Basic Biomedical Sciences 1

Case/Lecture 1: HEMOGLOBIN SYNTHESIS

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BLOCK
04

MODULE
01

OUTLINE:

1. **Review about the structure of hemoglobin**
2. **The function of hemoglobin**
3. **How do we synthesize hemoglobin**

OVERVIEW

The first breath of a baby inside the uterus

- Breathing is highly reliant on the **oxygen** in the **placenta**

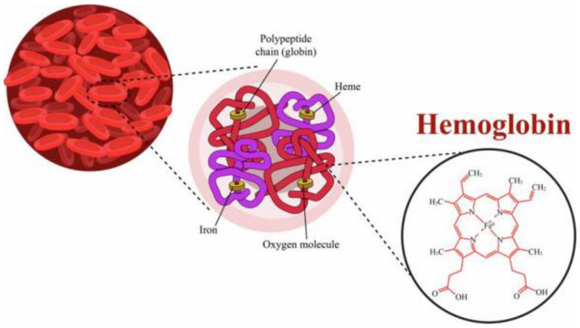
When the baby is born and has its first breath

- There is a change in the biochemical makeup, and that includes the hemoglobin
    - *Transitions from **fetal hemoglobin** to **adult hemoglobin***

Where do we see hemoglobin?

- It can be seen in the Erythrocytes or Red Blood Cells

HEMOGLOBIN



HEMOGLOBIN

- A biomolecule.
- It is a protein, with a **three-dimensional** structure
- Specifically, it is a **CONJUGATED PROTEIN**
    - Made up of **amino acids** to form a long chain and **heme** (non-protein or non-amino acid)

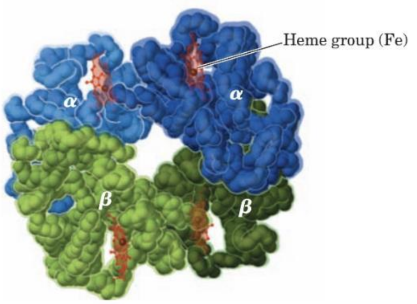
CONJUGATED PROTEIN:

- A protein that contains a non-amino acid moiety.

- It is also a **MULTIMERIC PROTEIN.**
    - Contains different chains of amino acids (represented by colors)
    - Forms together to form a large molecule
    - Each chains contain one **heme** (the prosthetic group)
    - **4 amino acid chains = 4 heme**

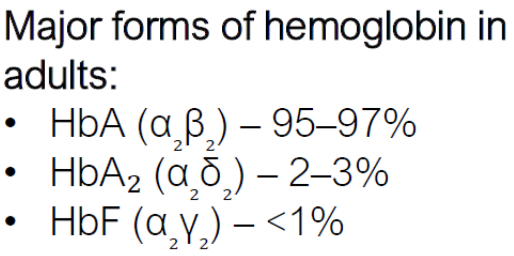
WHY IS HEME IMPORTANT?

- This carries oxygen
- In the middle of the heme, there is an iron (iron metal)
- Hemoglobin requires iron
- Iron can have a charge of 2 or 3
- For hemoglobin, it has a charge of 2



TYPES AND STRUCTURE OF HEMOGLOBIN

- **HEMOGLOBIN** is a protein composed of four polypeptide chains, and each of these chains contains a heme group. Every heme group can bind one oxygen molecule, which means that a single hemoglobin molecule can carry a total of four oxygen molecules at a time. The polypeptide chains are called globin chains, and this is where the name hemoglobin comes from - "**heme**" refers to the iron-containing component, and "**globin**" refers to the protein portion.

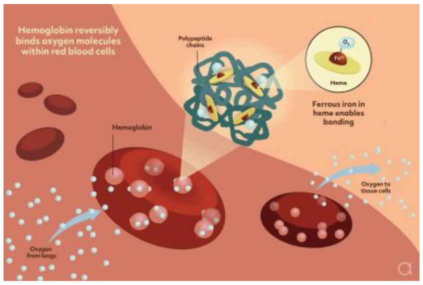


Major forms of hemoglobin in adults:

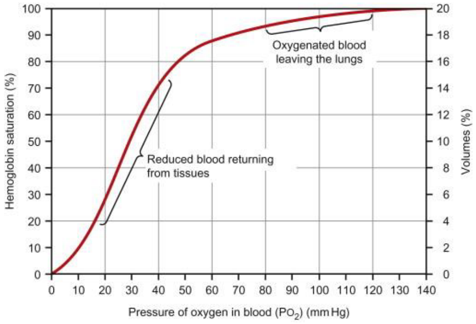
- HbA ($\alpha_2\beta_2$) – 95–97%
- HbA_2 ($\alpha_2\delta_2$) – 2–3%
- HbF ($\alpha_2\gamma_2$) – <1%

- The most common form of hemoglobin found in adults is known as **HbA**, and it consists of: **two alpha chains and two beta chains** (**$\alpha_2\beta_2$**).
- When globin chains are labeled with the same Greek letter, it indicates that these chains are similar in their amino acid sequence and primary structure. However, HbA is not the only form of hemoglobin present in the body. Adults also have small amounts of **HbA_2**, in which the beta chains are replaced by delta chains, giving it a composition of **$\alpha_2\delta_2$**. The difference between beta and delta chain is the sequence of amino acids.
- Meanwhile, **fetal hemoglobin**, called **HbF**, predominates during fetal life and consists of two alpha chains and two gamma chains (**$\alpha_2\gamma_2$**). Even though these forms differ in their globin chains, all types of hemoglobin contain heme groups where oxygen binding takes place.

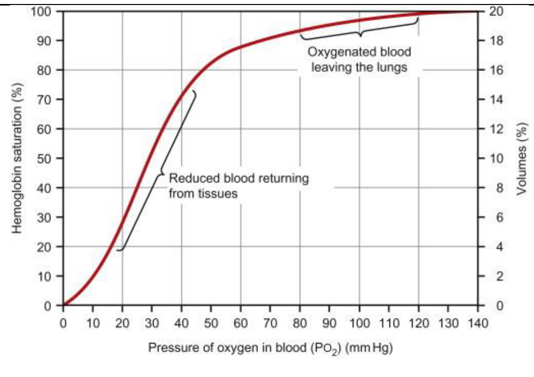
ALLOSTERIC BEHAVIOR & POSITIVE COOPERATIVITY



- Because Hemoglobin is multimeric, it has an **ALLOSTERIC PROPERTY**.
    - It has 4 Globin chains connected together; when you do something in one chain, it affects the other chains.
- This structural behavior explains the characteristic S-shaped oxygen dissociation curve.



- Hemoglobin also demonstrates **POSITIVE COOPERATIVITY**
    - Initially, O2 does not bind well to heme. Because heme is hidden with the Globin chains. Therefore, oxygen binding to heme is NOT spontaneous.
    - When one O2 successfully binds to one heme, it allows the entire molecule structure to change and allows other O2 molecules to then easily bind to the rest of the heme.
    - Low oxygen levels correspond to low hemoglobin saturation, but as oxygen concentration increases, binding becomes more rapid due to **positive cooperativity** (where one oxygen will successfully bind to hemoglobin)**.** In the lungs, the abundance of oxygen promotes strong oxygen binding, while in the tissues, where oxygen levels are lower, hemoglobin releases oxygen more readily. The overall structure of hemoglobin, therefore, perfectly complements its function, demonstrating how biochemical form directly supports physiological purpose.



"OXYGEN BINDING CURVE"

X-axis = Oxygen pressure
(how much O2 is available in the environment)

Y-axis = Hgb Saturation in percent
(how much O2 is found within the Hgb molecule)

NOTE: *With less O2 in the environment, there is less O2 binding for hemoglobin. With higher O2, there will be more O2 binding for the hemoglobin molecule.*

Hemoglobin delivers oxygen from the lungs to the tissues of our body.

Oxygen concentration in the LUNGS = HIGH
With high concentration of O2, Hgb is able to bind to it strongly.

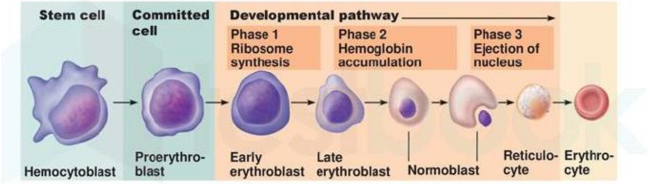
Oxygen concentration in the TISSUES = LOW / LESS
With lower concentrations of O2, Hgb is able to release it.

This is all possible due to the allosteric property of Hemoglobin (aka Structure-Function relationship).

HEMOGLOBIN SYNTHESIS

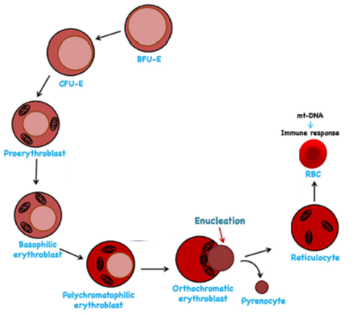
Cellular and Subcellular Site of Hemoglobin Synthesis

- Hemoglobin synthesis occurs in **erythroid precursors** (bone marrow).

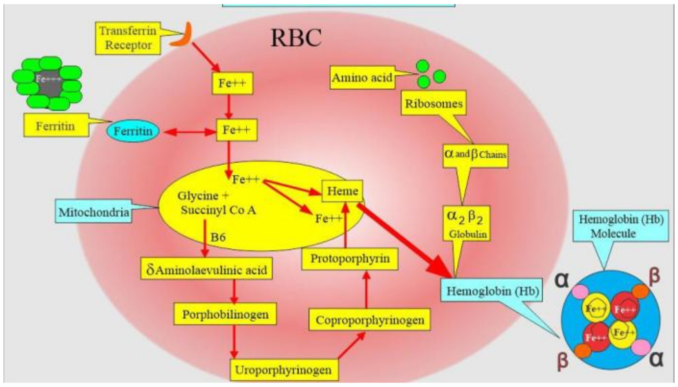


- **ERYTHROID PRECURSORS** – immature, developing RBCs in the bone marrow.
    - Mature RBCs are found in the peripheral / circulating blood, with the ability to carry O2.
    - Mature RBCs cannot synthesize Hemoglobin.

- Heme synthesis occurs in **mitochondrion** and **cytosol**
- Globin synthesis occurs in **ribosome**.

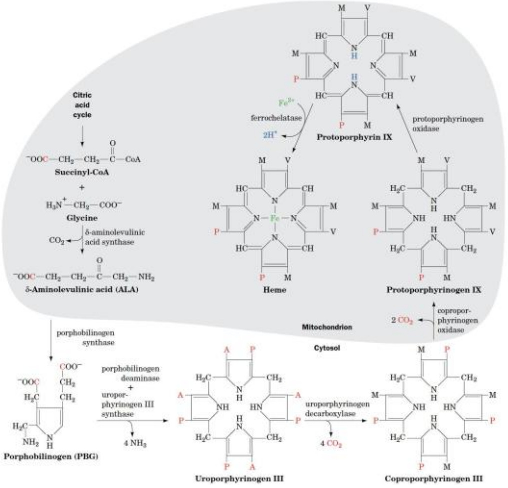


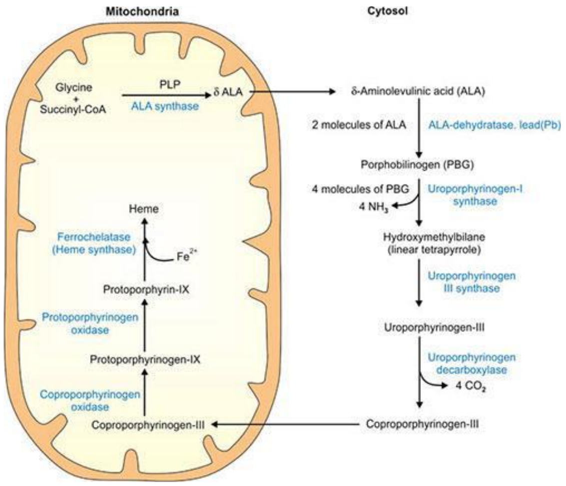
- This explains why mature RBCs cannot produce Hgb:
    - **Absence of mitochondria.**
    - **Absence of ribosomes and nucleus (anuclear).**
        - Globin being a protein is produced in the ribosomes, and for proteins to be synthesized instructions must be taken from the nucleus of the cell.



- **HEMOGLOBIN SYNTHESIS REQUIREMENTS:**
  - **Iron** – with a charge of +2
  - **Enzymes** – for the production of the metabolites in the Hgb synthesis pathway.
    - Defects or deficiency in certain enzymes involved will lead to metabolite accumulation and eventual pathologies and disorders.
  - **NOTE**: For Globin synthesis (coming from ribosomes and instructions from the nucleus), errors such as MUTATIONS can lead to defects in the overall synthesis of hemoglobin.

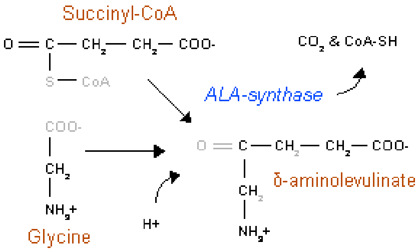
HEME SYNTHESIS





- Heme Synthesis have 8 enzymes involved, and the organelles involved are mitochondrion and cytosol.

PATHWAY FOR THE SYNTHESIS OF HEME



- It starts with an **ALA SYNTHASE**.
  - It condenses **glycine** and **succinyl CoA** forming **DELTA AMINOLEVULINIC ACID (ALA) $[C_5H_9NO_3]$.**
    - Functional group is the **carboxylic acid**
    - **Delta Aminolevulinic acid (ALA)** - The amino group is located in the last carbon, fourth (delta) carbon from your carboxylic acid
    - Other carbons:
      - Alpha (1st) carbon
      - Beta (2nd) carbon
      - Gamma (3rd) carbon
  - The reaction is called **CONDENSATION**.
  - Glycine contains 2 carbons $[C_2H_5NO_2]$ and Succinyl CoA contains 4 carbons (6 carbons in total); they combine ("condense") together.
    - **Glycine** is an amino acid.
    - **Succinyl CoA** is derived from the Krebs cycle (Citric acid cycle).
  - However, one carbon is lost as **carbon dioxide** so the remaining product is **5 carbons only**.
  - **THIS IS THE RATE DETERMINING STEP OF HEME SYNTHESIS**
    - It is the **slowest step** among the 8 enzymes; thus, it will determine the entire rate of the reaction.
  - It requires a **cofactor** which is a **PYRIDOXAL PHOSPHATE,** derived from vitamin B6 (Pyridoxine).
    - A deficiency in Vit. B6 will ultimately affect the overall synthesis of Heme, and the deficiency of Hemoglobin

- **Cofactor:** Pyridoxal phosphate (Vit B_6)
- **Regulation:**
  - ↓ by Heme (feedback),
  - ↑ by erythropoietin (EPO) & iron

  - **REGULATION**
    - Regulated by **Heme** (negative feedback), slowdown the production when there is sufficient amount.
    - **Erythropoietin** (**EPO**) and **Iron**, activating the enzyme
      - **EPO** – signals the production of more RBCs (more RBCs = more heme)
      - **IRON** – required in heme and Hgb synthesis

FIRST STEP OF HEME SYNTHESIS

- **Reactants**
  - Succinyl-CoA (from Krebs Cycle)
  - Glycine
- **These combine to form δ-Aminolevulinic Acid (ALA).**
- **Why this step matters:**
  - This is the rate-limiting step of the whole pathway.
  - It determines whether heme synthesis continues or stops.
- **Location:**
  - Occurs in the **mitochondrion**.

CONVERSION OF ALA → PORPHOBILINOGEN (PBG)

- 2 ALA molecules combine.
- 2 water molecules are removed.
- **Product:** Porphobilinogen, which has a cyclic ring structure.
- **Importance:**
  - Porphobilinogen is the building block of porphyrin.
  - The heme ring in hemoglobin comes from this structure.

REMEMBER:

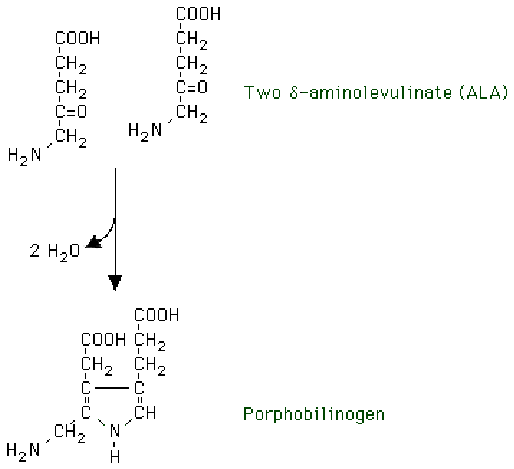
Initially, the structure is linear. However, when it reacts already to form porphobilinogen, it becomes cyclic. And this is **inhibited by lead.**

Quantities to remember:

- 4 PBG → 1 heme skeleton
- 8 ALA → 1 heme molecule

Lead toxicity:

- **Lead inhibits ALA dehydratase**, preventing formation of porphobilinogen → disrupts heme synthesis.
  - **Inhibitor**: Lead (Pb)



STRUCTURE OF PORPHOBILINOGEN

- Porphobilinogen has two side chains ("tails"):
  - **Propionyl** (3 carbons)
  - **Acetyl** (2 carbons)
    - These can be differentiated by counting carbon atoms.
- **To summarize:** Succinyl-CoA and Glycine are combined to create ALA, and 2 ALAs are combined together to form porphobilinogen. The reaction to produce ALA occurs in the mitochondrion, but that ALA needs to come out of the mitochondrion because the production of porphobilinogen occurs in the cytosol.
  - Succinyl-CoA + Glycine → ALA (mitochondria)
  - ALA exits the mitochondrion
  - 2 ALA → Porphobilinogen (cytosol)

FORMATION OF UROPORPHYRINOGEN

- 4 porphobilinogens combine to form uroporphyrinogen.
  - Porphobilinogen is just one ring, but once it forms the uroporphyrinogen, the four rings combine to form the skeleton of heme.
- This molecule is the first actual backbone (skeleton) of heme.
- The four individual rings from PBG join to create a tetrapyrrole backbone.

NOTE:

If asked: "**What is the first backbone formed in heme synthesis?**"

Answer: Uroporphyrinogen

- This step is a milestone because this is where we see how we utilize four porphobilinogen to form one single molecule of uroporphyrinogen which will serve as a backbone of heme. This backbone will then undergo a series of transformation/chemical reactions until we reach the heme.
- **Location:** Produced in the cytosol.
  - The **production of this backbone occurs in the cytosol.**

UROPORPHYRINOGEN → COPROPORPHYRINOGEN

- Uroporphyrinogen still contains the original acetyl (A) and propionyl (B) side chains contributed by each porphobilinogen.
- These are modified in later steps when it becomes coproporphyrinogen.
- Uroporphyrinogen decarboxylase removes carboxylic acid from Uroporphyrinogen, producing carbon dioxide.
- The backbone of the developing Heme structure contains acetyl, with a carboxyl group, that is what is removed in this step. Meaning that all of the acetyl (A) in the backbone is turned into methyl (M).
- This reaction produces a total of 4 CO2 molecules (**DECARBOXYLATION REACTION**).

COPROPORPHYRINOGEN III

- The next milestone in Heme synthesis involves the reintroduction of the molecule into the mitochondrion.

PROTOPORPHYNOGEN

- This step undergoes several **transformations**
  - Protoporphyrinogen is the result of the enzyme Coproporphyrinogen oxidase, producing 2 CO2 molecules instead of 4.
  - This change from 4 to 2 CO2 molecules is due to the removal of 2 Propionyl converting them to vinyl.

PROTOPORPHYRIN

- The enzyme protoporphyrinogen oxidase removes 2 Hydrogen ions, an oxidation reaction.

HEME

- The introduction of iron to Protoporphyrin creates Heme
- The enzyme Ferrochelatase introduces the iron while simultaneously removing the last 2 Hydrogen ions from the backbone of the Heme structure

HEME STRUCTURE

- 4 porphobilinogen (PBG); which forms a core with an Iron in the center.
- Iron is where the Oxygen (O2) will bind to.
- Formation of heme is partly cytosolic and partly mitochondrial; occurring in Erythroid precursors.

LIVER

- Also capable of Heme Synthesis
- But the heme produced in the Liver is NOT involved in the Hemoglobin synthesis in the blood

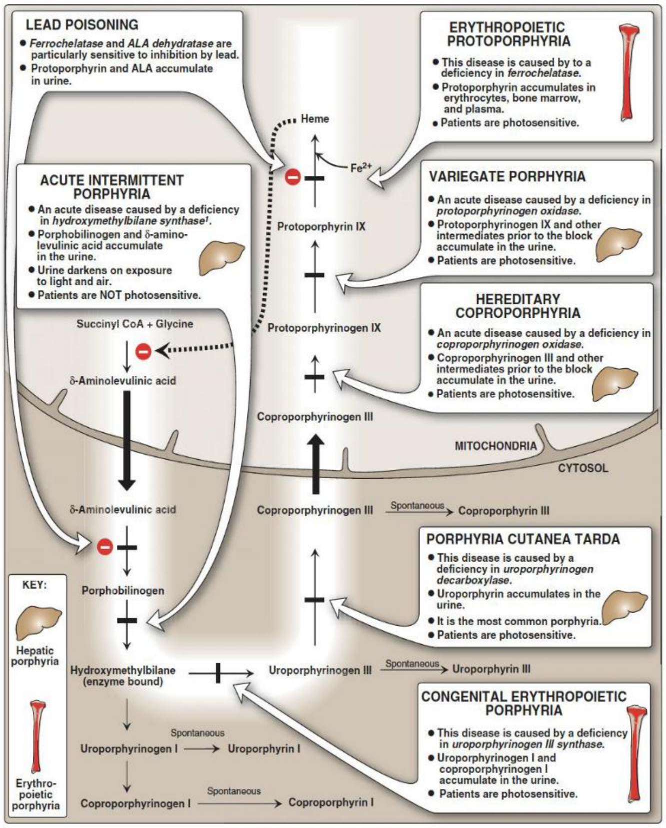
- Note that Heme can also be found in cytochromes (involved in the electron transport chain), and other important metabolic pathways

BONE MARROW

- Site of Heme synthesis specific for Hemoglobin

DEFECTS IN HEME SYNTHESIS: PORPHYRIA

- Involving mainly 8 enzymes
- 8 disorders are as follows:



- Defects in Heme Synthesis are called "**PORPHYRIAS**"
    - Note that Heme is made up of Porphyrin rings; when one of the enzymes for Heme synthesis is deficient or destroyed, the linear pathway is disrupted and therefore cannot continue - leading to the accumulation of these Porphyrin metabolites

- The enzyme affected will determine the type of porphyrin that accumulates, that ultimately determines the type of Porphyria.
- Porphyria may be **HEPATIC** (occuring in the **liver**) or **ERYTHROPOIETIC** (occuring in the **bone marrow**).

- **PORPHYRIA CUTANEA TARDA** is the most common type of Porphyria in humans, which is due to the accumulation of **Uroporphyrinogen**

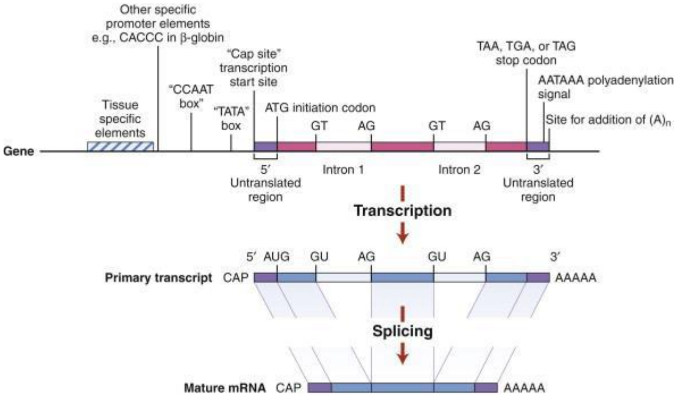

    - Note that Uroporphyrinogen is the product of the combination of Porphobilinogen; considered as a "milestone" as it becomes the backbone of Heme in the formation of Heme.
    - Uroporphyrinogen is ideally converted to Coproporphyrinogen; but in the absence of the converting enzyme, it accumulates leading to **Porphyria Cutanea Tarda**.
    - Characteristics include having photosensitive skin, and port wine red colored urine.

REVIEW:

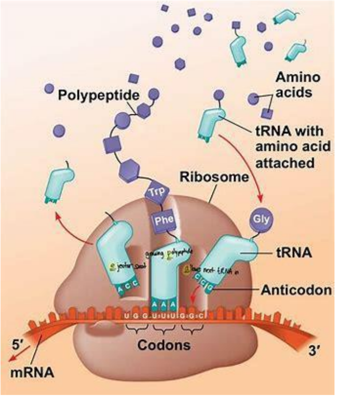
- **HEME PRECURSORS**: Glycine and Succinyl CoA (together with Fe / Iron)
- **SYNTHESIS LOCATION**: Cytosolic and Mitochondrial
- **DEFECTS**: "Porphyrias".

GLOBIN SYNTHESIS

- **GLOBIN** is the protein portion of Hemoglobin



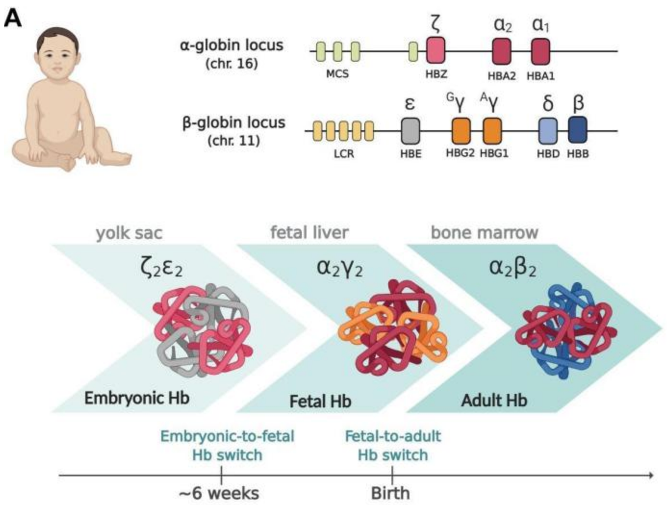
- **REVIEW ON PROTEIN SYNTHESIS:**
    - **(1) TRANSCRIPTION**
        - Copies DNA to form messenger RNA (mRNA).
        - Involves several processes (e.g. Introns, Exons, Splicing, Post-transcriptional modifications, TATA Box, etc.)
    - **(2) TRANSLATION**
        - mRNA undergoes translation within the cell's ribosomes
        - mRNA is read per codon, where each codon represents an amino acid.



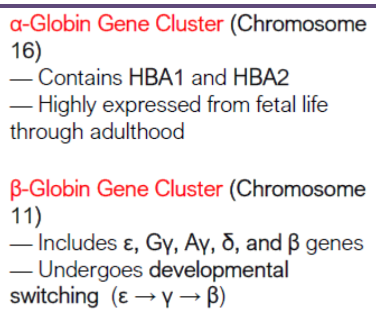
DNA, GENES, AND TRANSLATION OF GLOBINS

- DNA is located in the **nucleus** and contains numerous segments called **genes**, each with a specific function.
- During **translation**, the ribosome produces only a **linear polypeptide chain** (e.g., alpha or beta globin).

- After translation, the polypeptides undergo **chaperoning**, where helper proteins assist in proper folding and assembly to form functional globin chains.
- These globin chains later combine with heme to make **mature hemoglobin**.



CHROMOSOMAL LOCATION OF GLOBIN GENES


α-Globin Gene Cluster (Chromosome 16)
— Contains HBA1 and HBA2
— Highly expressed from fetal life through adulthood

β-Globin Gene Cluster (Chromosome 11)
— Includes ε, Gγ, Aγ, δ, and β genes
— Undergoes developmental switching (ε → γ → β)

- Humans have **23 pairs of chromosomes**, and specific globin genes are located on:
  - **Chromosome 16 – Alpha-Globin Cluster**
    - Contains:
      - **α1 and α2 genes** → produce **alpha chains**
      - **ζ (zeta) gene** → embryonic globin, not present in adult hemoglobin
  - **Chromosome 11 – Beta-Globin Cluster**
    - Contains:
      - **ε (epsilon)** – embryonic
      - **γ (gamma)** – fetal
      - **δ (delta)** – minor adult globin (HbA_2)
      - **β (beta)** – major adult globin
    - Because the beta cluster contains **more genes** and undergoes multiple transitions, it is **more prone to errors** (e.g., beta-thalassemia).

TWO MAJOR TYPES OF ADULT GLOBINS

- **Alpha globins**
- Encoded by the α1 and α2 genes
  - **Highly expressed from fetal life to adulthood**
  - Alpha chains are present throughout all developmental stages
- **Beta globins**
  - Undergo **hemoglobin switching** during development
  - Embryo → Fetus → Adult transitions involve multiple β-cluster genes
  - Adults need **β chains** for mature hemoglobin (HbA)

COMBINING HEME AND GLOBIN

- Once globin chains are synthesized and folded, they combine with **heme** to form **functional hemoglobin**.
- Defects can occur in either:
  - **Heme production → PORPHYRIA**
  - **Globin production → THALASSEMIA**

HEMOGLOBIN

HEMOGLOBIN SWITCHING THROUGHOUT DEVELOPMENT

A. Embryonic Stage

- Produces **embryonic hemoglobin**
- Composition:
  - **$\zeta_2\varepsilon_2$ (zeta$_2$ epsilon$_2$)**
- Still maintains the standard structure of:
  - **4 globin chains + 4 hemes → carries 4 O_2 molecules**

B. Fetal Stage

- Transition from embryonic to **fetal hemoglobin (HbF)**
- Composition:
  - **$\alpha_2\gamma_2$ (alpha$_2$ gamma$_2$)**
- Alpha begins to be expressed fully; beta is not yet active.

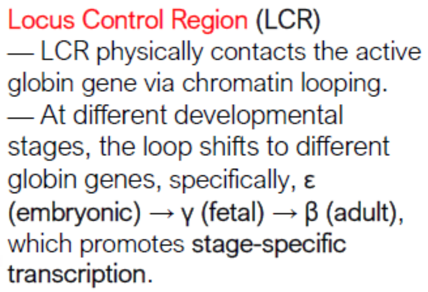
C. Adult Stage

- Switch from fetal to **adult hemoglobin (HbA)**
- Composition:
  - **$\alpha_2\beta_2$ (alpha$_2$ beta$_2$)**
- This is the final form needed for optimal post-natal oxygen delivery.

TRANSITION FROM FETAL TO ADULT HEMOGLOBIN

- At birth, when the baby takes its **first breath**, a major developmental shift occurs:
  - **HbF ($\alpha_2\gamma_2$) → HbA ($\alpha_2\beta_2$)**
- Adult hemoglobin (HbA) is essential because it:
  - Efficiently **binds oxygen in the lungs**
  - Effectively **releases oxygen to the tissues**
- This transition ensures proper oxygen delivery after the newborn is no longer dependent on placental oxygen.

LOCUS CONTROL REGION (LCR)


Locus Control Region (LCR)
— LCR physically contacts the active globin gene via chromatin looping.
— At different developmental stages, the loop shifts to different globin genes, specifically, ε (embryonic) → γ (fetal) → β (adult), which promotes stage-specific transcription.

- The **beta-globin gene cluster** contains a regulatory segment called the **LCR – Locus Control Region**.
- DNA is not fixed linearly; it can **fold and loop**.
- During different developmental stages, the LCR physically loops to activate specific globin genes:

  - **Embryo** → LCR loops to **ε (epsilon)** gene → produces ε-globin
  - **Fetus** → LCR loops to **γ (gamma)** gene → produces γ-globin (HbF)
  - **Adult** → LCR loops to **β (beta)** gene → produces β-globin (HbA)
- This dynamic looping underlies **hemoglobin switching**.

DO ADULTS STILL HAVE FETAL GLOBIN?

- Yes, but only in **very small amounts**.
- Majority becomes **adult hemoglobin**, needed for proper lung-based oxygenation.

FUNCTIONAL DIFFERENCES: FETAL VS. ADULT HEMOGLOBIN

Fetal Hemoglobin (HbF: $\alpha_2\gamma_2$)

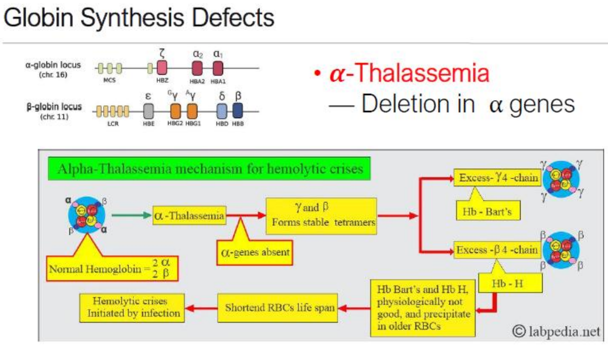
- **Higher oxygen affinity**
- Necessary because:
  - The fetus receives oxygen **indirectly through the placenta**
  - Must pull oxygen away from maternal blood → requires tighter binding

Adult Hemoglobin (HbA: $\alpha_2\beta_2$)

- **Lower oxygen affinity** compared to HbF
- Has **positive cooperativity**:
  - Essential for efficient **oxygen loading in the lungs**
  - And **oxygen unloading to tissues** after birth
- After birth, once the umbilical cord is cut, oxygen comes from the **lungs**, so hemoglobin must optimize release—not tight binding.

DISORDERS OF GLOBIN SYNTHESIS: THALASSEMIAS

ALPHA THALASSEMIA

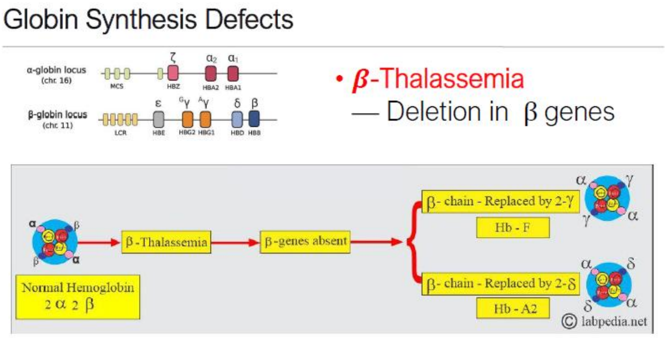


Globin Synthesis Defects

- *α*-Thalassemia
  - — Deletion in α genes

- Cause: Defect or deletion of α-globin genes (on chromosome 16).
- Consequence:
  - Reduced alpha-chain production → Excess beta chains
  - This forms abnormal β_4 tetramers (HbH).
  - Since normal hemoglobin should be $\alpha_2\beta_2$, lack of alpha chains leads to:
    - Hemolytic crises
    - Low hemoglobin
    - Severe anemia
  - Diagnosis: Hemoglobin electrophoresis
    - Shows decreased HbA
    - Increased abnormal hemoglobin types

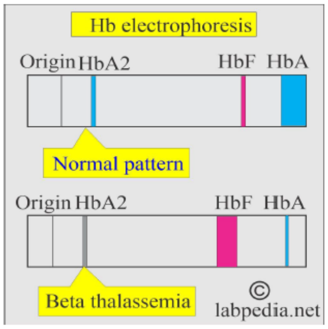
BETA THALASSEMIA



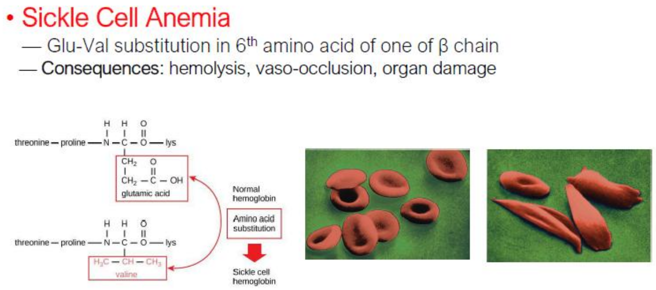
Globin Synthesis Defects

- *β*-Thalassemia
  - — Deletion in β genes

- Cause: Deletion or mutation of β-globin gene (chromosome 11).
- Consequence:
  - Reduced or absent β-chain synthesis
  - Body compensates by producing more γ-globin (fetal) → increased HbF
  - Thus, instead of forming adult HbA, patients produce:
    - Excess fetal hemoglobin (HbF: $\alpha_2\gamma_2$)
  - Electrophoresis Findings:
    - Decreased HbA
    - Elevated HbF and sometimes HbA_2



SICKLE CELL ANEMIA (STRUCTURAL HEMOGLOBINOPATHY)



- Sickle Cell Anemia
  - — Glu-Val substitution in 6th amino acid of one of β chain
  - — **Consequences:** hemolysis, vaso-occlusion, organ damage

- Unlike thalassemia (deletion or reduced production), sickle cell is caused by:
  - A single amino acid substitution in the β-globin chain.
- Normal amino acid at position 6:
  - Glutamic acid
- Mutated form:
  - Valine
- This "Glu → Val substitution" is enough to produce:
  - Abnormal hemoglobin HbS
  - Polymerization under low O_2 → sickling of RBCs
- This mutation is a point substitution, not a deletion.
- Unluckily, you will encounter a patient that will have an abnormal hemoglobin because that abnormal hemoglobin will

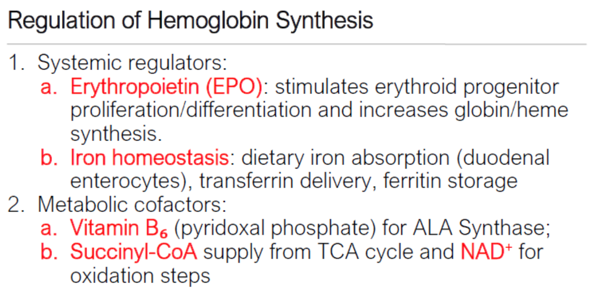
have a valine instead of a glutamic acid in a six amino acid residue.

- What's going to happen?
    - It will produce a **sickle cell hemoglobin.**
- Why is the substitution very fatal?
    - There are different kinds of amino acid:
        - **Polar**
            - Polar acidic
            - Polar basic
            - Polar neutral
        - **Non-polar**
    - **Glutamic acid:** Polar acidic
    - **Valine:** Non-polar
- Glu-Val substitution in 6th amino acid of one of beta chain (from slides)
    - From **polar** *(glutamic acid - normal)* to **non-polar** *(valine - abnormal)*, it's a major change that could change the structure of the entire protein because they have differences in polarity.
    - And what's going to happen?
        - The red blood cell will have the shape of a sickle.
    - Hence, sickle cell anemia due to the characteristic or form of the red blood cell which will be shaped like a sickle.
- Effects:
    - **Hemolysis**
    - **It cannot carry oxygen.**
        - Abnormally carries less oxygen because the hemoglobin molecule is abnormal.
    - **Blockage in the capillaries (vaso-occlusion) and organ damage**
        - *Remember:* Normal red blood cells are disc shaped which allows for it to flow through capillaries.
        - However, if you have sickle cell red blood cells, it would result in blockage in the capillaries.
        - Blockage = necrosis
            - Because there will no longer be enough oxygen flowing through there.
            - It will kill the organ. And so when the organ is killed, you're also killed.
            - Hence, it is a very fatal disease.
- Treatment:
    - No treatment for it yet.
    - **Gene therapy (tried):** used to correct the defect in the Glu-Val substitution.
        - However, there is no therapeutically approved gene therapy that could correct this.
    - **CRISPR technology (mentioned by sir):** is a gene-editing technology that allows scientists to precisely modify DNA in living organisms by using a system adapted from bacteria.
        - It uses a guide RNA molecule to direct a Cas protein, often Cas9, to a specific DNA sequence to cut, remove, or insert new genetic material.
        - This is used to try to correct sickle cell anemia (no updates on its progress yet).
- Lifespan of patients with sickle cell anemia:
    - Can **live** only up until *childhood.*
    - They **cannot live** until *adulthood*.
    - This is because the condition can cause necrosis.
    - Increased iron intake is needed.
        - Patients can experience iron overload because of continuous blood transfusion which could be fatal.
        - So, the management of the symptoms itself can also cause death.

SUMMARY OF HEMOGLOBIN SYNTHESIS

- Synthesize heme, then globin, and then synthesize hemoglobin.
- Analysis:
    - If you don't have heme, what's going to happen?
        - You will have a lot of globin.
    - What's going to happen to globin if you don't have hemoglobin?
        - Proteins will precipitate.
        - Excess protein always precipitates.
    - What's going to happen once this precipitates?
        - It will eventually kill the erythrocytes.
    - If you do not have globin and always create heme, it will also result in prophyria.
- Also remember: Heme synthesis requires **iron**.
    - Iron is:
        - Stored in the protein, **ferritin (storage form of iron)**.
        - Transported into the blood through **transferrin**.
    - Heme synthesis is affected if there is a defect in either/both ferritin and transferrin.
    - If heme synthesis is affected, it will also affect hemoglobin synthesis.
    - First cell affected if hemoglobin is effected:
        - **Red blood cell** – since it stores hemoglobin.
    - Less hemoglobin = less oxygen in the blood **(hypoxia).**
    - Hypoxia can result in eventual death or other complications.

REGULATORS OF HEMOGLOBIN SYNTHESIS



Regulation of Hemoglobin Synthesis

1. Systemic regulators:
    a. Erythropoietin (EPO): stimulates erythroid progenitor proliferation/differentiation and increases globin/heme synthesis.
    b. Iron homeostasis: dietary iron absorption (duodenal enterocytes), transferrin delivery, ferritin storage
2. Metabolic cofactors:
    a. Vitamin B_6 (pyridoxal phosphate) for ALA Synthase;
    b. Succinyl-CoA supply from TCA cycle and NAD^+ for oxidation steps

SYSTEMIC REGULATORS

- **Erythropoietin (EPO)** stimulates hemoglobin synthesis.
    - It is because EPO produces more **erythroid precursors.**
        - More erythroid precursors = more hemoglobin
- **Iron homeostasis** also affects hemoglobin synthesis.
    - Iron is absorbed in the duodenum.
    - If you have a damaged duodenum, iron will be malabsorbed.
    - **Malabsorption of iron** = cannot create hemoglobin.

METABOLIC COFACTORS

- **Vitamin B6 (pyridoxal phosphate)** is required for the first enzyme (ALA synthase) in heme synthesis.
    - Vit. B6 deficiency affects hemoglobin synthesis.
- **Succinyl-CoA** is a supply from the TCA cycle.
    - Any defects in the Krebs cycle affects hemoglobin synthesis.
    - Hence, Krebs cycle is a central pathway.

SUMMARY OF TOPICS:

- **Hemoglobin** = heme + globin
- **Heme:** produced in the mitochondria and cytosol.
    - **ALA synthase:** first enzyme, key enzyme, rate-limiting determining step because it tells you whether to create heme or not (regulator and inhibitor).
- **Globin:** produced in the ribosome.
    - Beta globin undergoes developmental switching – from Epsilon to Gamma to Beta ($\epsilon \rightarrow \gamma \rightarrow \beta$) – controlled by the LCR (Locus Control Region).
- **Regulation:** heme, iron, EPO, Vitamin B6
- Disorders:
    - **Porphyria**: affects *heme* synthesis
    - **Lead poisoning:** affects 2nd and last enzyme (heme synthesis)
    - **Thalassemias and sickle cell:** affects *globin* synthesis.

QUICK CHECK:

Rate-limiting enzyme in heme synthesis?

- **ALA synthase**

Enzyme/s in heme synthesis affected by Pb poisoning?

- *2nd enzyme:* **ALA-dehydratase**
- *Last enzyme:* **Ferrochelatase**

Order of developmental switching in beta-globulin gene?

- **Epsilon to Gamma to Beta ($\epsilon \rightarrow \gamma \rightarrow \beta$)**

If Hb electrophoresis shows increased HBA2 – diagnosis?

- **Alpha (α)-thalassemia**

Glu → Val mutation in β-globulin leads to?

- **Sickle cell anemia**