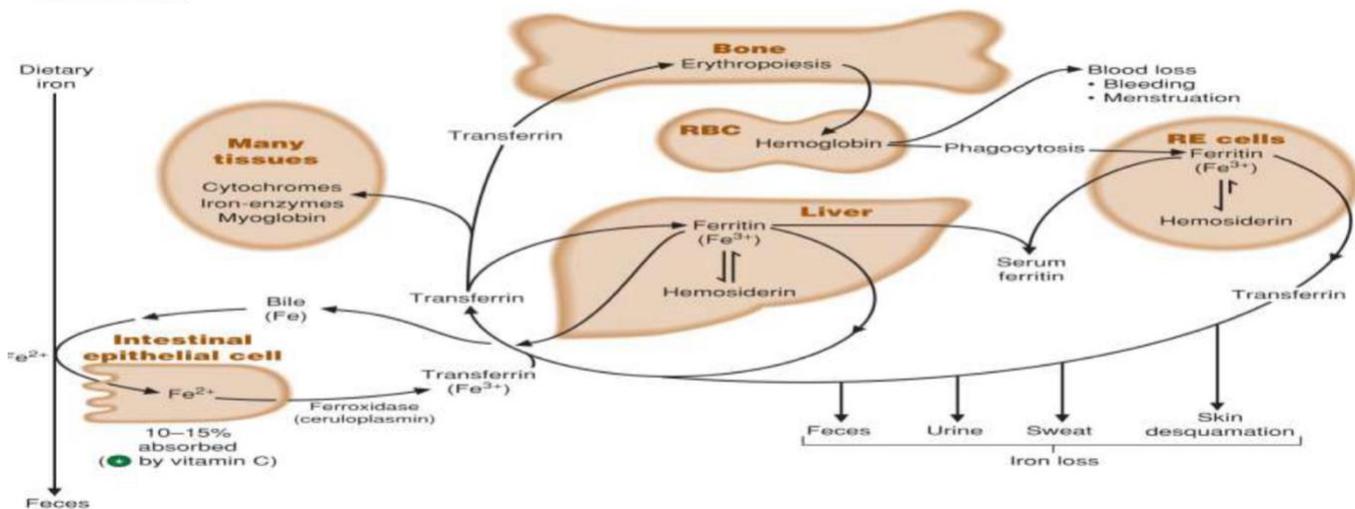
- In erythroid cells all of the heme is synthesized for incorporation into hemoglobin and occurs only upon differentiation when synthesis of hemoglobin proceeds.
- When red cells mature both heme and hemoglobin synthesis ceases.
- The hemoglobin must, therefore, survive for the life of the erythrocyte.
- In reticulocytes (immature erythrocytes) heme stimulates protein synthesis.
- Additionally, control of heme biosynthesis in erythrocytes occurs at numerous sites other than at the level of ALA synthase.
- Control has been shown to be exerted on ferrochelatase, the enzyme responsible for iron insertion into protoporphyrin IX, and on porphobilinogen deaminase.

Regulation of Porphyrin Synthesis



Regulation of iron absorption and transport

- Iron for synthesis of heme, Fe-S centers and other non-heme
- Iron is obtained from:
 - 1- The diet
 - 2- Release of recycled iron from macrophages of the reticuloendothelial system that ingest old & damaged erythrocytes.
- There is no known mechanism for iron excretion.
- Iron is significantly lost only by bleeding, including menstruation in females.



When erythrocytes degraded as after its life span, iron will exit from them. Iron is not excreted except very little amount in feces, urine or sweat.

The main items through which the iron can be lost is the skin & during menstrual cycle in females (it varies according to amount of blood lost during different stages of cycle)

There is no role of excretion of iron so in hemolysis of erythrocytes the iron will accumulate in the liver and cause hemosiderosis.

If this occur we have to make periodic blood transfusion, and to use iron chelator (a substance that binds to iron and enhances its excretion). in order to avoid hemochromatosis.

Iron metabolism and proteins

- Many proteins have been identified playing roles in iron metabolism such as ferritin or transferrin are the main cargos of blood iron, whereas peptides such as iron regulatory proteins, hepcidin, and matriptase2 are **key determinants of iron regulation at different physiological levels.**
- A set of different proteins, notably divalent metal transporter-1, ferroportin, and transferrin receptors in association with ferroxidases such as duodenal cytochrome B, ceruloplasmin and heme carrier protein, are involved in **the cellular membrane transportation of iron.**
- Others proteins such as myoglobin, Hb, and many different enzymes are the 'end' products of iron metabolism, because **they require iron for their functions.**

The treatment & diagnosis of iron metabolism & deficiency are very complicated because it has different types of proteins playing very important roles in the metabolism of iron, it is subdivided into 3 groups :

- **Key determinants of iron regulation at different physiological levels** >

This protein determines what are the requirements of iron under physiological conditions ??, is it absorbed ?? and determining their level in blood either increasing or decreasing ?? etc

Examples : ferritin & transferrin

** ferritin : when we investigate iron deficiency anemia, not Hb & iron are the only measurements .. we have to complete full investigation to treat IDA in a full term .

** transferrin : carrier protein which carries ferritin from side to side .

** hepcidin : secreted from liver as hormone .

- **Transportation of iron across cellular membrane .**

Examples : slide

- **Iron utilized in synthesis requirements you need absorption it will increase, you don't need absorption it will decrease .**

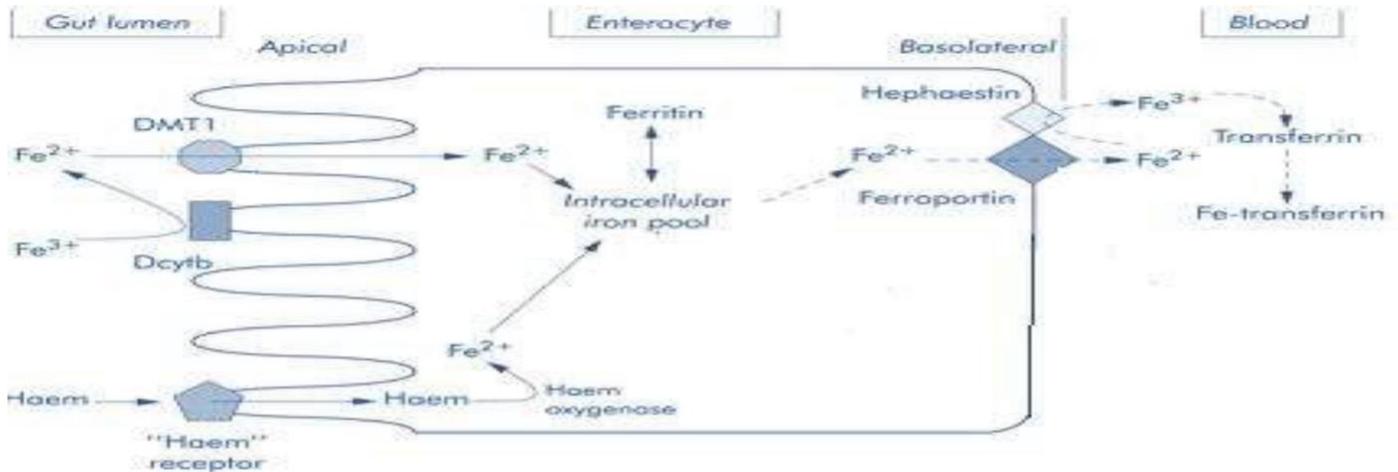
But under normal physiological conditions, the iron absorption does not exceed 5% although the cause of increasing demand of it .

** so absorption of more iron = formation of more heme = formation of more RBCs = polycythemia

+

Iron in circulation if doesn't carry it is toxic

Absorption of Iron



- 1- Iron stores within cells as a complex with apoferritin (ferritin), the main storage site is liver
- 2- Pass across basolateral membrane to be carried to transferrin through a protein ferroportin and hephaestin
- 3- Fe^{2+} is converted to Fe^{3+} by ferroxidase (Cu^{2+})
- 4- Hepcidin act as down regulator peptide secreted by liver.

**** The iron which we take have to be bounded = salt .**

**** Salt is not allowed to be absorbed , so the ionization occur , this ionization occurs by gastric acid (HCl) so you have to be careful , don't drink coffee at the early morning because it will lead to acute gastritis , which will lead to chronic gastritis , so parital cells function will decrease and HCl production decrease .**

**** so before treatment of IDA , I have to make sure what is the situation of the gastric mucosa because in the state of gastritis you will not use tablet or capsules , you will use I.V injections because of problems on absorbtion**

**** ionization may also occur by glutathion indirectly .**

**** most of doctors during treatment of IDA by tablets or capsules add vit. C to increase state of ionization .**

**** iron to cross any membrane must to be ferrous form (Fe+2)**

**** iron to bind to any type of protein must to be in ferric form (Fe+3)**

**** If iron take in diet as heme (not free iron) there are no ionization in this state , there are special receptor**

for heme , which increase ability of heme to cross inside , then inside the heme oxygenase enzyme system(which are more than one enzyme , it is group of enzymes not one enzyme)

either iron cross by DMT1 or by heme oxygenase enzyme system , inside cell it will bind to apoferritin and convert into ferritin which are known stored iron

When the body need this iron for utilization , iron exit from the cell by protein known as ferroportinwhen it bind to any protein (as ferroportin) it have to be converted into ferric form , how it will be converted from ferrous into ferric in this state ??

By effect of protein known as (copper dependant ferroxidase)

**** This process regulated by hepcidin protien / hormone .**

**** Outside the intestinal cell , iron have to be converted into ferrous form by effect of ferroxidase (Cu+2) =copper dependent**

Regulation of iron absorption and exportation by enterocytes

How hepcidin regulate the absorption of iron ??

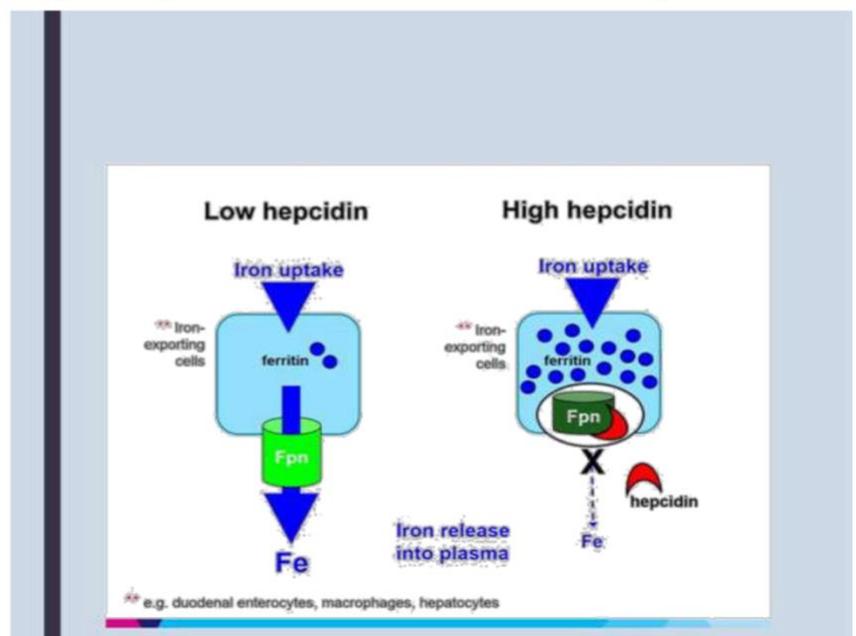
With another protein inside cells known as (Hemojuvelin protein), which also known as iron sensing protein , which sense the level if iron high or low

If iron level high and we are in no need of iron , iron will not go from stored site to blood , but the Hemojuvelin protein will increase the transcription of the gene encoded for the hepcidin to prevent exit of iron outside of cells by degradation of ferroportin so iron can not go outside .

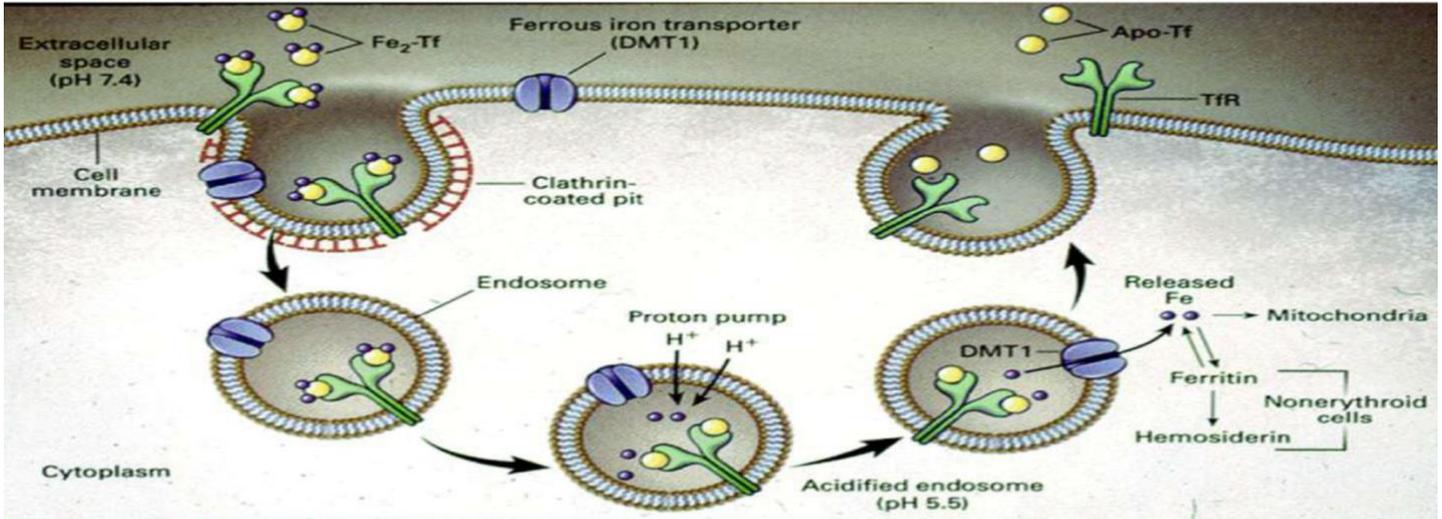
If are level low , there are no need for increase transcription of gene which encoded for hepcidin , so ferroportin activity don't stopped , and iron can cross outside to blood to the site of utilization .

** so hepcidin has antimicrobial effect , because alot of microbes require iron , so because it regulate the iron absorption it also has antimicrobial effect .

- Transcription of the gene for the iron transporter ferroportin is responsive to iron.
- When iron levels are high or in response to cytokines produced at sites of inflammation, hepcidin is secreted to induce ferroportin internalization and degradation, thus, leads to decreased absorption of dietary iron and decreased serum iron.
- Inversely, in the absence of hepcidin, ferroportin is maintained on the cell membrane, and iron transportation is facilitated.
- The plasma membrane protein ferroportin mediates:
 - 1- Release of absorbed iron from intestinal cells to blood.



- 2- Release of iron from hepatocytes (liver cells) and macrophages.
- Control of dietary iron absorption and serum iron levels involves regulation of ferroportin expression.
- Hepcidin is considered an antimicrobial peptide because by lowering serum iron it would limit bacterial growth.



- The plasma membrane transferrin receptor mediates uptake of the complex of iron with transferrin by cells via receptor mediated endocytosis.

When iron bounded to transferrin and what to go inside the cells , how it will enter inside the cells ??

Transferrin with iron will bind to receptor of the transferrin on the cell membrane , the transferrin - iron complex bind , then the coated pit convert to endosome (which are separated from cell membrane)

Endosome contains (transferrin - iron complex & transferrin receptors)

Then , how iron will be separated from transferrin ??

There are proton pump , which pumping H⁺ inside the endosome , which lead to decrease Ph to be acidic inside the endosome , this leads to separation of iron from transferrin , but transferrin still bounded with transferrin receptors (iron will leave transferrin , transferrin will not leave its receptor)

(يعني الوحيد اللي بينفصل عن المجموعة هو iron)

Then iron will go outside by DMT1 (dimetal transporter 1) for utilization , while the endosome will go to bind to cell membrane , and then transferrin will separate from its receptor to take up more iron again .

- Hereditary hemochromatosis is a family of genetic diseases characterized by excessive iron absorption, transport & storage.
- Genes mutated in these disorders include those:
 - 1- Transferrin receptor
 - 2- A protein HFE (Human hemochromatosis protein) that interacts with transferrin receptor to regulate iron absorption by inhibiting transferrin-receptor interaction
 - 3- Hemojuvelin, an iron-sensing protein required for transcription of the gene for hepcidin.
 - 4- Impaired synthesis or activity of hepcidin leads to unrestrained ferroportin activity, with high dietary intake and high % saturation of serum transferrin with iron.
- Organs particularly affected by accumulation of excess iron include liver and heart.

**** Hemochromatosis = more absorption & storage of iron .**

**** hemochromatosis may also occur with persons with hemolysis of erythrocytes , then blood transfusion is required , and we will add iron chelator to reduce iron storage by increasing iron excretion .**

**** The hemochromatosis that is related to gene mutation can not be treated .**

**** the liver (site of iron storage) are not the only organ which will be affected , other organs may be affected as heart , brain , and some irons go to Beta cells inside islets of langerhans and make destruction inside it which affect production of insulin .**

So the cases of human hemochromatosis due to genetic factor mostly will be diabetic , which known as (broods diabetis) because of destruction of beta cells by accumulated iron in the islets of langerhans .

Genetic polymorphism of proteins involved in iron metabolism

- In humans, genome-wide association studies found linkage of various gene polymorphism (single nucleotide polymorphism; SNP) and iron status, notably polymorphism of the gene coding for matriptase2.
- There is an evidence that genetic polymorphism of the matriptase2 gene is associated with the risk to develop iron deficiency anemia.
- Also, the investigators found a significant association of SNPs at the transferrin gene as well as at the HFE gene with iron deficiency.

**** IDA in some cases may be caused not from blood loss or inadequate intake of iron or inadequate absorption ... which is related to SNP .**

Globin synthesis

- Humans normally carry 8 functional globin genes, arranged in two duplicate gene clusters:
 - A- The β -like cluster on the short arm of chromosome 11.
 - B- The α -like cluster on the short arm of chromosome 16.
- These genes encode for 6 different globin chains: $\alpha, \beta, \gamma, \delta, \epsilon$ and ζ .

Type of Hb	Type of Globin Gene	Region	Time
Hb Gawer1 ($\zeta \epsilon$) ₂	ζ & ϵ	Yolk Sac	3 weeks of Gestation
Hb Portland($\zeta \gamma$) ₂	ζ & γ	Yolk Sac	5 weeks of Gestation
Hb Gawer II ($\alpha \epsilon$) ₂	α & ϵ		
Hb F ($\alpha \gamma$) ₂	α & γ	Liver & spleen	6-30 weeks of Gestation
Hb A ₂ ($\alpha \delta$) ₂	α & δ	Liver	30 weeks of Gestation
HbA($\alpha \beta$) ₂	α & β	Bone marrow	At Birth

Hemoglobin in adults

	Hb A	Hb A ₂	Hb F
Structure	$\alpha_2\beta_2$	$\alpha_2\delta_2$	$\alpha_2\gamma_2$
Normal %	96-98 %	1.5-3.2 %	0.5-0.8 %

الدكتور ركز على اوقات والجينات لكل وقت كثير في الشرح .

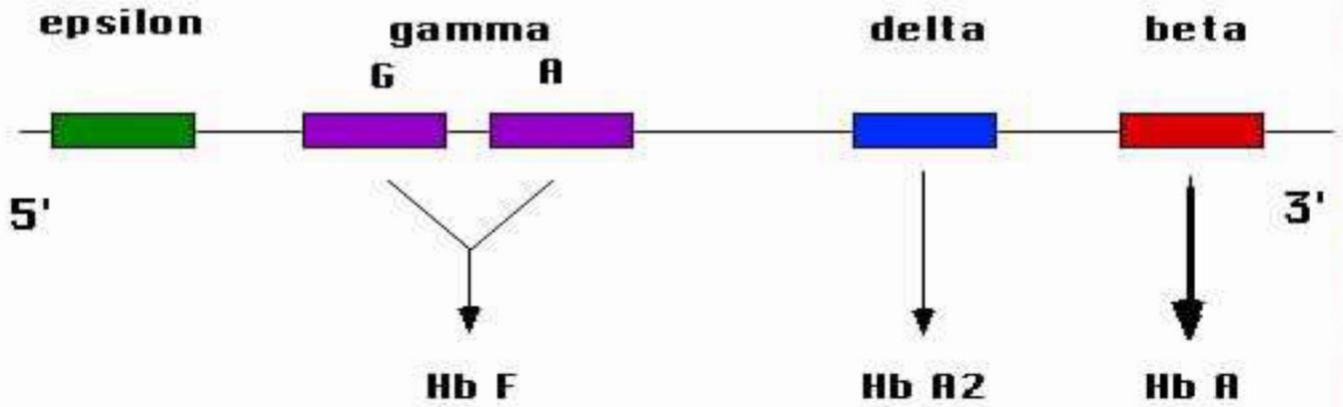
**** At the embryonic life time for the first 5 weeks of gestation , there are 4 types of globin genes which make Hb chains (alpha , gamma , zeta , epsilon)**

**** after first 5 weeks, when fetal Hb forms. Zeta & epsilon genes switches off , and the activated genes are for the globin chains (alpha & gamma chains) .**

**** after first 30 weeks = 6 months , when HB A2 is formed , gamma gene switches off and the delta gene switches on**

**** at birth , when HB A forms , delta gene switches off and Beta gene switches on ... the globin chains then will be (alpha & beta)**

Beta Globin Gene Cluster Chromosome 11



Alpha Globin Gene Cluster Chromosome 16

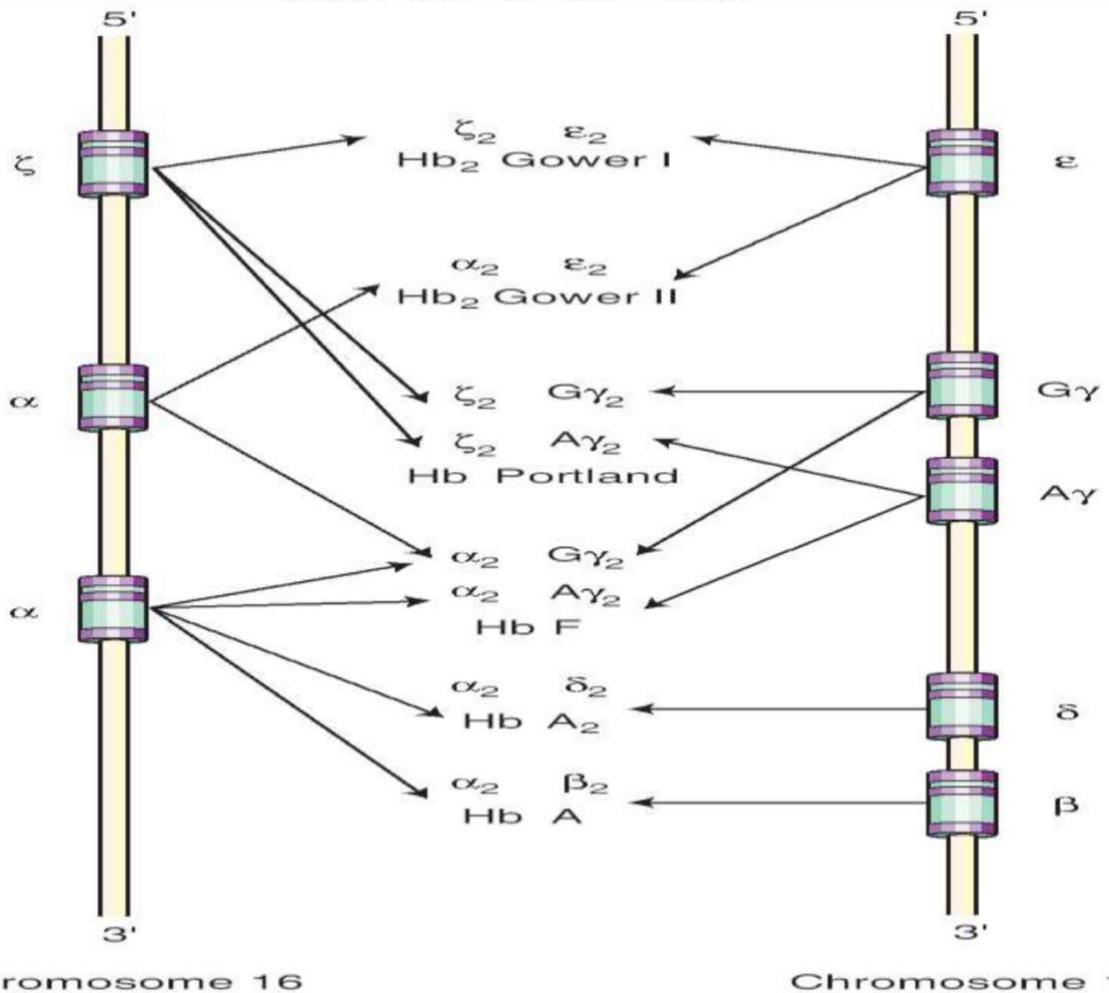


Figure 4.2 Specific chromosomes relative to human hemoglobin formation.