Metabolism of RBC & Hb structure & functions

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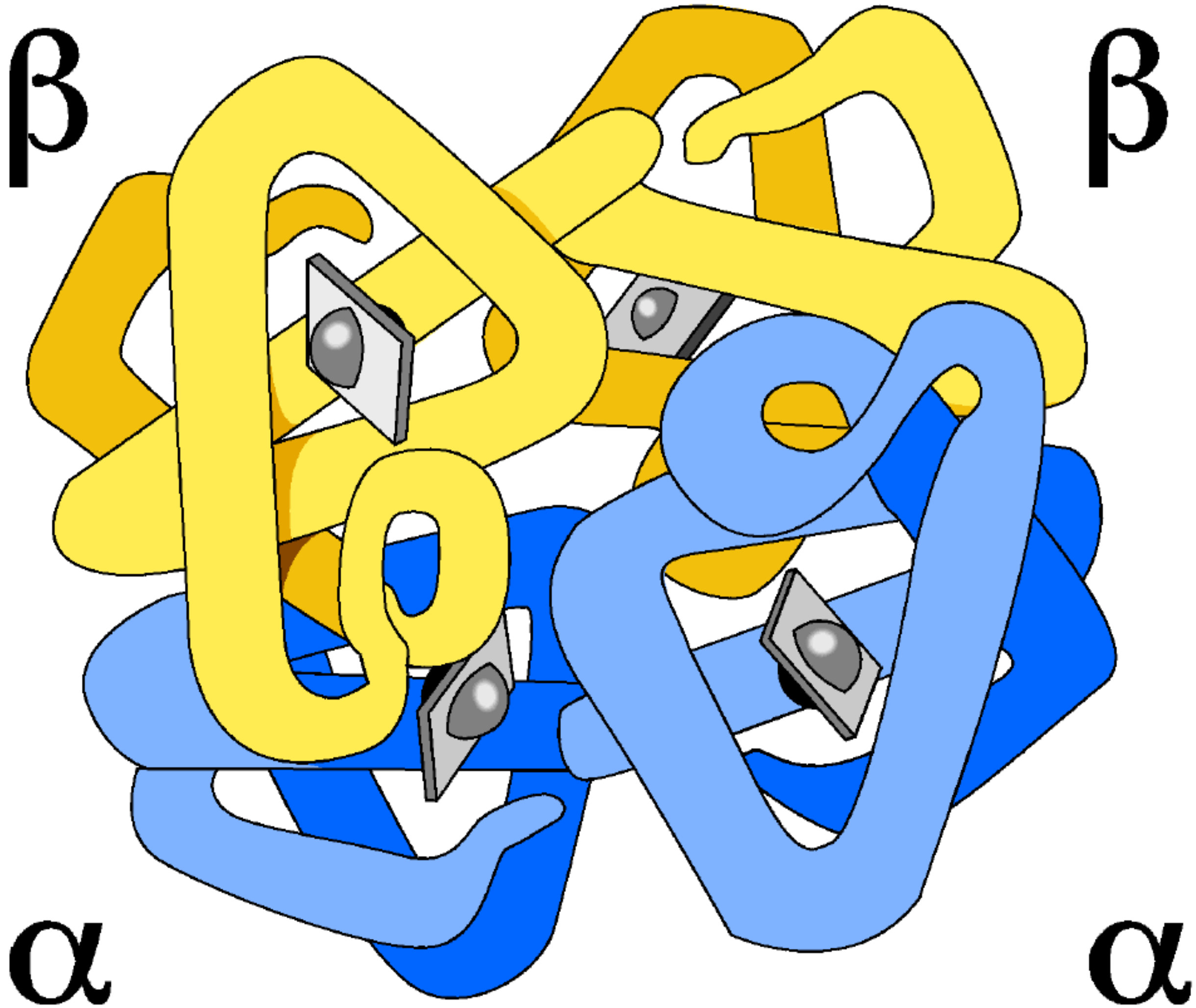
Introduction

- Hemoglobin- Tetrameric protein
- Hemoglobin

✓ Globin- unbranched chain of amino acid residues

✓ Heme- Tetrapyrrole ring with a central iron atom

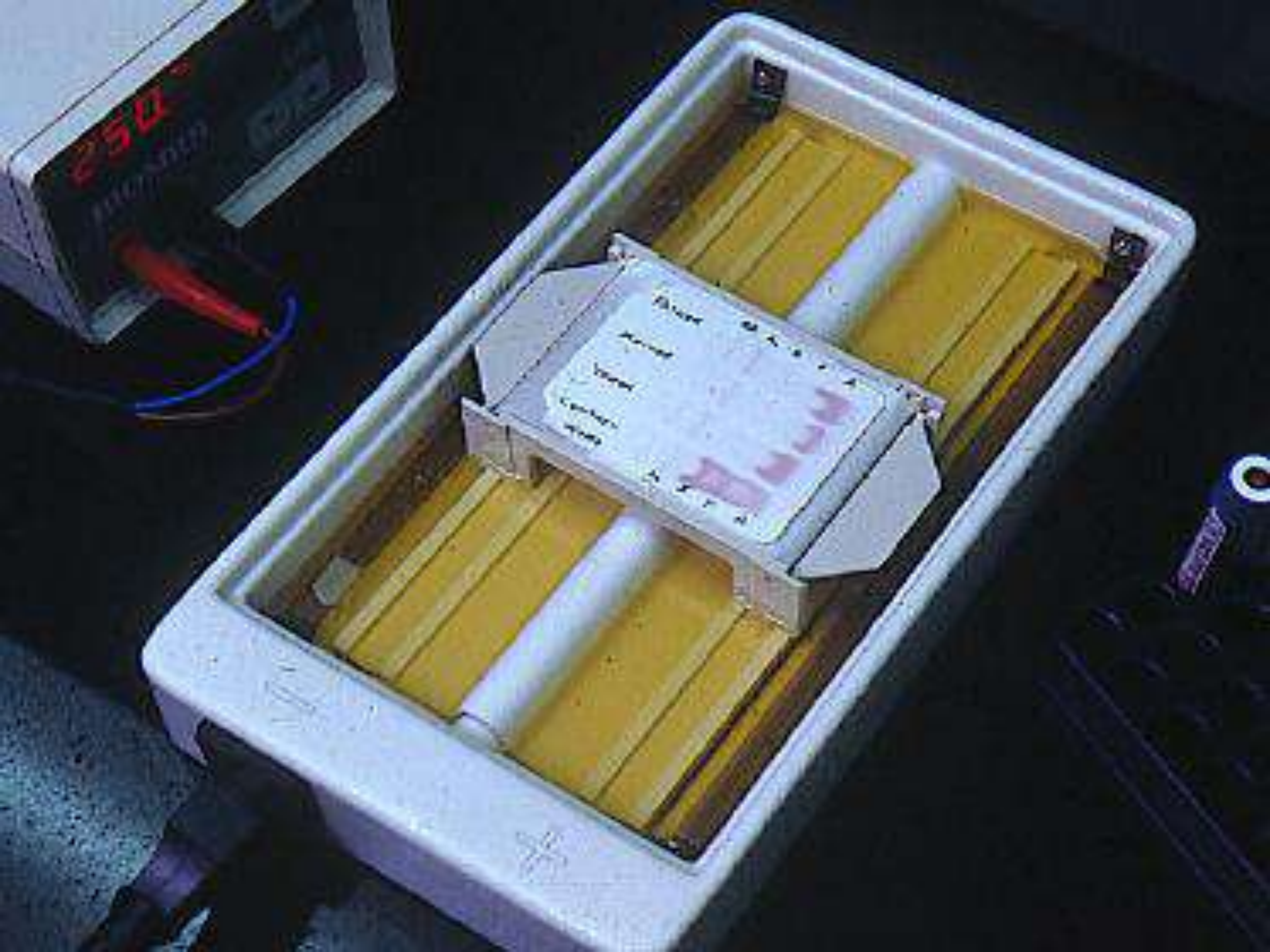
Formation of Hemoglobin



Heme

+

Globin

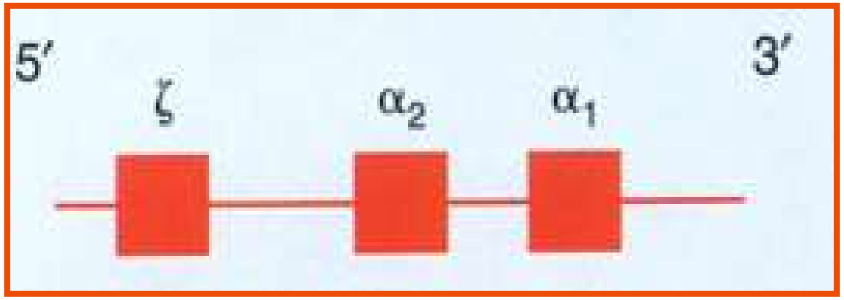


Globin Synthesis

- Occurs in the cytoplasm of the normoblast and reticulocyte.
- The polypeptide chains- on the ribosomes.
- Synthesis depends on transcription of precursor mRNA from DNA from the erythroid nucleus.

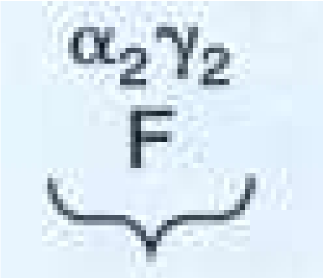
Globin Chain Synthesis

Chromosome 16

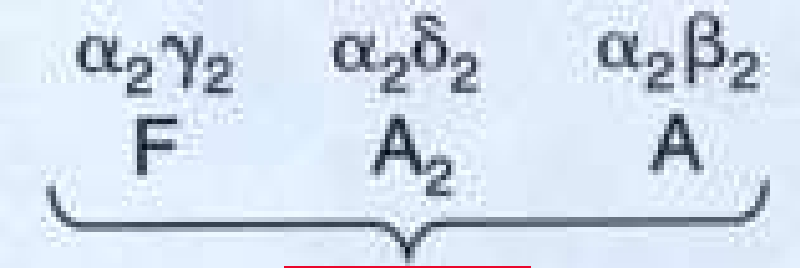


Chromosome 11

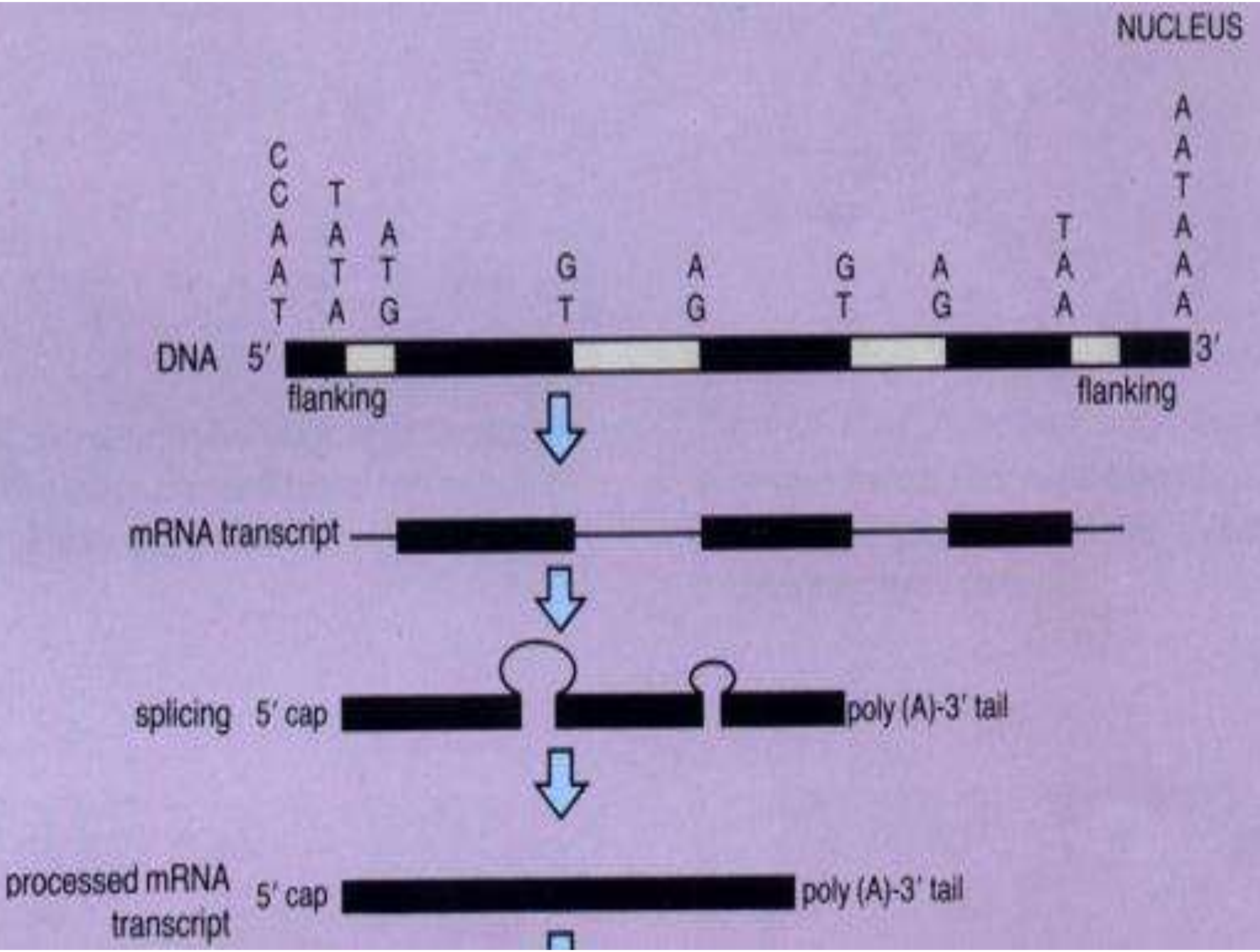
Embryo



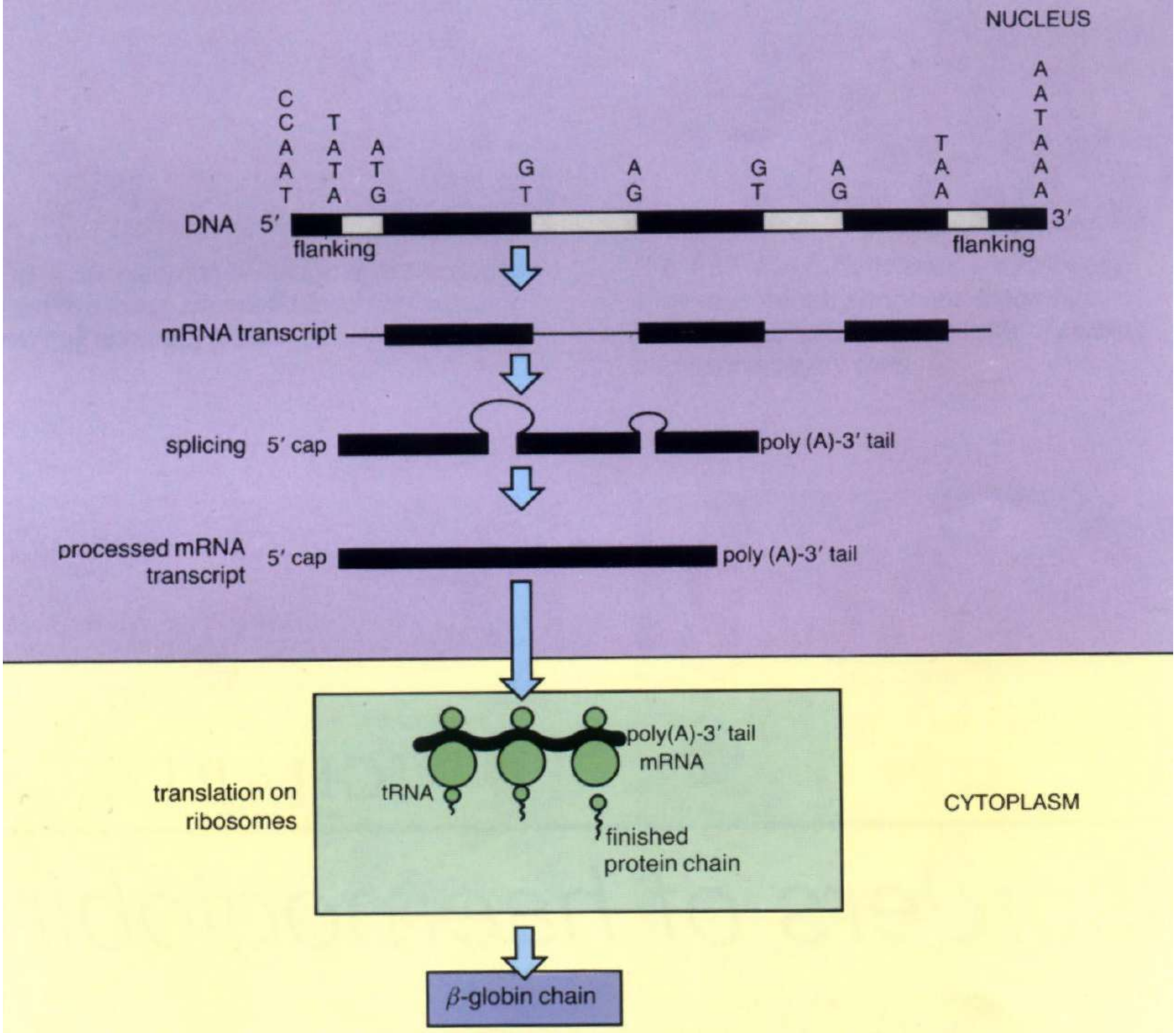
Fetus

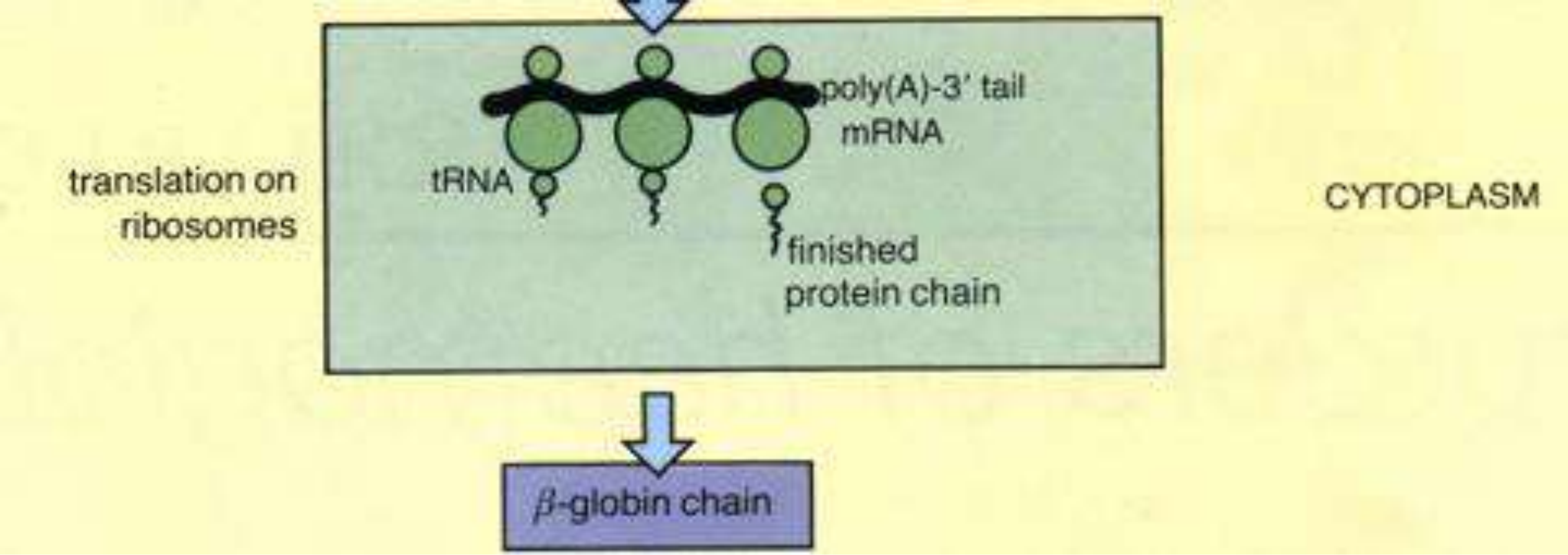


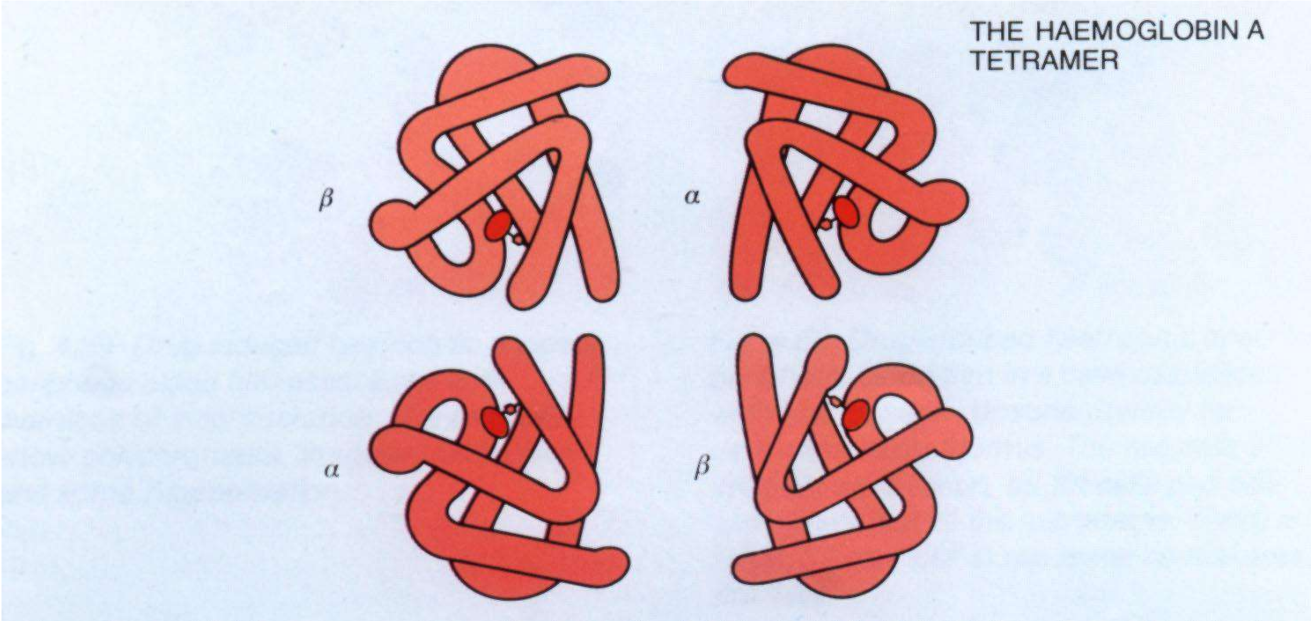
Adult



NUCLEUS
CCAAT
TATA
ATG
GT
AG
GT
AG
TAA
AATAAA
DNA
5′
3′
flanking
flanking
mRNA transcript
splicing
5′ cap
poly (A)-3′ tail
processed mRNA transcript
5′ cap
poly (A)-3′ tail


NUCLEUS
C C A A T
T A T A
A T G
G T
A G
G T
A G
T A A
A A T A A A
DNA 5′
3′
flanking
flanking
mRNA transcript
splicing 5′ cap
poly (A)-3′ tail
processed mRNA transcript
5′ cap
poly (A)-3′ tail
poly(A)-3′ tail
mRNA
tRNA
finished protein chain
translation on ribosomes
CYTOPLASM
β-globin chain


poly(A)-3' tail
mRNA
translation on ribosomes
tRNA
CYTOPLASM
finished protein chain
β-globin chain


THE HAEMOGLOBIN A TETRAMER
β
α
α
β

Heme Synthesis

- Heme is a tetrapyrrole ring with a central iron atom linked with four nitrogen atom

Heme Synthesis (2)

- Succinyl coenzyme A + Glycine
  Vit B6 ↓ ALA Synthetase(ALAS2)-
  Mitochondria
  α Amino β keto adipate (Unstable)
  ↓ Decarboxylation
  δ amino Levulinic acid (ALA)

- 2δ amino Levulinic acid (ALA)
  ↓ ALA dehydratase
  Porphobilinogen

Heme Synthesis (3)

Porphobilinogen

↓ Porphobilinigen deaminase

pyrole intermediate

↙ ↘

Uroporphyrinogen III   Uroporphyrinogen I

↓

Coproporphyrinogen III

↓

Protoporphyrinogen III

↓

Protoporphyrin IX (Mature RBC)+ Fe^{++}

↓ Ferochelatase

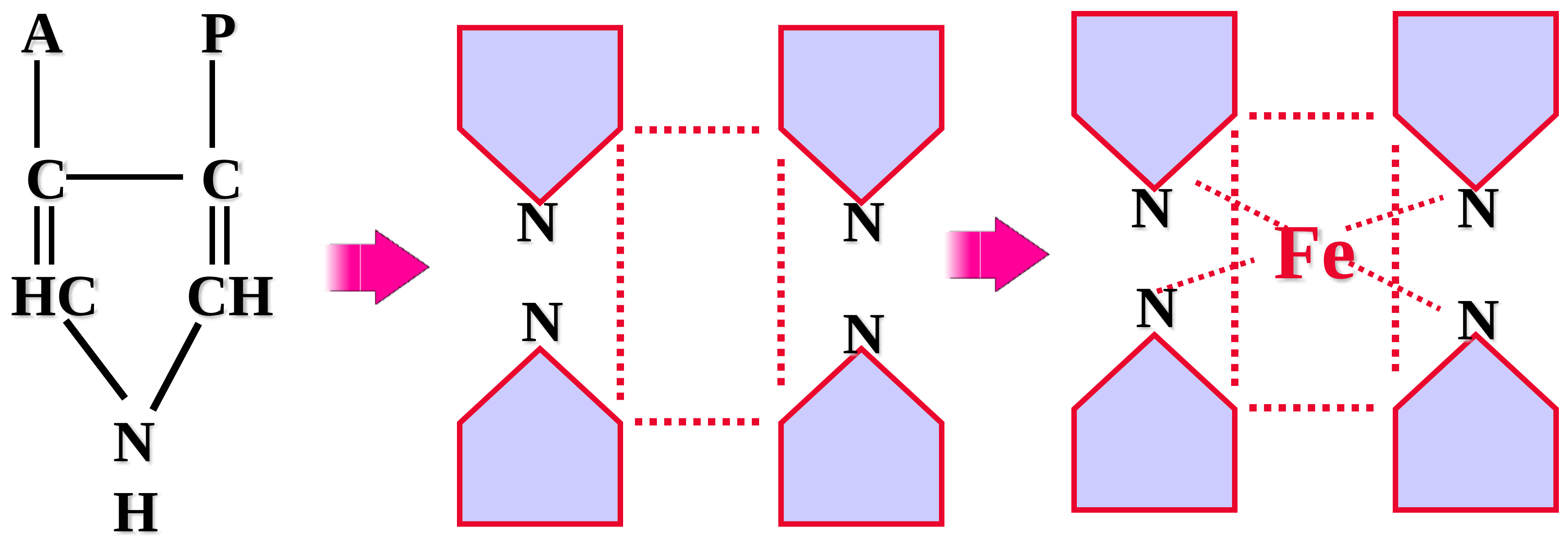
Heme

Heme Synthesis (4)

- Uroporphyrin III, Coproporphyrin III, Uroporphyrin I, Coproporphyrin I – Excreted in urine & feces in small amt
- Porphobilinogen- normally smaller amt in urine but markedly elevated in acute intermittent porphyria – detected by Ehrlich's aldehyde reagent

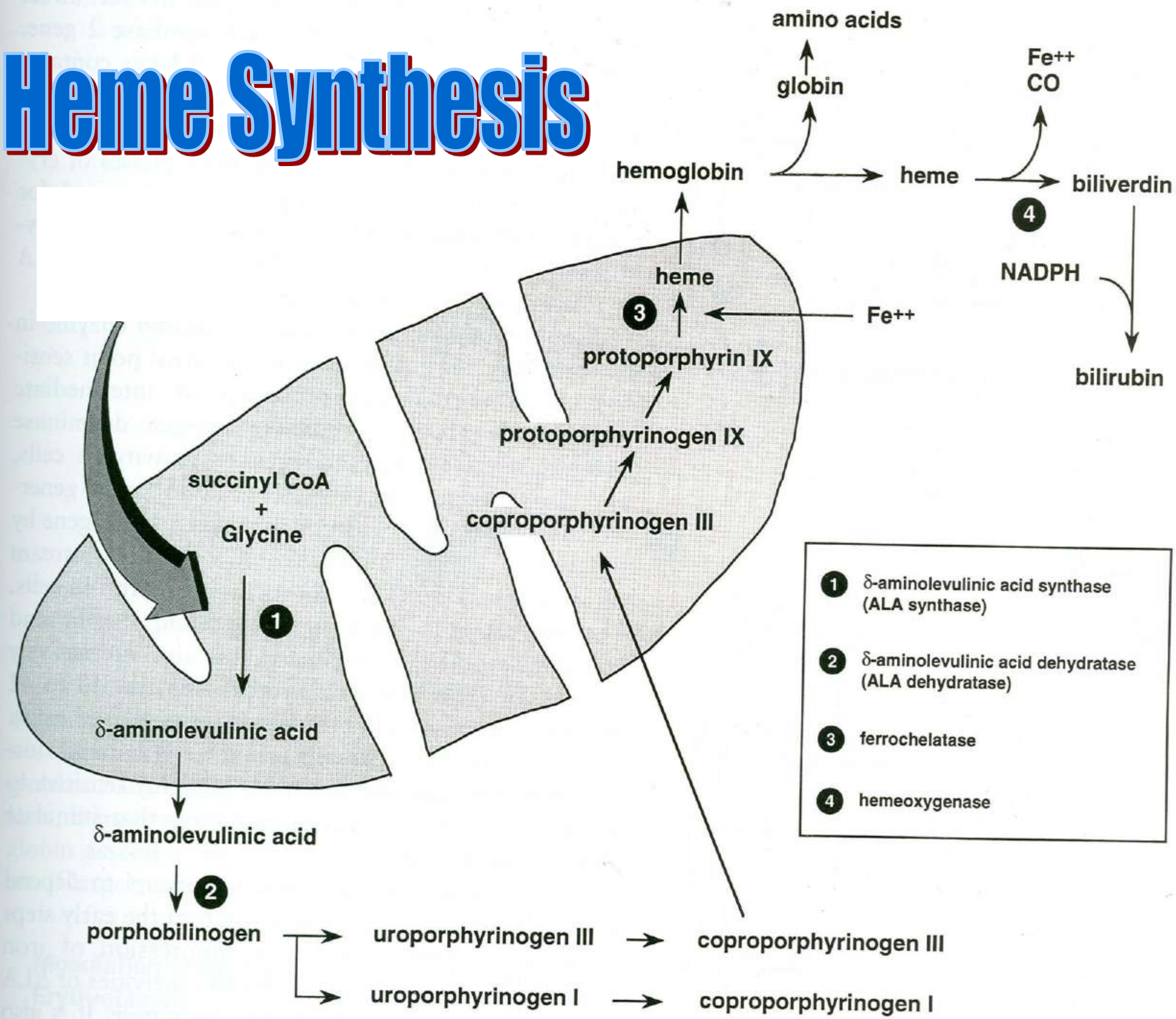
Heme Synthesis

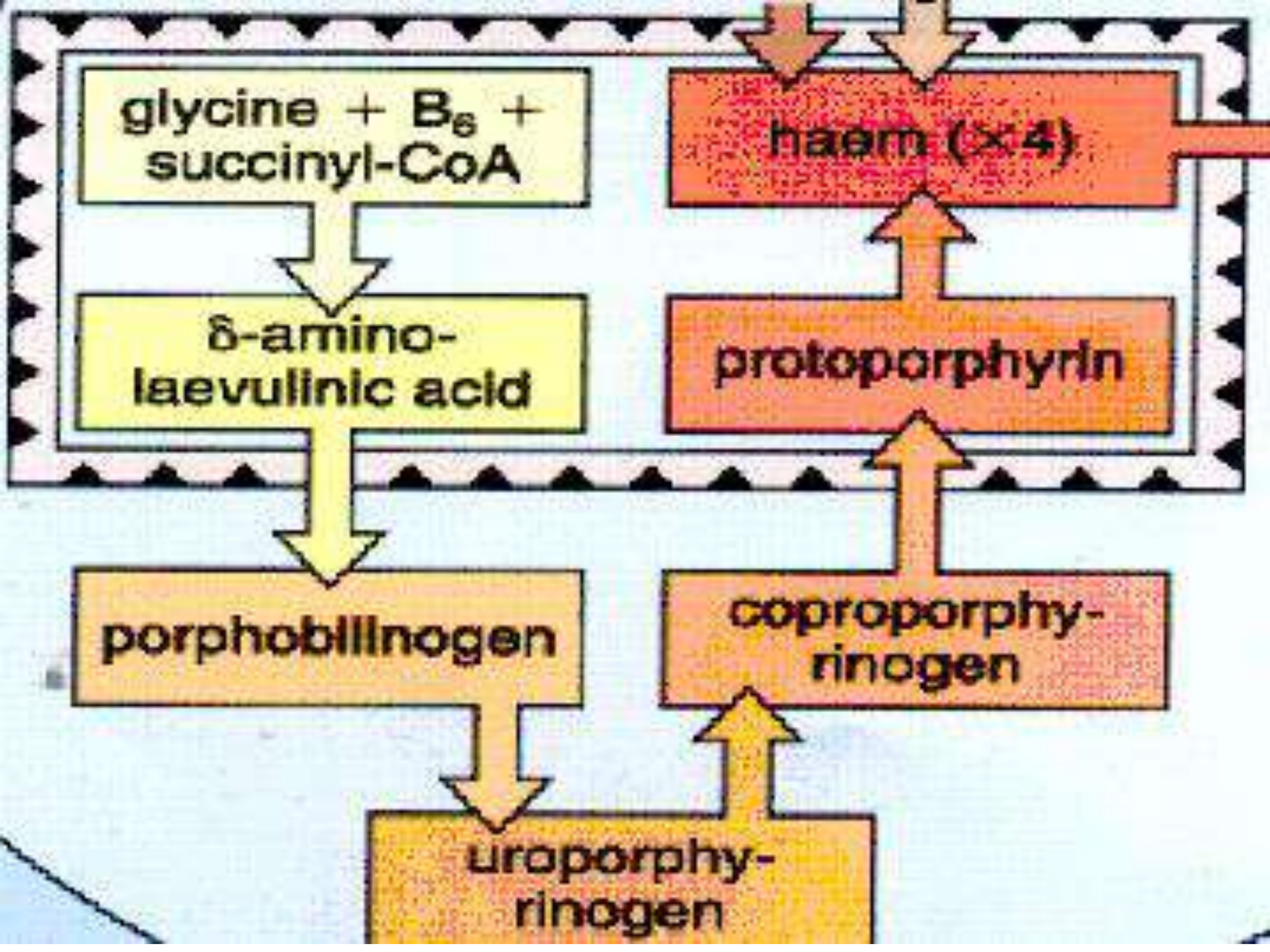
2 Sccinyl CoA + 2 Glycine → Pyrrole Ring
B_6



Pyrrole Ring Protoporphyrin IX Heme

Heme Synthesis




glycine + B_6 +
succinyl-CoA
δ-amino-
laevulinic acid
porphobilinogen
uroporphy-
rinogen
coproporphy-
rinogen
protoporphyrin
haem (×4)

Heme Synthesis (5)

- Erythroid heme production is tightly linked to iron availability and globin synthesis
- Control of heme production-

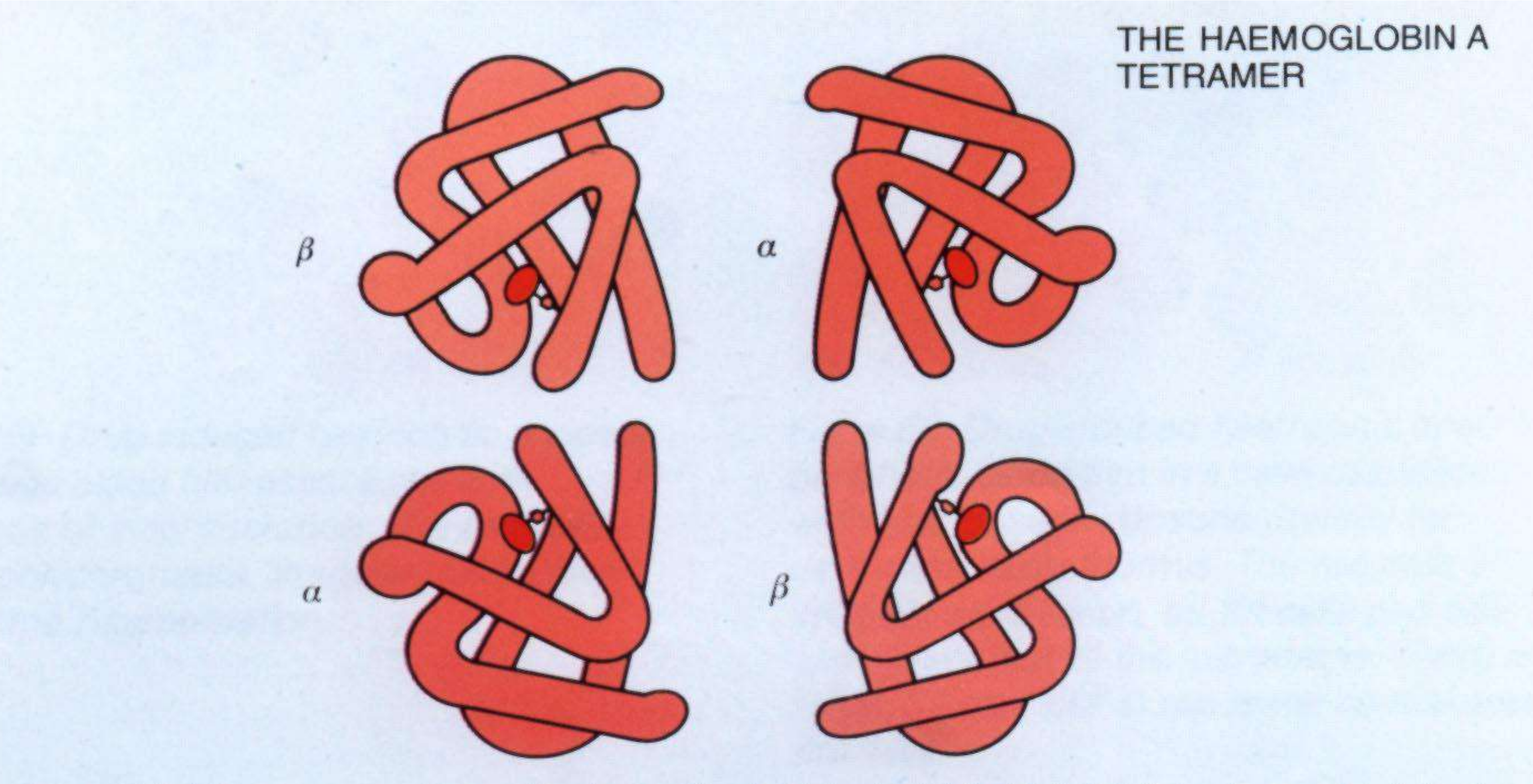
✓ Heme, when excessive, inhibits cellular iron uptake and may inhibit *ALAS2* transport to mitochondria

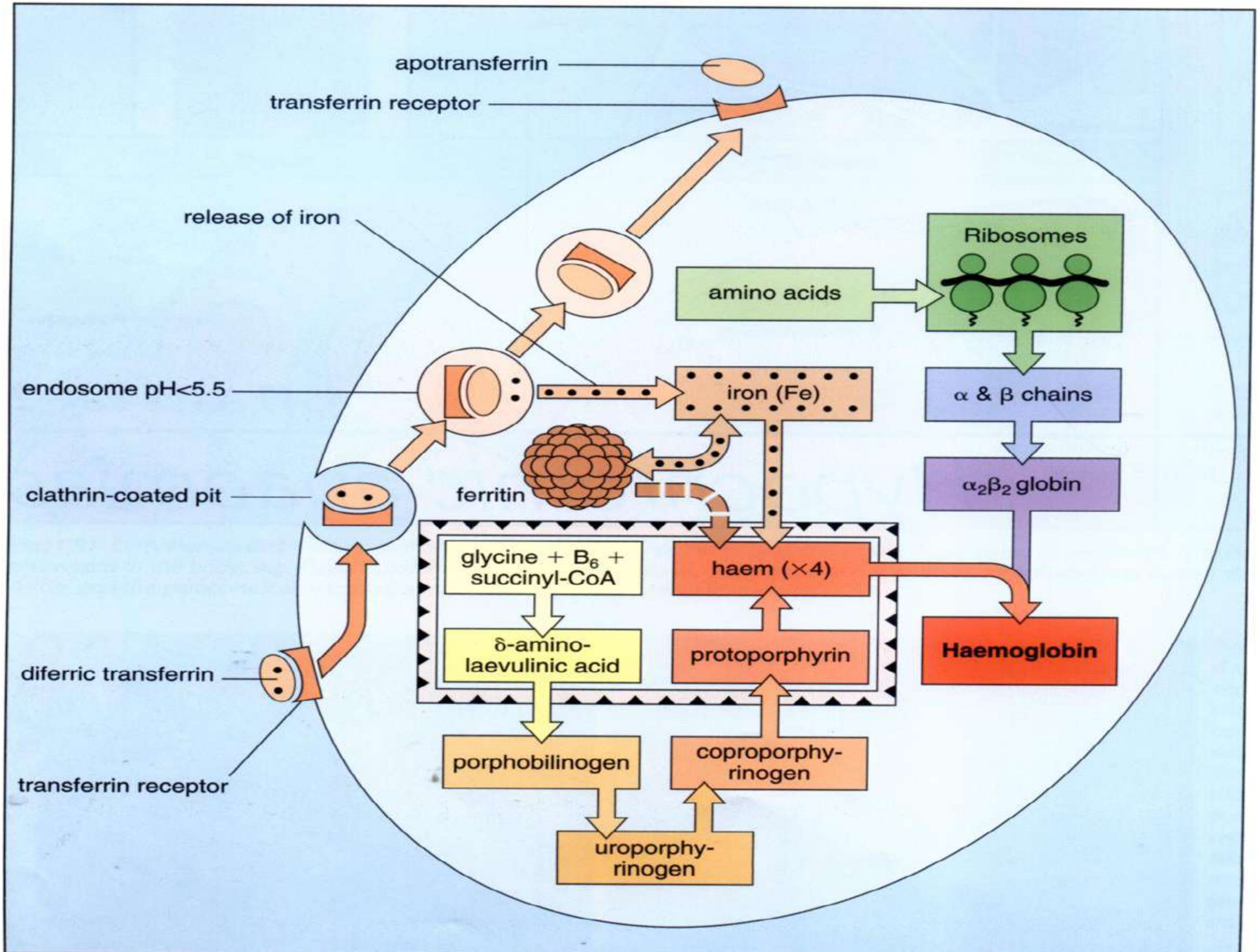
✓ porphobilinogen deaminase- increases with acceleration of erythropoesis

Tetramer Assembly

- Final assembly-spontaneously in the cytoplasm
- Binding of heme with the globin monomer occur during or just after globin transcription as chain is adopting secondary or tertiary configuration
- Dimers- α and non-α chain
- Formation- Fecilitated by difference in electrostatic charges at physiologic pH (α +ve charge, β -ve charge) and by the large number of specific contacts along the α-β interface.

Globin Chain Synthesis





apotransferrin
transferrin receptor
release of iron
Ribosomes
amino acids
endosome pH<5.5
iron (Fe)
α & β chains
clathrin-coated pit
ferritin
α2β2 globin
glycine + B6 + succinyl-CoA
haem (×4)
δ-amino-laevulinic acid
protoporphyrin
Haemoglobin
diferric transferrin
porphobilinogen
coproporphy-rinogen
transferrin receptor
uroporphy-rinogen

Hb species in humans

NAME	FORMULA	FUNCTION
A	$\alpha 2\ \beta 2$	Predominant Adult Hb
A2	$\alpha 2\ \delta 2$	Minor adult Hb(~2.5%)
F	$\alpha 2 \gamma 2$	Major fetal Hb(<1%in adult)-binds O2 more tightly
Gower	$\alpha 2\ \varepsilon 2$	Embryonic Hb
Portland	$\varepsilon 2\ \gamma 2$	

Hb Function

- Oxygen transporter
- Reversible oxygenation with cooperativity, bohr effect, carbon dioxide effect, the chloride effect, 2,3-BPG effect, effects associated with nitric oxide and temperature effect

Reversible Oxygen Binding

- $Hb + 4O_2 \rightleftharpoons Hb(O_2)_4$
- Theoretical maximal binding- 1.39 ml O_2 per gram of Hb
- Oxygen Equilibrium curve (OEC) – Sigmoidal.
- Sigmoidicity- cooperativity or heme-heme interaction, a phenomenon in which the oxygenation of one subunit of Hb tetramer alters the O_2 affinity of other subunit

Reversible Oxygen Binding(2)

- Cooperativity- depends on the presence of two different types of globin chain. (Exception-Hb Bart β4)
- Oxygenation of Hb begins in one of the α subunits, which under physiological conditions are thought to have higher affinity
- Binding of the first O2 causes the affinity of other subunit tend to increase.
- Shift to right- more oxygen is released for any given decrease in pressure

Molecular Basis

- Cooperative effect in an enzyme (or Hb) arise from an equilibrium between two different physical structure that occurs as a result of ligand binding
- Hb- molecular wt 64,500 daltons

✓Gobular shape

✓Diameter- 5 -6.5 nm

Linked Functions of Hb

- Oxygenation and deoxygenation
- Effect of ligands, oxygenation and temperature on Hb-O2 affinity

Increased affinity	Decreased affinity
O2 binding by deOxy	O2 release from oxyHb
pH <6	pH >6
↓ in temperature	↑ in temperature
Carbon monoxide	2,3 BPG

Bohr Effect

- Christian Bohr
- The change in oxygen affinity caused by a change in carbon dioxide concentration
- The alkaline Bohr effect- ↑ in hydrogen ions (pH >6) shifts the OEC to the right and a ↓ shifts it to left

Factors affecting the Oxygen Affinity

- CO_2 effect- ↑ in CO_2 binding decreases the affinity to O_2 & oxygen uptake causes CO_2 binding to ↓
- Chloride Effect-Accounts for approximately one half of the Bohr effect. Hydrogen and chloride ions ↓ oxygen affinity.

Factors affecting the Oxygen Affinity (2)

- 2,3 BPG effect- It lowers intra erythrocytic pH and thereby ↑ P50.
- Nitric Oxide Effect
- Temperature Effect- An ↑ in temperature shifts the OEC to the right.

Stored Blood

- OEC- shifted to left (level of 2,3 BPG in the RBC decreases to very low level by about 10 days)
- Rate of decrease depends on rate of cooling to room temp, duration of room temp holding, solution pH
- Massive transfusion with units stored for more than 7 to 10 days produces a marked ↑ in oxygen affinity in vivo.

Novel Therapies

- RSR13, a compound, derivative of antilipemic drug bezafibrate, crosses the RBS membrane & interact with Hb at a locus different and shifts OEC to right.
- Modification of RBC by means of incorporation of inositol hexaphosphate. It has a powerful effect on the OEC of human Hb.

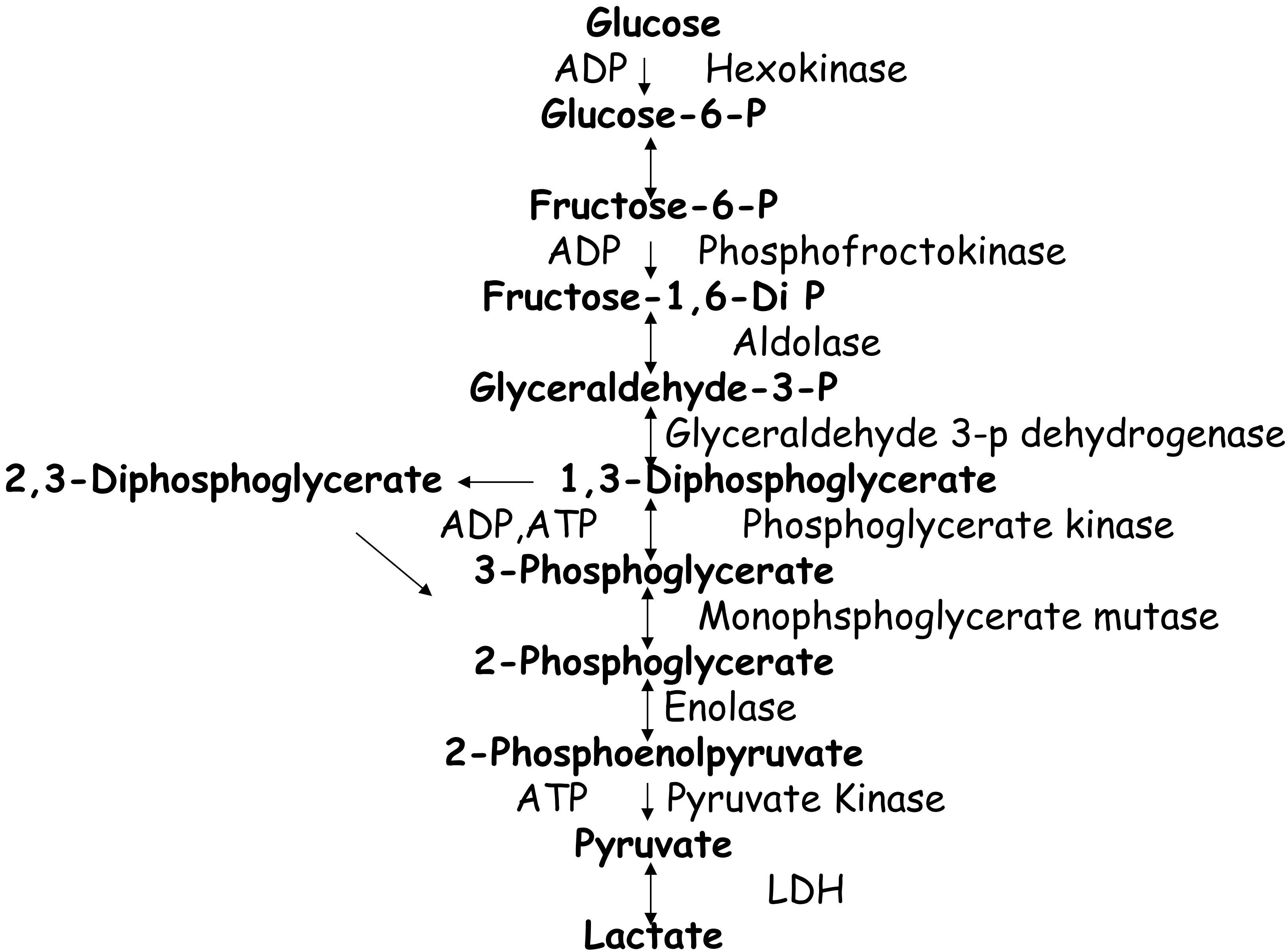
RBC Metabolism

Introduction

- Lacks nucleus and mitochondria
- Cannot divide
- Life-120 days
- Cannot synthesis protein
- Use oxygen for extraction of energy
- Transportation of oxygen and carbon dioxide

Metabolism of Glucose

- Energy is derived mainly from the breakdown of glucose to lactate or pyruvate.
- Known as the Glycolytic or the Embden-Meyerhof pathway


Glucose
ADP
Hexokinase
Glucose-6-P
Fructose-6-P
ADP
Phosphofroctokinase
Fructose-1,6-Di P
Aldolase
Glyceraldehyde-3-P
Glyceraldehyde 3-p dehydrogenase
2,3-Diphosphoglycerate
1,3-Diphosphoglycerate
ADP,ATP
Phosphoglycerate kinase
3-Phosphoglycerate
Monophsphoglycerate mutase
2-Phosphoglycerate
Enolase
2-Phosphoenolpyruvate
ATP
Pyruvate Kinase
Pyruvate
LDH
Lactate

Rapoport-Luebering Shunt

- The production of large quantities of 2,3-DPG is a unique feature of glycolysis in the RBC.
- Interaction of 2,3-DPG with Hb accounts for its special role in RBC.
- Binding to β subunit of deoxyHb, stabilize low-affinity conformation.
- Production of 2,3-DPG is the function of a special side pathway

Hexose Monophosphate Pathway

- Under normal condition, most glucose is metabolized by RBC by the main glycolytic pathway.
- HMP Pathway or Shunt

Glucose 6-P

↓ G6PD

6-Phosphogluconolactone

↓

6-Phosphogluconic acid

↓

Ribulose 5-P

↙ ↘

Fructose-6-P   Glyceraldehyde-3-P

Hexose Monophosphate Pathway (2)

- Important to red cells primarily as a source of NADPH.
- The pentose formed in the HMP pathway also plays important role in the economy of RBC by providing ribose-5-P needed for phosphoribosyl pyrophosphate (PRPP), an essential substrate for the synthesis of adenine nucleotide.
- G6PD def. RBC does not maintain ATP levels in adenine containing media as well as inability to generate sufficient amount of pentose phosphate.

Alternative Substrates for RBC Metabolism

- Fructose and mannose
- Galactose
- Dihydroxyacetone
- Inosine
- Erythrocyte are generally regarded as impermeable to phosphorylated metabolic intermediate but phosphoenolpyruvate (PEP) is the exception.

Regulation of Energy Metabolism
1. Glucose Metabolism

- In most tissues, metabolic regulation can be achieved by ↑ing or ↓ing the rate of transcription of DNA or the translation of messanger RNA.
- RBC- not by this option but by a series of elegant feedback mechanism
- Hexokinase- inhibited by glucose-6-P
- Phosphofructokinase- by ATP & 2,3 DPG
- Both-by hydrogen ions (Rate of glycolysis slows markedly during storage due to accumulation of lactic acids lowers the pH)

Regulation of Energy Metabolism(2)

2. HMP Pathway: Regulated by availability of NADP and the level of NADPH
3. 2,3 DPG: At low pH, phosphatase activity is stimulated and mutase inhibited. A high pH favors good 2,3 DPG maintenance during storage.
4. ATP: ATP is used in a number of metablic pathways particularly by kinase.
5. Guanine Nucleotides