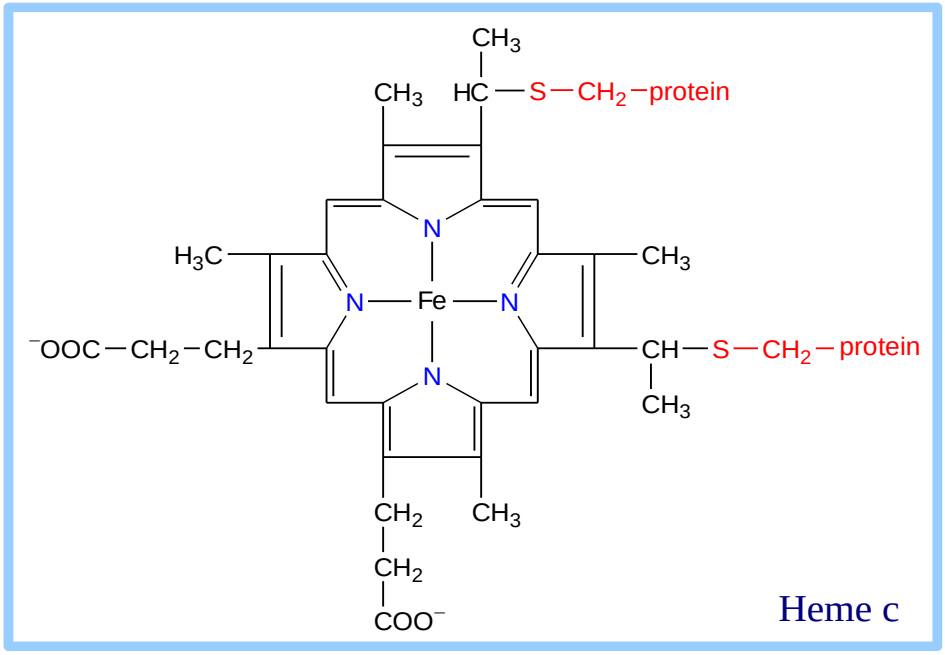
Hemoglobin synthesis

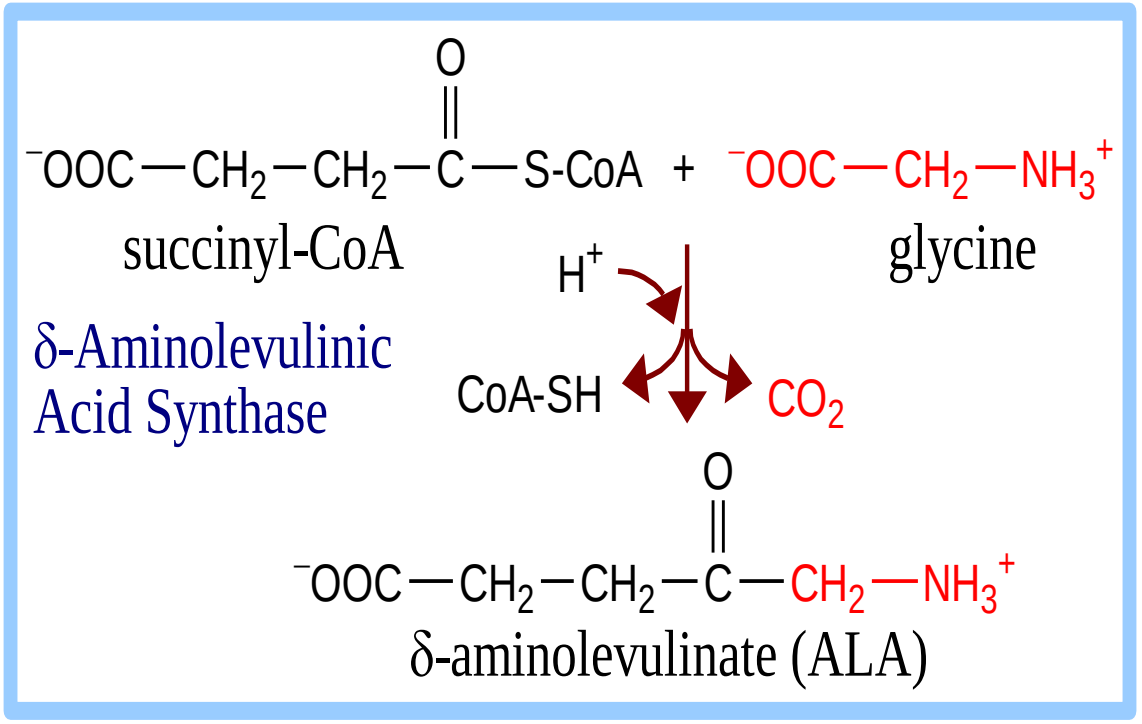
- Heme is the prosthetic group of hemoglobin, myoglobin, & cytochromes.
- Heme is an asymmetric molecule.



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- aminolevulinate synthase (ALA synthase), an enzyme related to transaminases.



- 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.

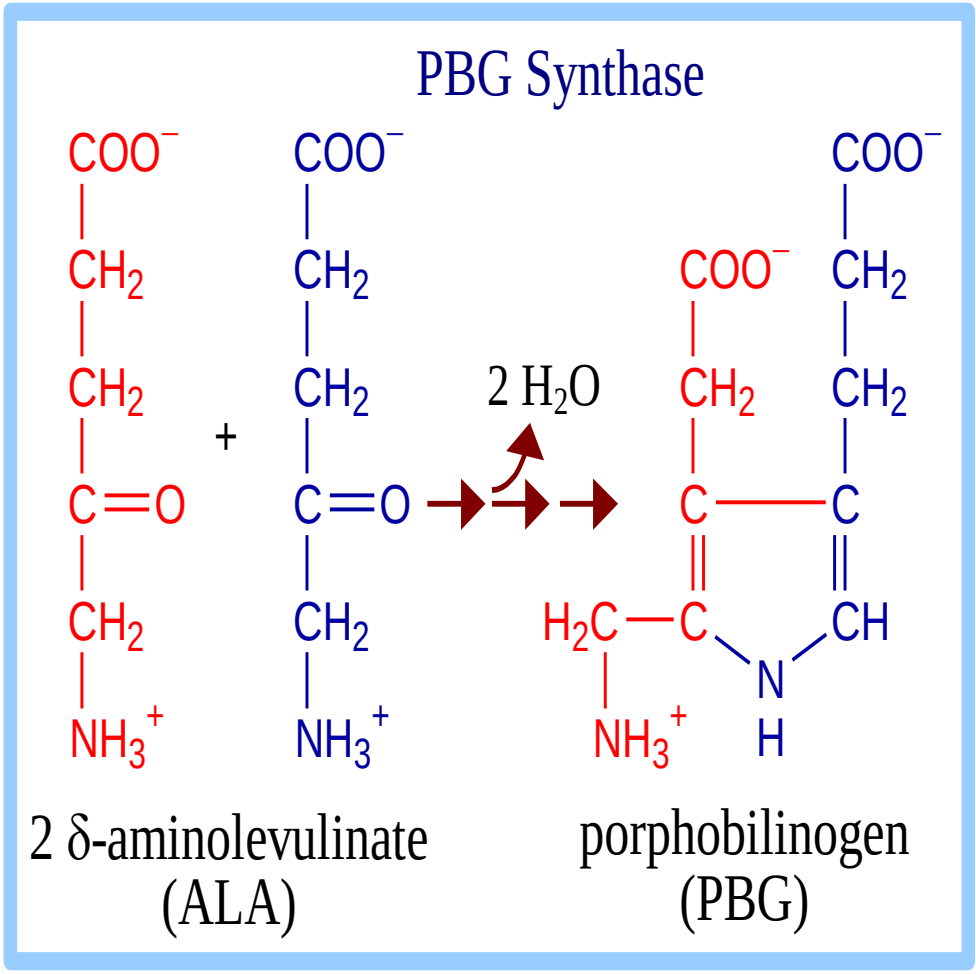
- A variant of ALA synthase expressed only in developing erythrocytes is regulated instead by availability of iron in the form of iron-sulfur clusters.

There are two forms of ALAS:

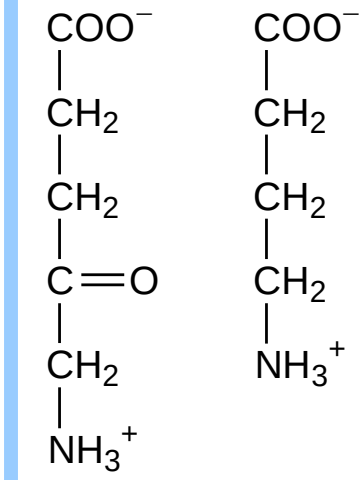
1-ALAS1 is considered a house-keeping gene and is expressed in all cells (located on chromosome 3).

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

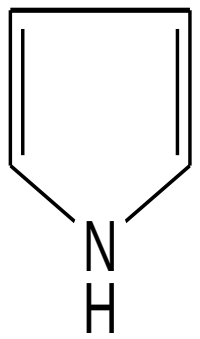


- High ALA is thought to cause some of the neurological effects of lead poisoning, although Pb^{++} also may directly affect the nervous system.
- ALA is toxic to the brain, perhaps due to:
  1- Similarity in the structures between ALA and GABA (γ- aminobutyric acid).
  2- ALA autoxidation generates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



ALA GABA

- 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.

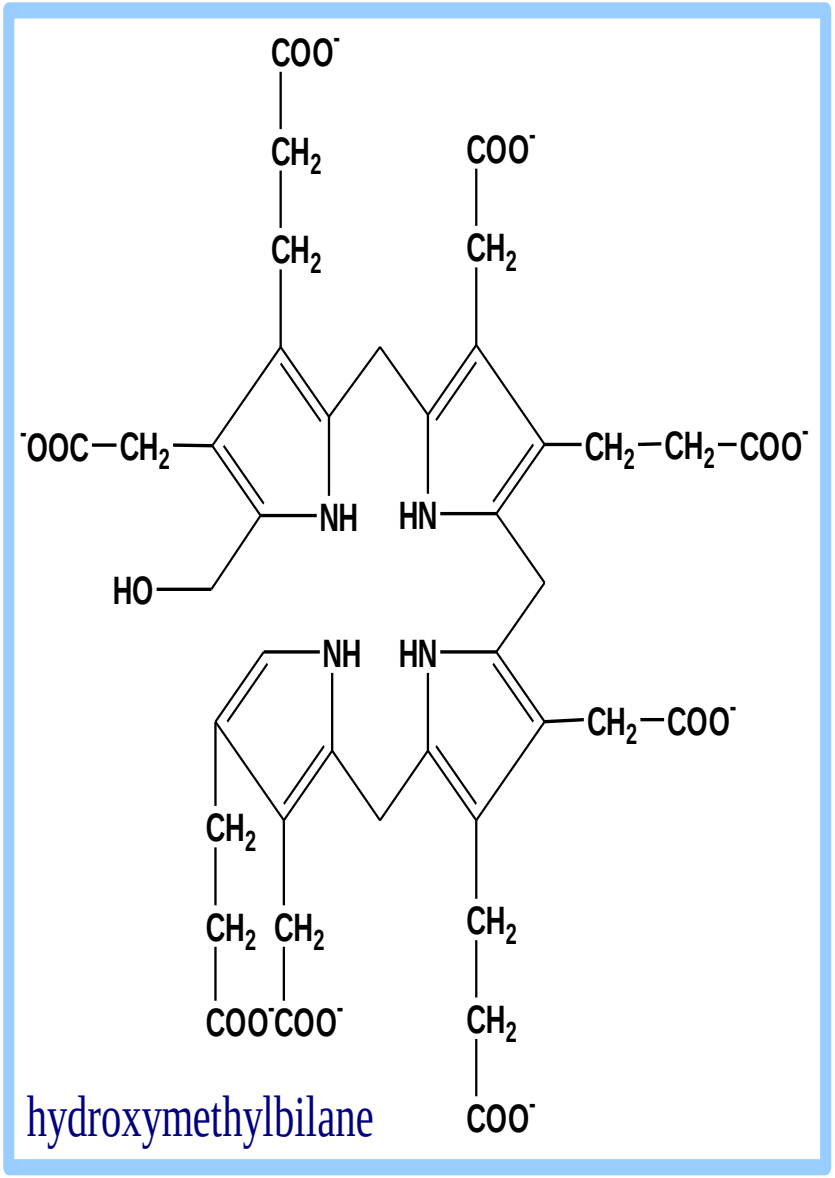


pyrrole

Hydroxymethylbilane has two fates:

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.

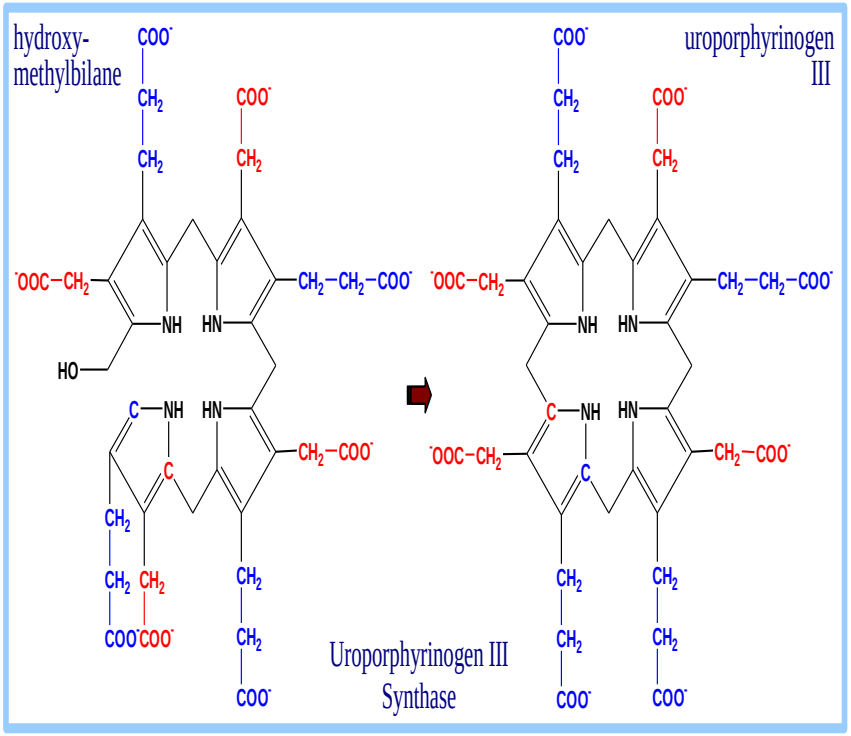


hydroxymethylbilane

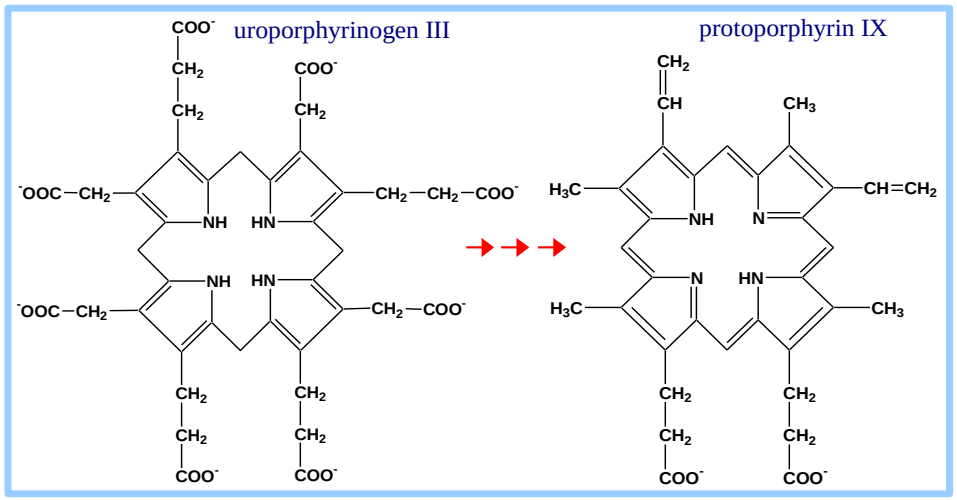
- Uroporphyrinogen III synthase converts the linear tetrapyrrole hydroxymethylbilane to the macrocyclic uroporphyrinogen III.
- Uroporphyrinogen III synthase catalyzes ring closure & flipping over of one pyrrole to yield an asymmetric tetrapyrrole.

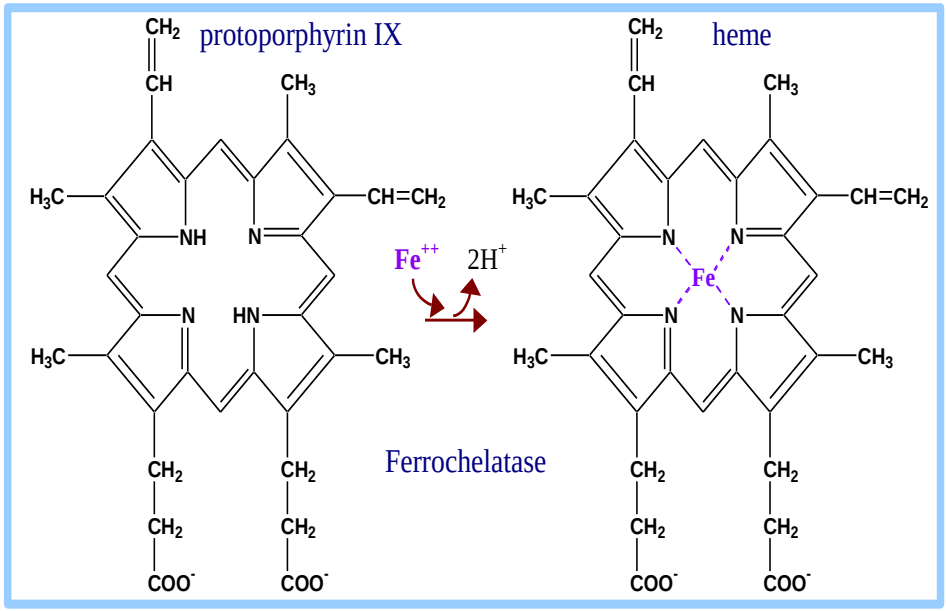
-The distribution of acetyl & propionyl side chains, as flipping over of one pyrrole yields an asymmetric tetrapyrrole.

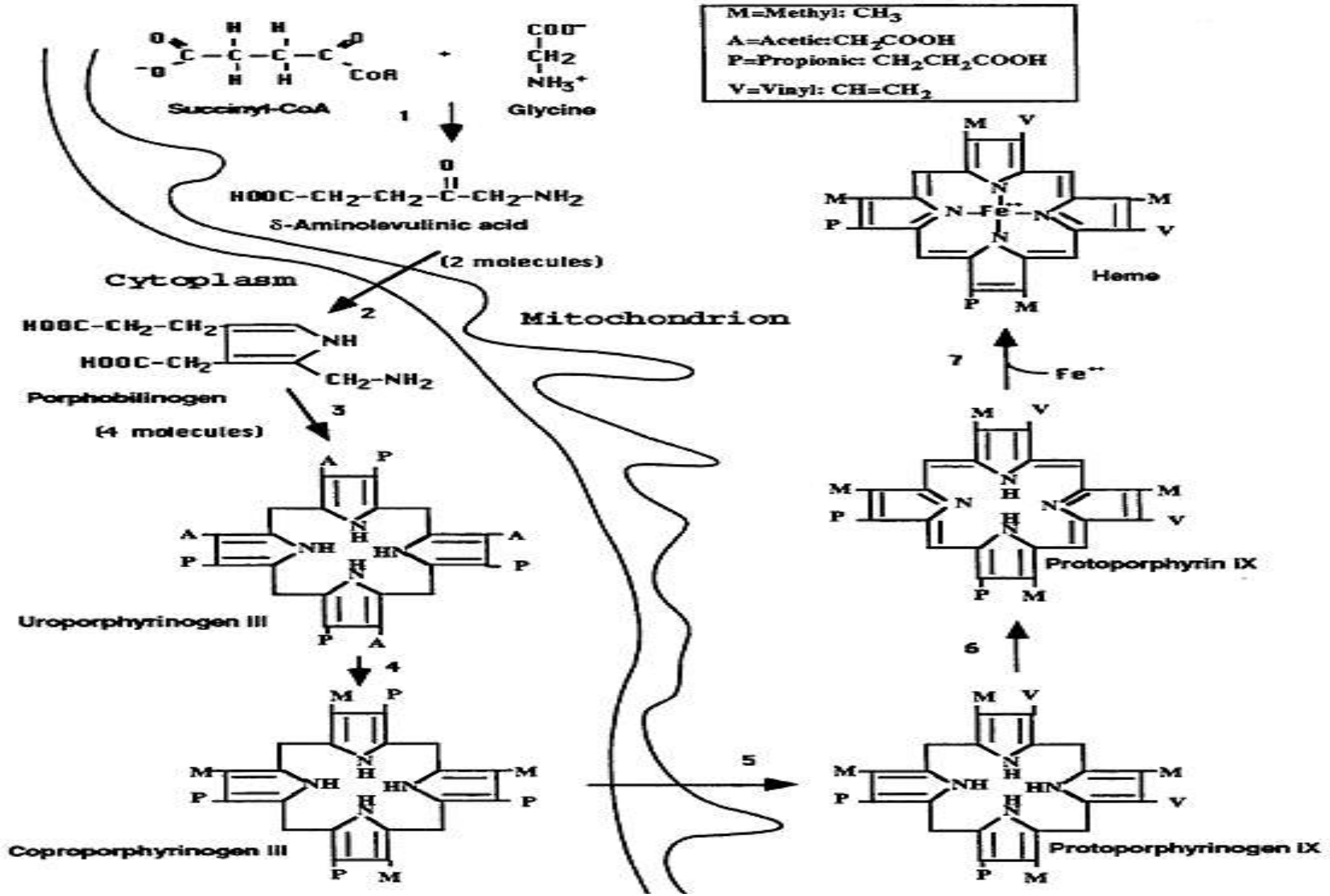
- Uroporphyrinogen III is the precursor for synthesis of vitamin B12, chlorophyll, and heme, in organisms that produce these compounds.



- 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 Ferrocheletase, 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.



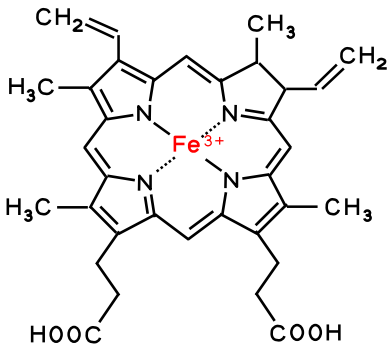




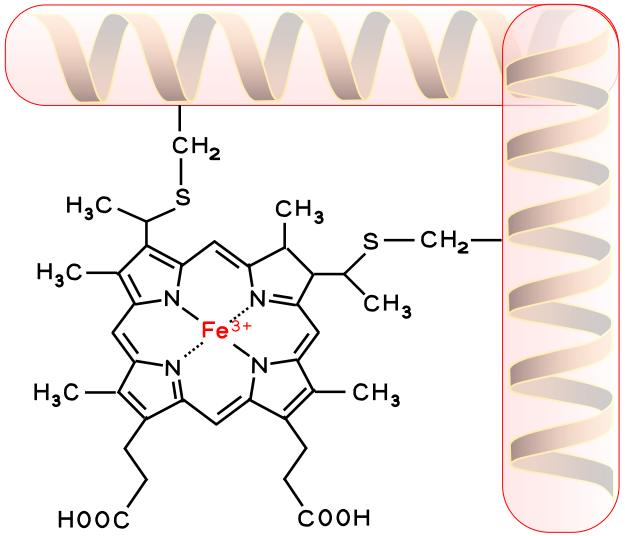
Pathway of Heme Biosynthesis

- 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.

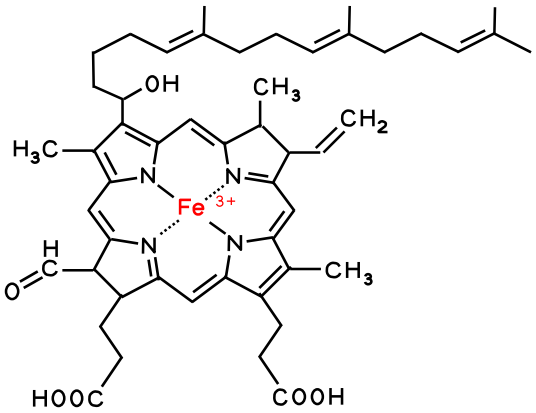
Heme *b*



Heme *c*



Heme *a*



- 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.

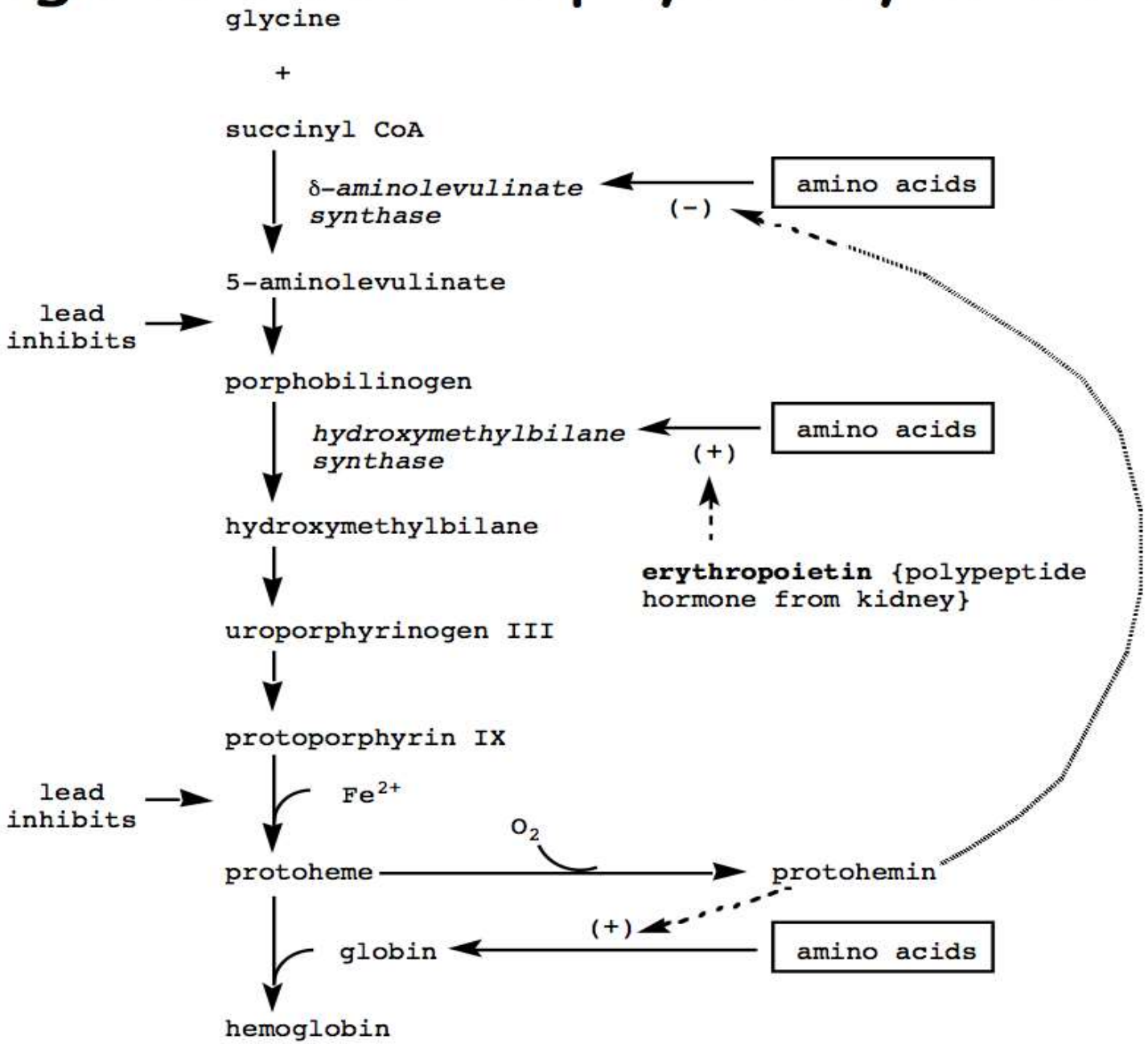
-When red cells mature both heme and hemoglobin synthesis ceases.
-The hemoglobin must, therefore, survive for the life of the erythrocyte.

- 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.

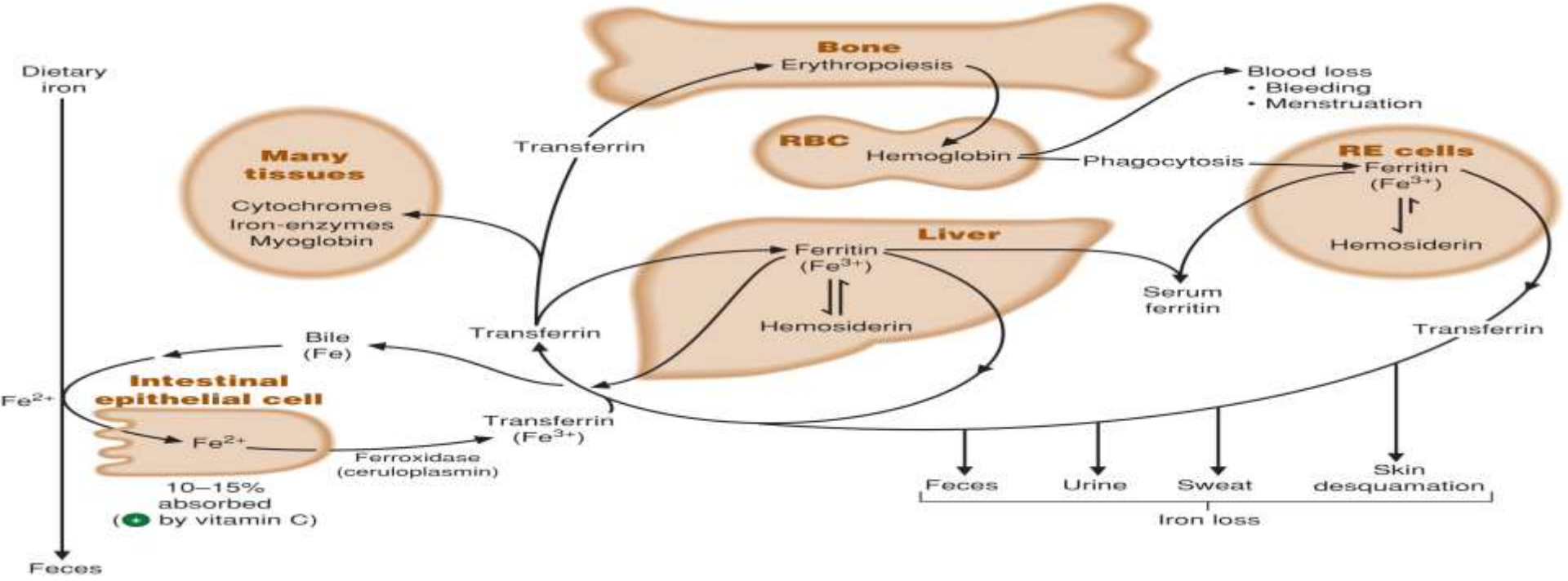
- Control has been shown to be exerted on ferrochelatase, the enzyme responsible for iron insertion into protoporphyrin IX, and on porphobilinogen deaminase.

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.



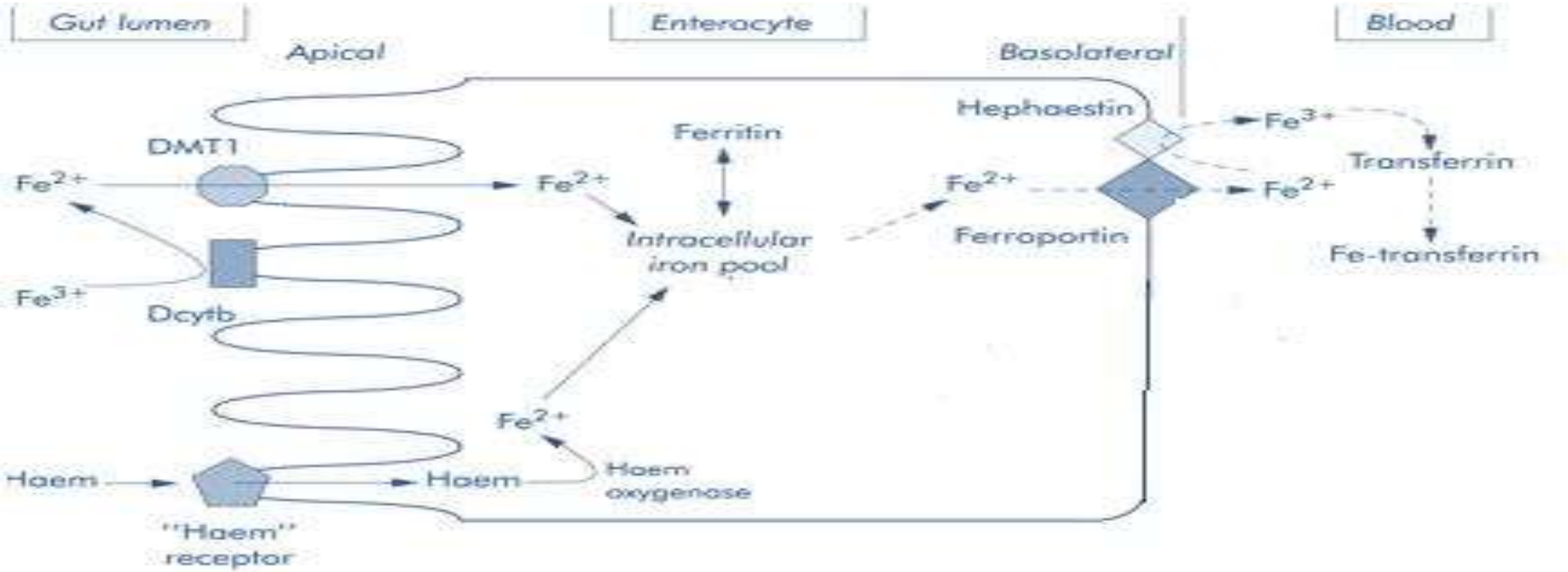
Iron metabolism and proteins

- 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, ceruloplamin 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.**

Absorption of Iron

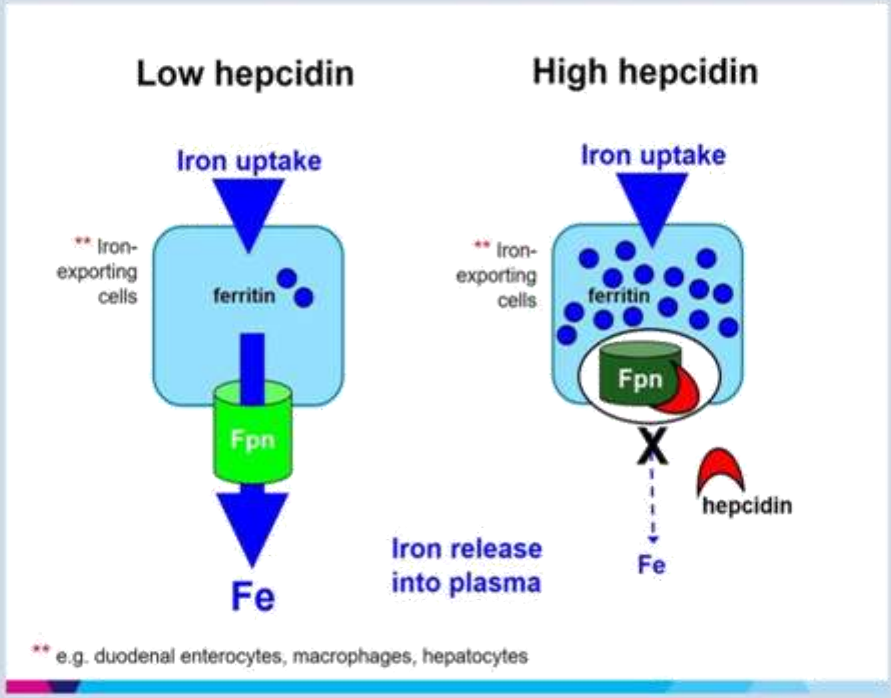


1- Iron stores within cells as a complex with apoferritin (ferritin), the main storage site is liver
2- Pass across basolateral membrane to be carried to transferrin through a protein ferroportin and hephaestin
3- Fe+2 is converted to Fe+3 by ferroxidase (Cu+2)
4- Hepcidin act as down regulator peptide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

Regulation of iron absorption and exportation by enterocytes

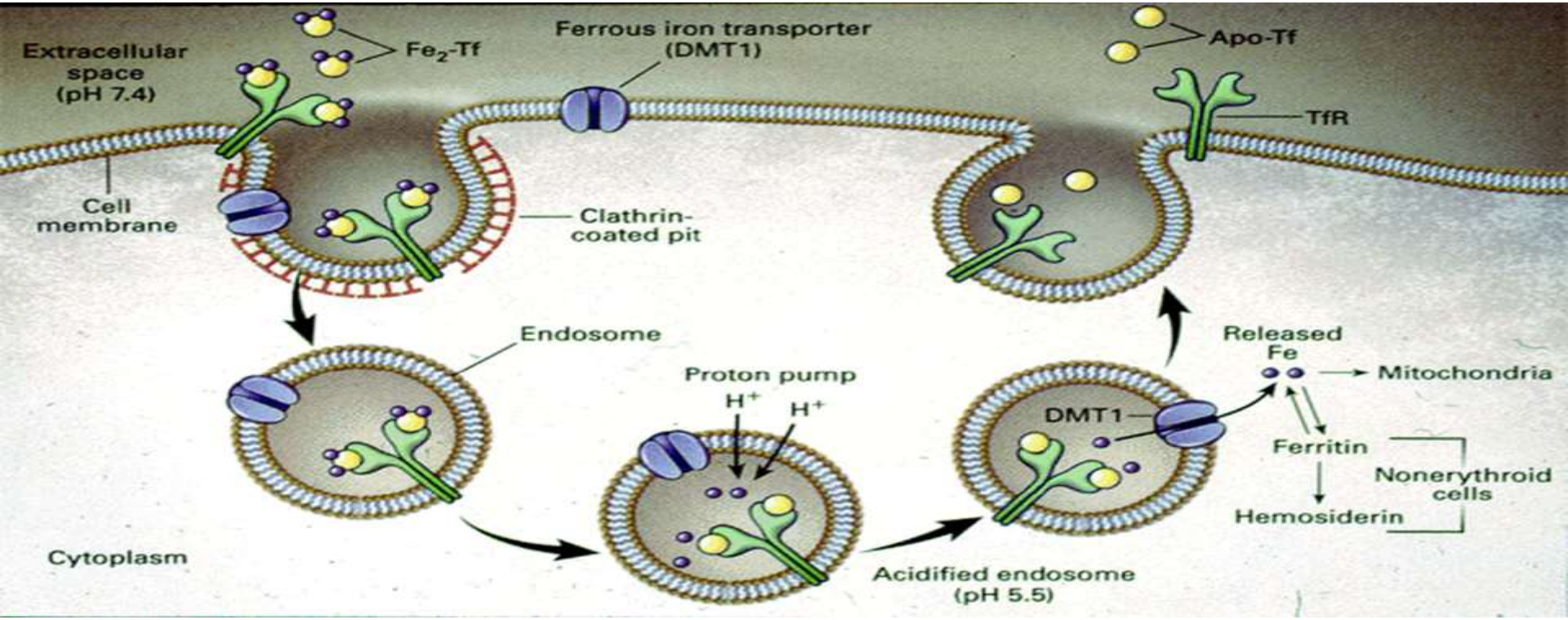
- Transcription of the gene for the iron transporter ferroportin is responsive to iron.
- When iron levels are high or in response to cytokines produced at sites of inflammation, hepcidin is secreted to induce ferroportin internalization and degradation, thus, leads to decreased absorption of dietary 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.

- 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.

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.

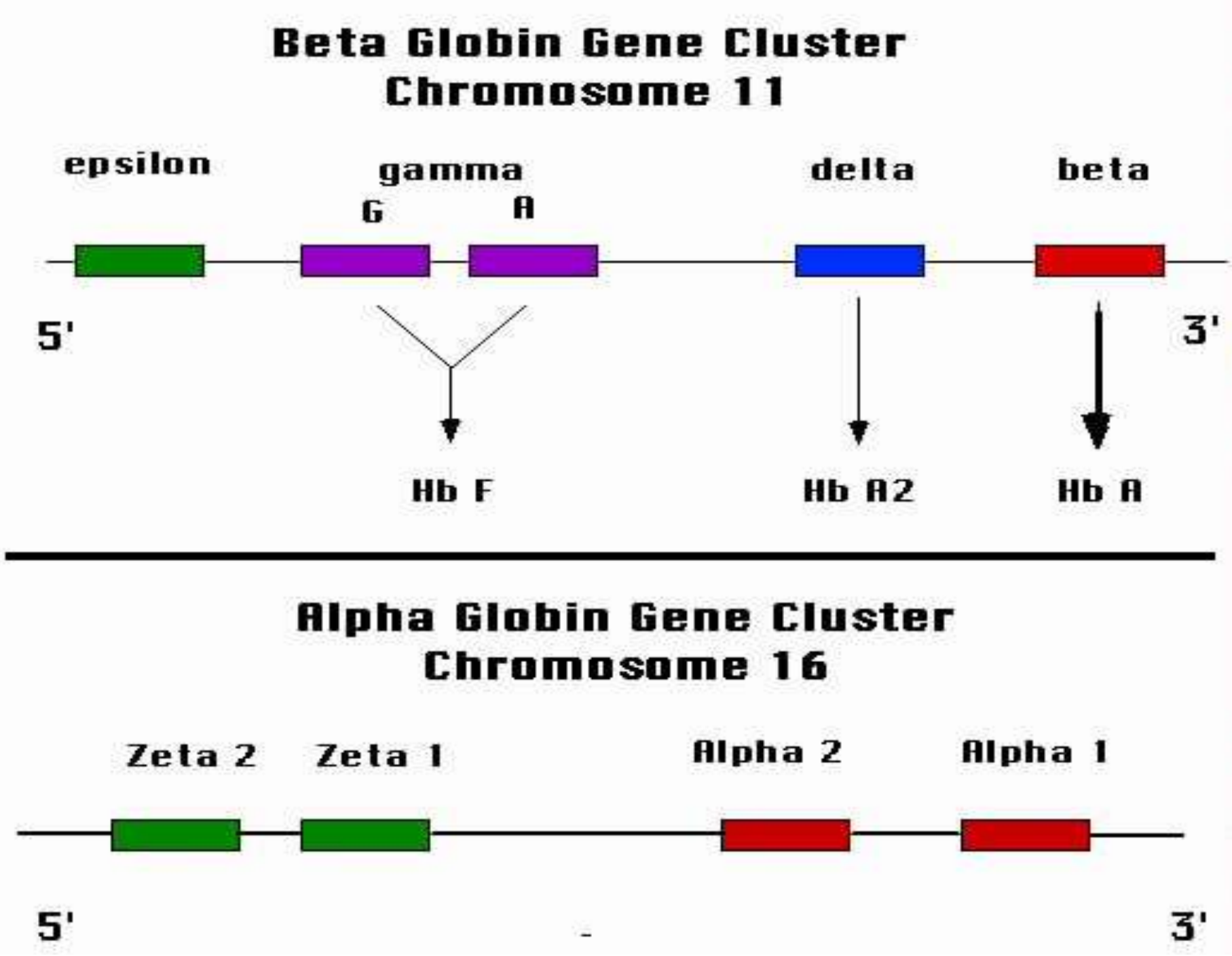
Globin synthesis

- 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: α,β,γ,δ,ε and ζ.

Type of Hb	Type of Globin Gene	Region	Time
Hb Gawer1 $(\zeta\ \varepsilon)_2$	ζ & ε	Yolk Sac	3 weeks of Gestation
Hb Portland$(\zeta\ \gamma)_2$	ζ &γ	Yolk Sac	5 weeks of Gestation
Hb Gawer II $(\alpha\varepsilon)_2$	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	$a_2\beta_2$	$a_2\delta_2$	$a_2\gamma_2$
Normal %	96-98 %	1.5-3.2 %	0.5-0.8 %


Beta Globin Gene Cluster
Chromosome 11
epsilon
gamma
G
A
delta
beta
5'
3'
Hb F
Hb A2
Hb A
Alpha Globin Gene Cluster
Chromosome 16
Zeta 2
Zeta 1
Alpha 2
Alpha 1
5'
3'

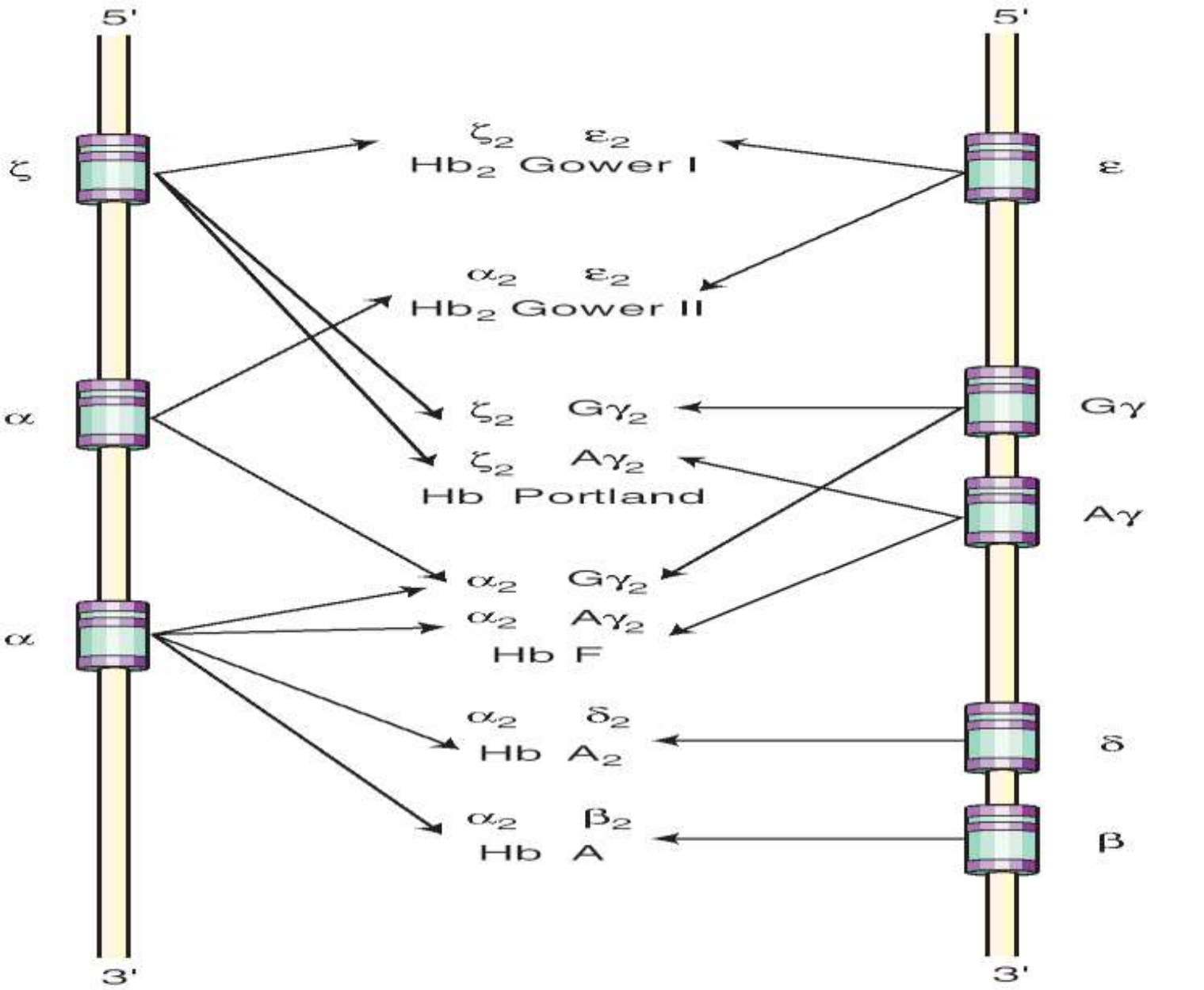


Figure 4.2 Specific chromosomes relative to human hemoglobin formation.