بسم الله الرحمن الرحيم

Biochemistry

MID | Lecture 3

Hemoglobinopathies

﴿ وَقُل رَّبِّ أَدْخِلْنِي مُدْخَلَ صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَل لِّي مِن لَّدُنكَ سُلْطَانًا نَّصِيرًا ﴾

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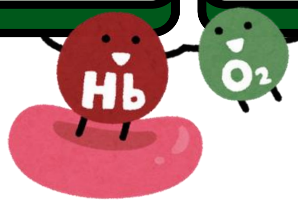
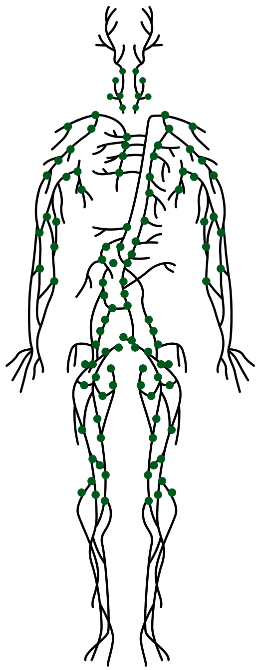




What we are covering in this lecture

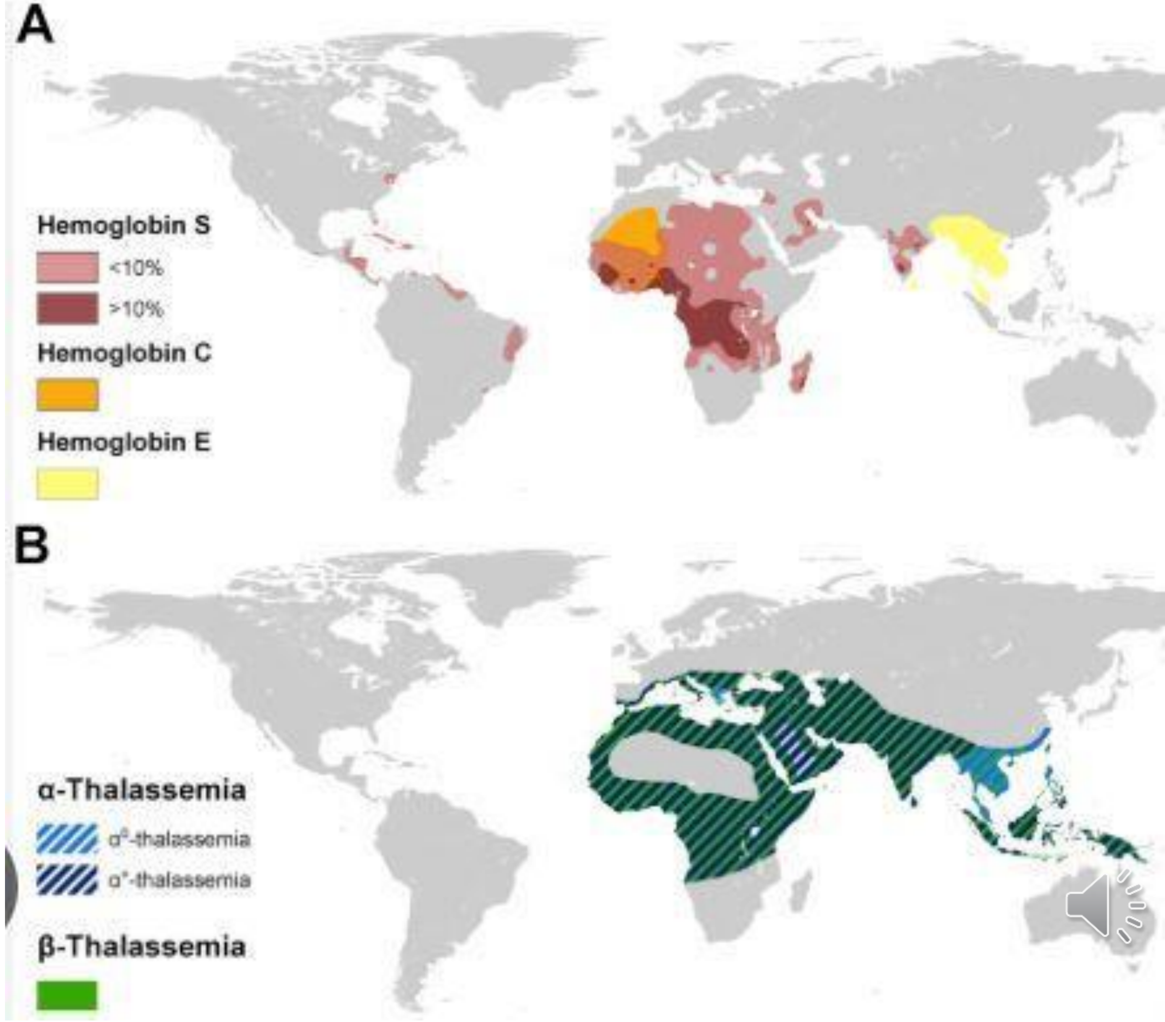
- In this lecture we will cover:
- Introduction

1. Quantitative abnormalities in the hemoglobin (Thalassemia):
    A. Alpha Thalassemia and its subtypes
    B. Beta Thalassemia and its subtypes
2. Qualitative abnormalities in hemoglobin:
    A. Mutations in surface residue
    B. Mutations in internal residues
    C. Mutations at $\alpha 1$-$\beta 2$ contacts
    D. Mutations stabilizing methemoglobin
3. Hereditary persistence of fetal hemoglobin

Introduction

What are hemoglobinopathies?

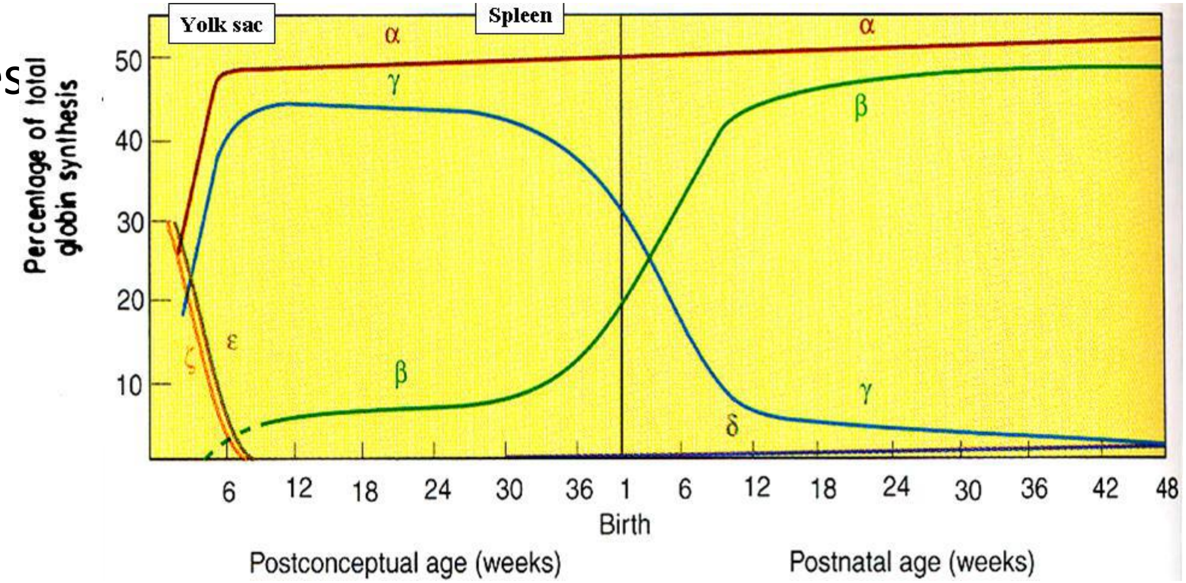
- Hemoglobinopathies: Disorders of human hemoglobin.
- The most common genetic disease group in the world (5% of people are carriers) with substantial morbidity (about 300,000 born each year with the disorder).
- Hemoglobin disorders account for 3.4% of deaths in children < 5 years.



Hereditary hemoglobins disorders

- Hemoglobin disorders are:

1. Quantitative abnormalities are abnormalities in the relative amounts of α and β subunits (thalassemias).
2. Qualitative abnormalities: mutations resulting in structural variants.
   - Over 800 variants have been identified.
3. Hereditary persistence of fetal hemoglobin (HPFH): impairment of the perinatal switch from g to b globin.
   - Fetal hemoglobin has a higher affinity for oxygen than adult hemoglobin. In conditions where fetal hemoglobin persists after birth, symptoms are usually mild or absent.



1- Quantitative abnormalities (Thalassemia)

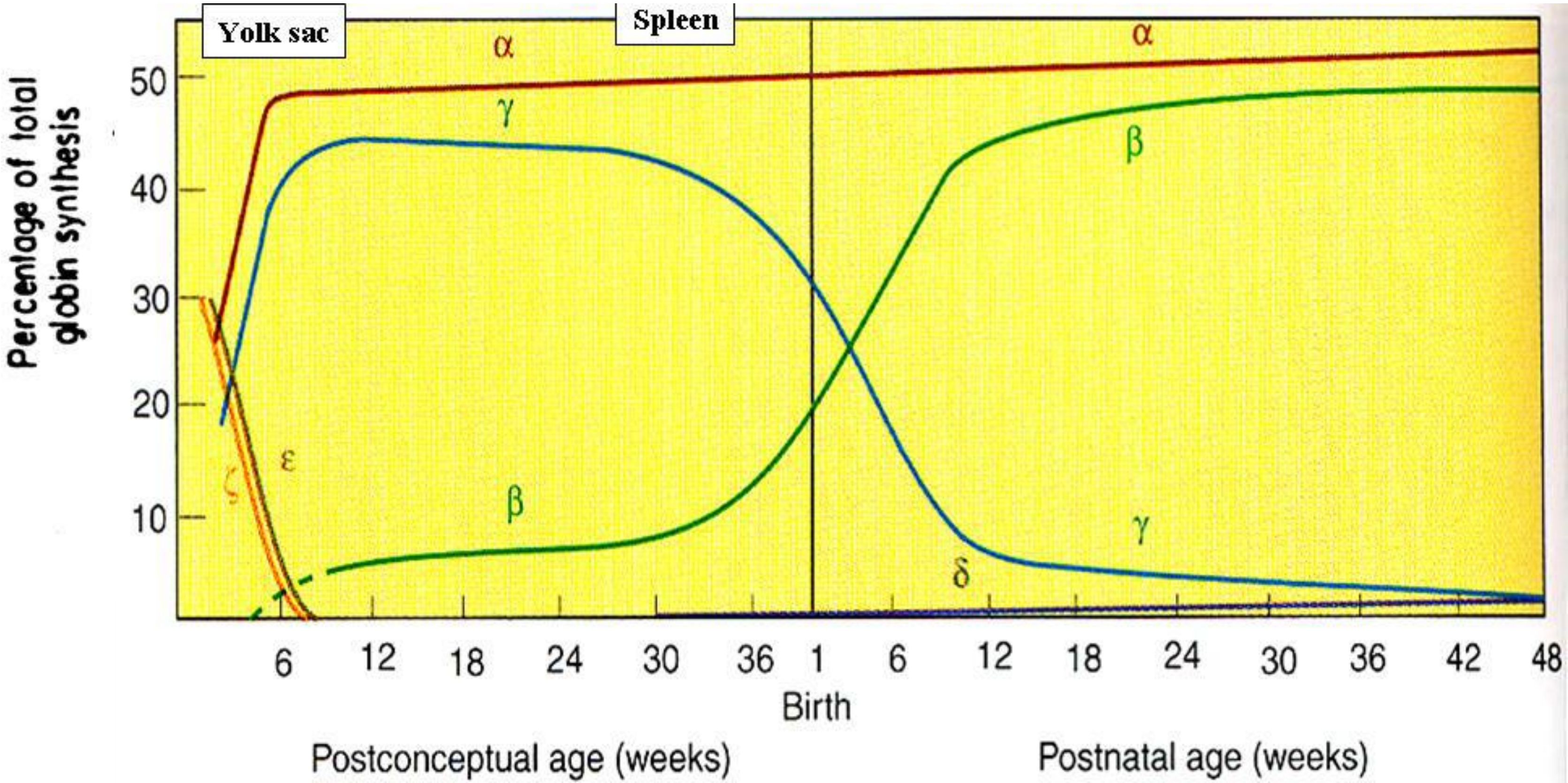
A. α-Thalassemia and its subtypes

Thalassemias

- Thalassemias: the most common human single-gene disorder. Either in alpha or beta
- They are caused by a reduced amount of either the α or β protein, which alters the ratio of the α:β ratio.

The α-Thalassemias

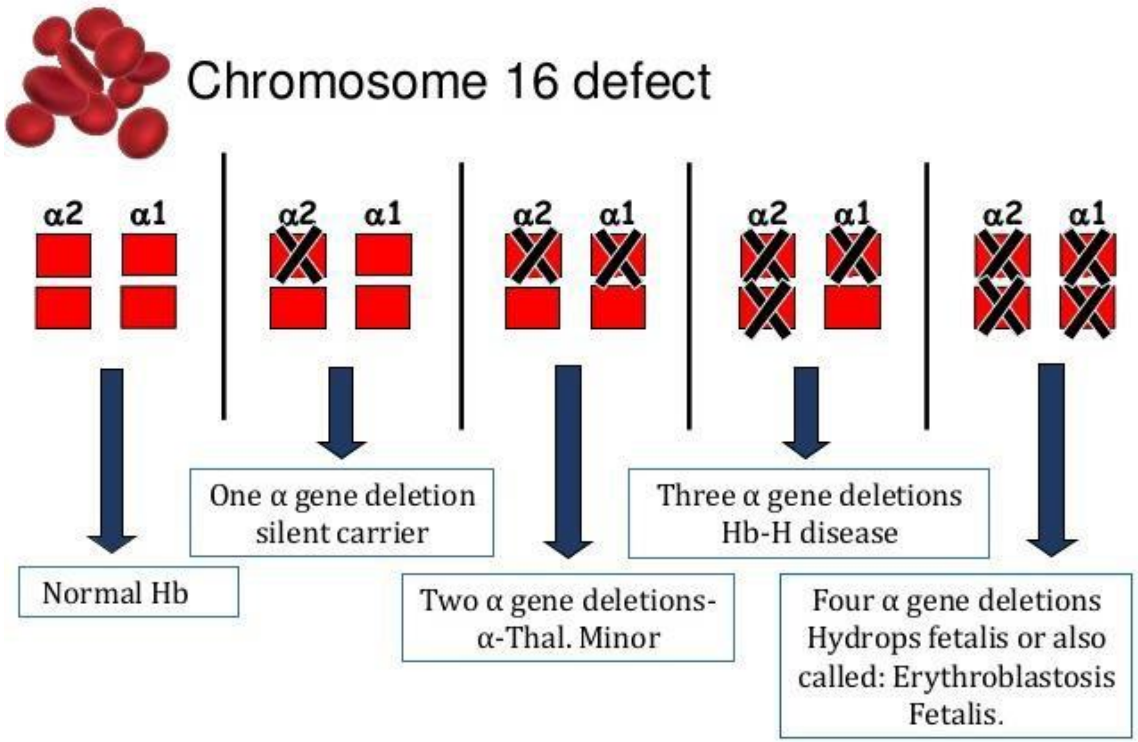
- Alpha-thalassemia: underproduction of the α-globin chains.
- Mainly a deletion mutation that causes the gene to be non-functional.



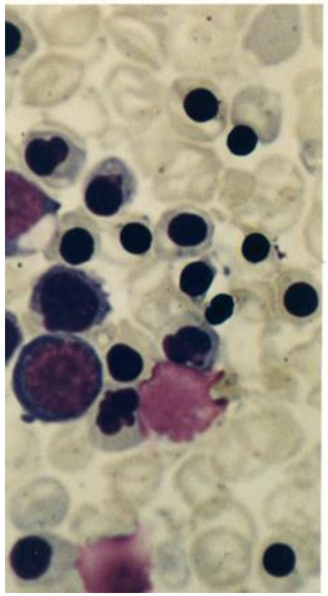
Alpha Thalassemia subtypes from the most severe to the least

Variable severity

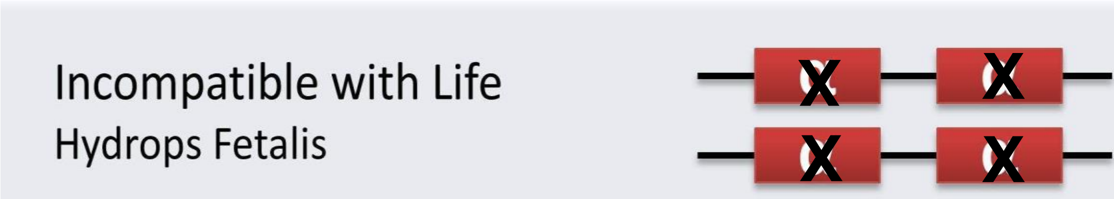
- With α-thalassemia, the level of α-globin production can range from none to very nearly normal levels.
- This is due to the fact that each individual has 4 genes, 2 on each chromosome.



α-thalassemia major Hydrops fetalis or Hb Bart (most severe)

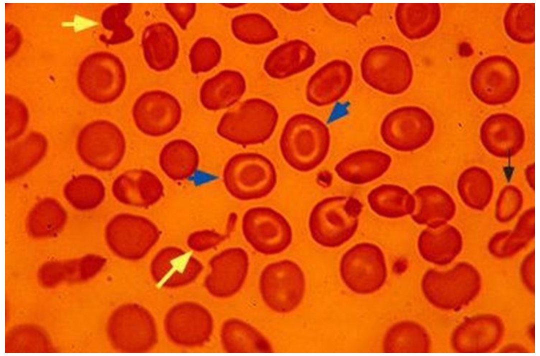


- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a tetramer of g-chains.
- g4 or Hb Bart: a homotetramer of g.
- Hb Bart has a <u>high</u> affinity towards oxygen.
- This condition is called hydrops fetalis.
- Stillbirth (death in the womb)
- or death shortly after birth occurs.

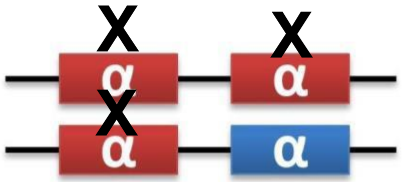


- This condition causes severe anemia and fluid accumulation under the skin (hydrops fetalis), often resulting in fetal death.
- In this case, the loss of all alpha chains leads to the formation of hemoglobin composed of four gamma chains (Hb Bart's), which cannot release oxygen effectively. Death usually occurs before or shortly after birth.

α-thalassemia intermedia
or Hemoglobin H (HbH) disease

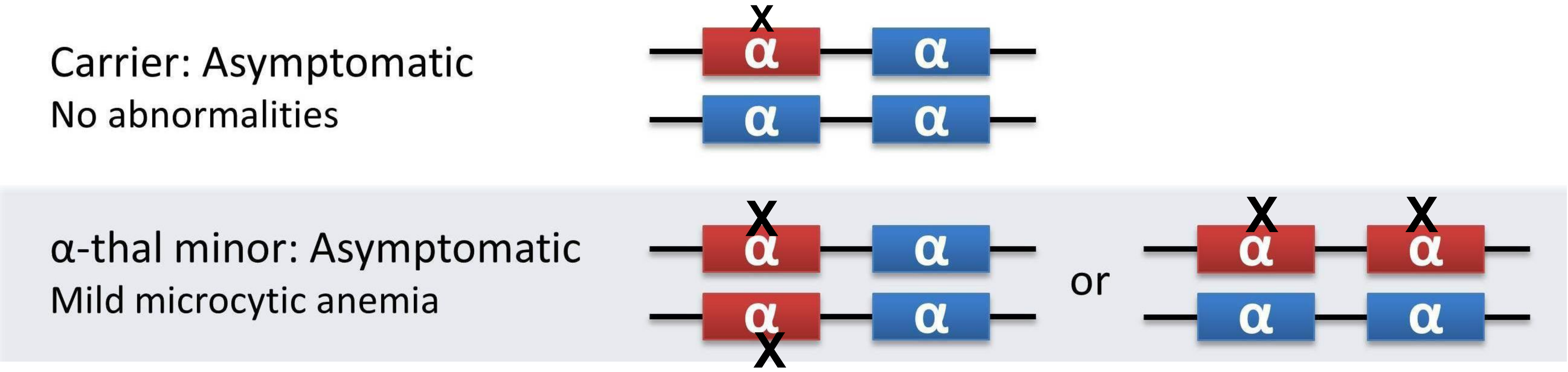


- 3 of 4 genes deleted.
- Clinically, it is known as hemoglobin H disease.
- With the reduction of a chain production, and b-chain production is established, there is a chance that homotetramers of b (b4 or HbH) can form, with low normal hemoglobin.
- The HbH tetramers have a high affinity towards oxygen and are highly unstable (meaning that they denature, aggregate and precipitate resulting in the formation of Heinz bodies).
- Mild to moderate, not severe, hemolytic anemia in adults, may need blood transfusions. May shift to the severe side.
- The disease is not fatal.
- The main type of mutation is deletion.



α-thalassemia minor and silent carrier

- α-Thalassemia trait: If 2 of the 4 genes are inactivated.
  - The individuals have mild microcytic anemia.
- Silent carrier: 1 of 4 genes deleted.
  - Individuals are completely asymptomatic.



Summary of α-thalassemias

Genotype	α-globin gene number[a]	Name	Phenotype
αα / αα	4	Normal state	None
αα / α–	3	Silent carrier	None (values for Hb and MCV may be near the lower limits of normal)
– – / αα or α– / α–	2	Thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic anemia
– – / α–	1	Hb H disease	Thalassemia intermedia: mild to moderate microcytic anemia
– – / – –	0	Alpha thalassemia major	Thalassemia major: hydrops fetalis

[a]Number of normal alpha globin genes

Note microcytic anemia has to do with the shape of the RBC while hemolytic has to do with lysis of the RBCs.

1- Quantitative abnormalities (Thalassemia)
B- β-Thalassemia and its subtypes

Beta thalassemia has two types because there is one beta gene on each chromosome.

Either there is no beta chain production at all, or there is partial production.

The β-thalassemias

- β-globins are deficient and the α-globins are in excess and will form α- globin homotetramers.
- The main types of mutation are <u>point mutations</u> that lead to non- functional protein.
    - The mutations occur within the promoter or LCR (enhancer), the exon, translation initiation codon, splicing positions, or poly-adenylation termination signal.
- The α-globin homotetramers are extremely insoluble, which leads to premature red cell destruction in the bone marrow and spleen.
- Delta and gamma are common globin become apparent.

β-thalassemia Major and Minor

- <u>**β-thalassemia major (Cooley's anemia)**</u>
- A complete lack of HbA is denoted as b^0-thalassemia or b-thalassemia major.
- Affected individuals suffer from severe anemia beginning in the first year of life and need blood transfusions.
- Long-term transfusions lead to the accumulation of iron in the organs, particularly the heart, liver and pancreas and, finally, death in the teens to early twenties. Can be treated with iron chelators which improve the prognosis.
- **This condition is survivable but causes extremely severe anemia. This may cause the body to continue producing fetal hemoglobin since beta chains are produced later while alpha chains are produced from the start. And patient can live.**

- <u>**β-thalassemia minor**</u>
- Individuals heterozygous for β-thalassemia with one normal β-globin gene and a mutated gene are termed b-thalassemia minor.
- Individuals with beta-thalassemia minor are generally asymptomatic with mild anemia.

Classification and types of β-thalassemia

Common genotypes	Name	Phenotype
β/β	Normal	None
β/β^0 β/β^+	Beta thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia
β^+/β^+ β^+/β^0 β^E/β^+ β^E/β^0	Beta thalassemia intermedia	Variable severity Mild to moderate anemia Possible extramedullary hematopoiesis Iron overload
β^0/β^0	Beta thalassemia major (Cooley's Anemia)	Severe anemia Transfusion dependence Extramedullary hematopoiesis Iron overload

β^0: complete lack of β chain
β^+: some expression of β chain
β: normal expression of β chain
β^E: point mutation that is characterized by substitution of a.a. 26 from Glu to Lys on the surface of the b molecule that causes lower production of B chain.

Note:

a. that in the 2nd raw the thalesemia is minor when half the beta is functional. The same as in the alpha thalassemia table when half of the alpha were functional the symptoms were mild.

b. In the third raw there is some partial functional b thalesemia produced B+, however β^E β^0 is more on the severe end.

C- homozygous β^E β^E leads to mild microcytic anemia as it causes the production of B chain to be reduced.

Summary of Thalassemias

Genotype	α-globin gene number[a]	Name	Phenotype
αα / αα	4	Normal state	None
αα / α–	3	Silent carrier	None (values for Hb and MCV may be near the lower limits of normal)
– – / αα or α– / α–	2	Thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic anemia
– – / α–	1	Hb H disease	Thalassemia intermedia: mild to moderate microcytic anemia
– – / – –	0	Alpha thalassemia major	Thalassemia major: hydrops fetalis

[a]Number of normal alpha globin genes

Common genotypes	Name	Phenotype
β/β	Normal	None
β/β^0 β/β^+	Beta thalassemia trait	Thalassemia minor: asymptomatic, mild microcytic hypochromic anemia
β^+/β^+ β^+/β^0 β^E/β^+ β^E/β^0	Beta thalassemia intermedia	Variable severity Mild to moderate anemia Possible extramedullary hematopoiesis Iron overload
β^0/β^0	Beta thalassemia major (Cooley's Anemia)	Severe anemia Transfusion dependence Extramedullary hematopoiesis Iron overload

2- Qualitative abnormalities in hemoglobin:

- They are caused by point mutations, and most of them occur in the beta globin chain.

A. Mutations in surface residue
B. Mutations in internal residues
C. Mutations at α1-β2 contacts
D. Mutations stabilizing methemoglobin

- Note: Qualitative abnormalities refers to abnormal functionality rather than quantity.

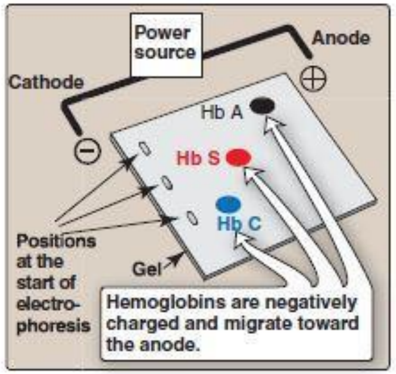
Classification of molecular mutations

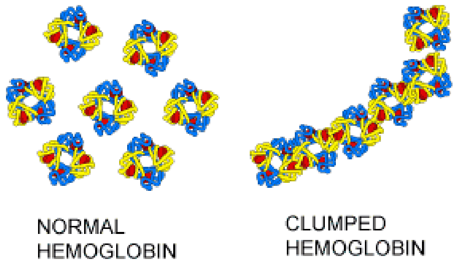
A- Mutations in surface residues

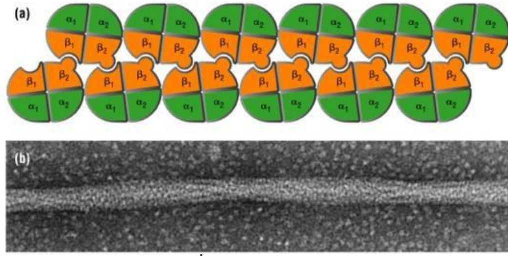
– HbS, HbC, HbSC and HbE

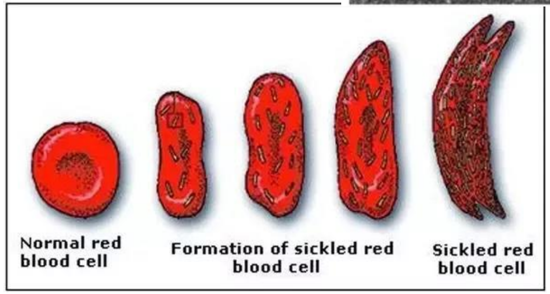
Sickle cell hemoglobin (HbS)

- It is caused by a change of amino acids in the 6th position of β globin (Glu negatively charged to Val hydrophobic).
- This change causes the hemoglobin molecules to stick together and form long fibers. Therefore, during electrophoresis at alkaline pH, because of the less negative charge of Hb S, it migrates more slowly toward the anode (positive electrode) than does Hb A. The hemoglobin is designated $\alpha 2 \beta s2$ or HbS.
- In Hb C, glutamic acid is replaced by lysine, giving it a more positive charge, so it migrates less toward the anode.



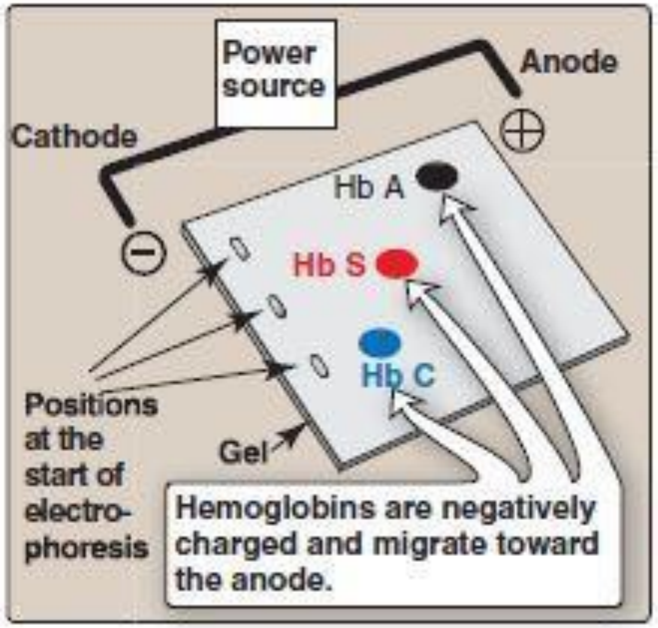


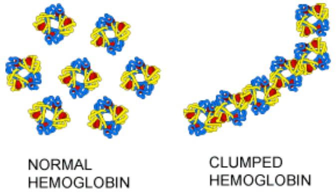


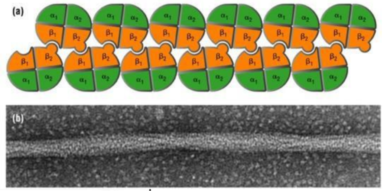


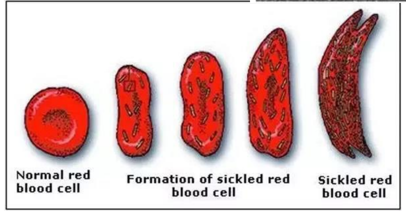
Sickle Cell Hemoglobin (HbS)

- The hemoglobin tetramers aggregate into arrays upon deoxygenation in the tissues and increase their sickle trait upon conditions that increase the deoxygenation such as high altitudes. When hemoglobin binds to CO_2 or 2,3-bisphosphoglycerate, all these interactions increase sickling.
- This aggregation leads to deformation of the red blood cell.
- It can also cause hemolytic anemia (life span of RBCs is reduced from 120 days to <20 days).
- Sickle cell anemia is characterized by lifelong episodes of pain ("crises"), the pain in sickle cell anemia occurs due to occlusion of small arteries and veins, which can also lead to stroke, and increased susceptibility to infections, usually beginning in early childhood. Other symptoms include acute chest syndrome, stroke, splenic and renal dysfunction, and bone changes due to marrow hyperplasia.







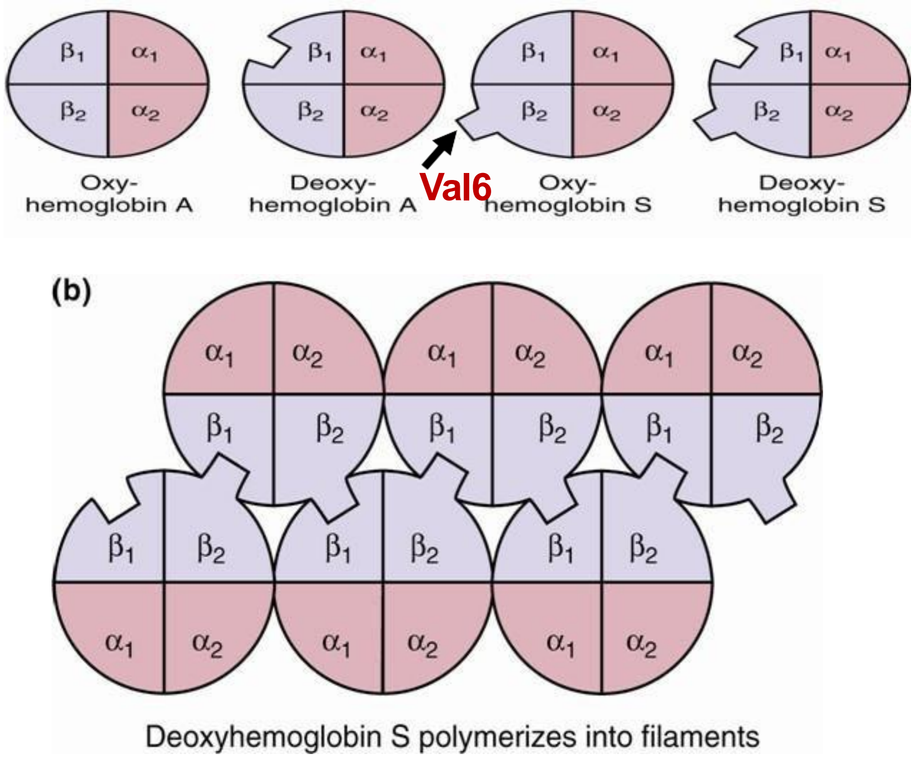


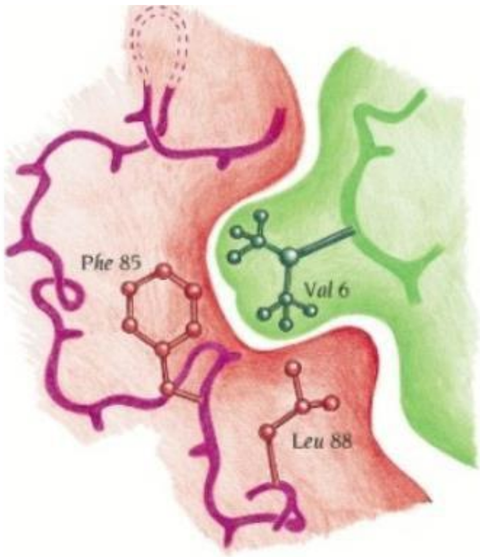
Sickle cell hemoglobin (HbS) control

- Therapy involves adequate hydration, analgesics, aggressive antibiotic therapy if infection is present or transfusion is done. Intermittent transfusions with packed red cells reduce the risk of stroke, but the benefits must be weighed against the complications of transfusion, which include iron overload (hemosiderosis), bloodborne infections, and immunologic complications.
- Hydration is an important management measure, as dehydration increases blood viscosity and the likelihood of red blood cell sickling (clumping).
- Hydroxyurea, an antitumor drug, is therapeutically useful because it increases circulating levels of Hb F, which decreases RBC sickling

How does the fiber form?

- Fiber formation only occurs in the **deoxy- or T-state**.
- The mutated valine of the $\beta 2$ chain is protruded and inserted into a hydrophobic pocket on the surface of $\beta 1$ chain.



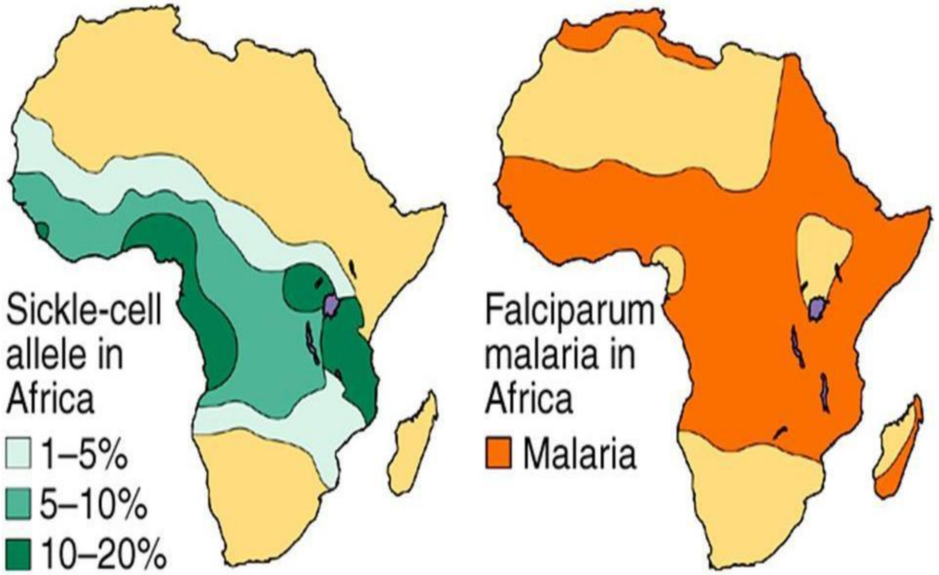


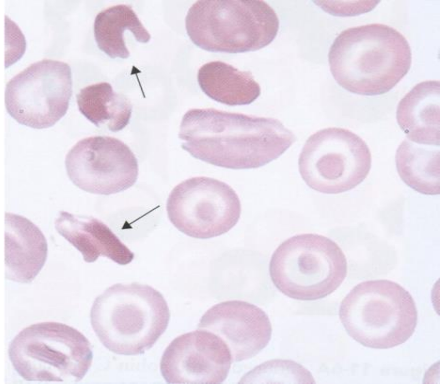
<u>Variables that increase sickling:</u> (simply anything that leads to deoxygenation)

- Decreased oxygen pressure (high altitudes)
- Increased pCO_2
- Decreased pH
- Increased 2,3-BPG
- Dehydration (why?)
- Because dehydration increases the concentration of the hemoglobin

Sickle cell trait

- It occurs in **heterozygotes** (individuals with both HbA and HbS), who are clinically normal or show less severe symptoms relative to those with homozygous trait, but they have few cells that sickle when subjected to low oxygen. They have lower potential to sickle than the homozygote trait.
- Generally, sickle cells have selective advantage against malaria because they have a **shorter life span**, so the parasite, **plasmodium falciparum will not have enough time** to complete the intracellular stage of its development.
- The figure shows both sickle-cell hemoglobin and malarial spread patterns in the African continent. Note that with higher sickle cell spread there is less malarial spread.



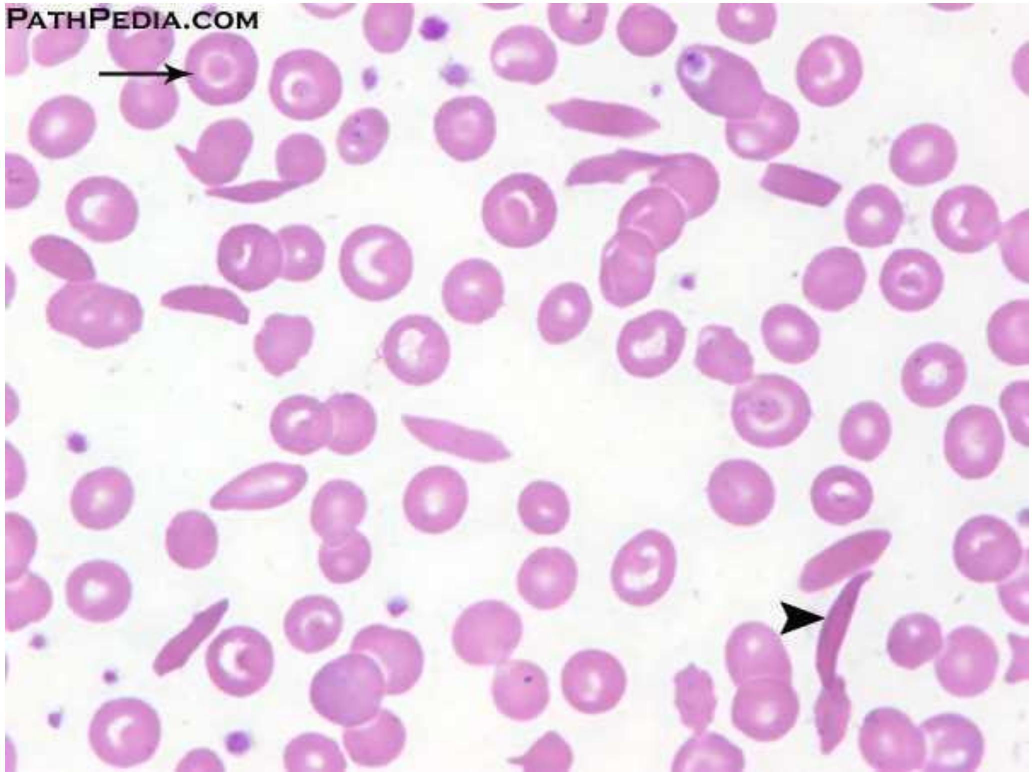


Hemoglobin C (HbC)

- (HbC) is also due to a change at the **6th** position of β globin replacing the **glutamate** with **lysine** (designated as βc).
- This hemoglobin is less soluble than HbA, so it **crystallizes** in RBCs reducing their deformability in capillaries.
- HbC also leads to water loss from cells leading to higher hemoglobin concentration. This dehydration occurs because the HbC mutation in the beta-globin chain changes the **charge and solubility** of hemoglobin inside red blood cells, this activates a potassium-chloride cotransporter (KCC) leading to increased cellular loss of potassium and water.
- This problem causes only <u>a minor hemolytic disorder</u>.

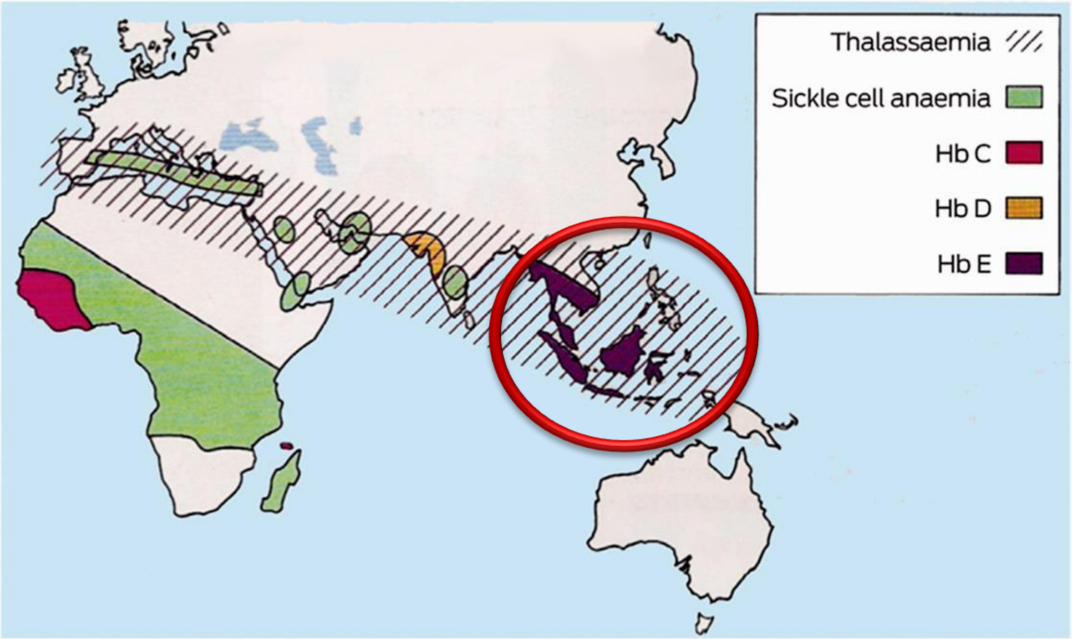
HbSC disease

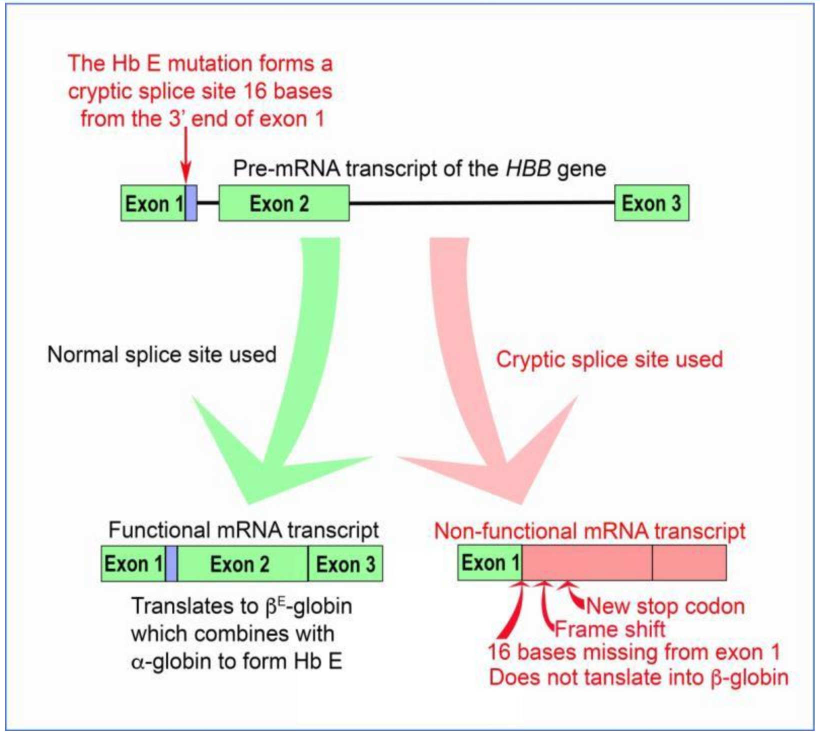
- Individuals with both βc and βs mutations have HbSC disease, a mild to severe hemolytic disorder that may have no clinical consequences but is clinically variable.



Hemoglobin E

- It is common in Southeast Asia
- It has both quantitative (lead to less HB production) and qualitative characteristics (the point mutation affects the functionality; however, it is towards the milder end).
- It can cause alternative RNA splice site and an earlier stop codon which causes lower production of hemoglobin leading to microcytic anemia (Quantitative).
- It can cause a point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating a slightly different hemoglobin (Qualitative).
- Individuals with this mutation make only around 60% of the normal amount of b-globin protein making it some sort of thalassemia.
- Mild disease but can be severe if co-inherited with beta-thalassemia, where the genotype is $\beta^E\beta^0$, leading to low/mutated production and no production respectively.

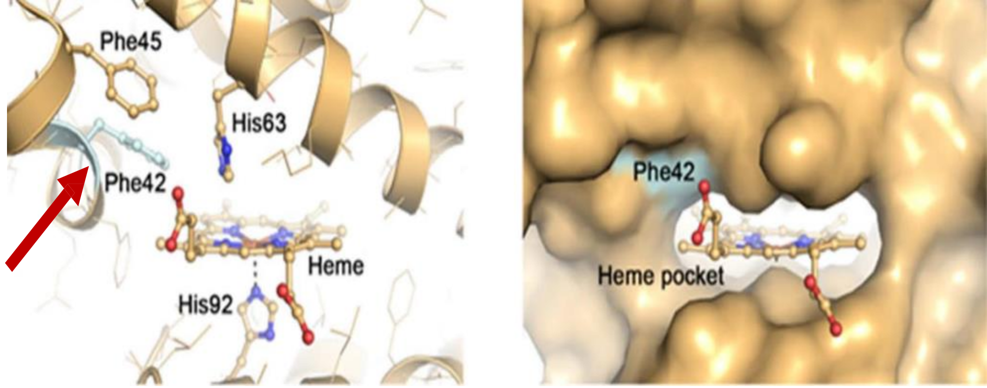




B- Mutations in internal residues

– Hb Hammersmith, Hb Constant Spring (Hb CS)

Hb Hammersmith

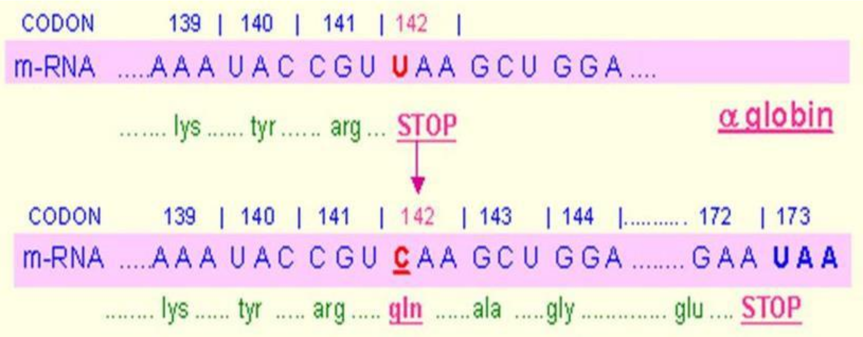


- Hb Hammersmith results from a point mutation that leads to formation of unstable hemoglobin and denaturation of the globin protein.
- The most common point mutation of Hb Hammersmith substitutes an internal phenylalanine (hydrophobic A.A) with a serine within the β globin, reducing the hydrophobicity of the heme-binding pocket, heme positioning, and lower oxygen binding affinity causing cyanosis.

Underlined word is hyperlinked.

Hb Constant Spring (Hb CS) Not HbSC

- Hemoglobin Constant Spring (Hb CS) is an abnormal Hb caused by a mutation at the termination codon of the **α2-globin** gene leading to the production of longer than normal unstable mRNA and protein products.
  - The anemia is usually moderate.
- Heterozygotes have the **genotype** ($\alpha\alpha/\alpha\alpha^{CS}$) and have **α°-thalassemia trait phenotype,** like if it was (αα/α°α°).
- It is commonly found among Southeast Asian and Chinese people.
- If co-inherited with α-thalassemia, it leads to an **α°-thalassemia intermedia syndrome** like if it was (αα°/α°α°).

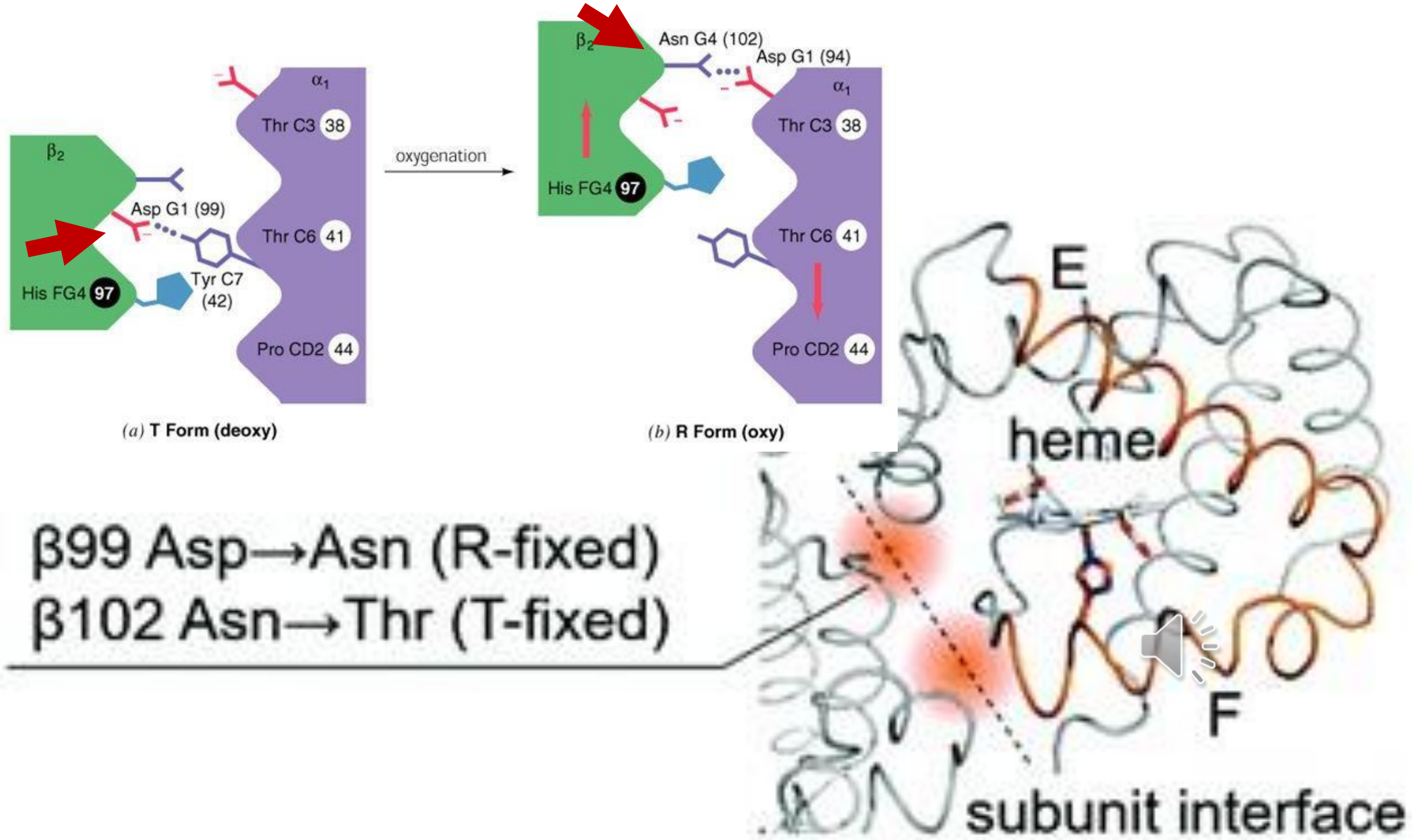


C- Mutations at α1-β2 contacts

– e.g: Cowtown, Yakima and Kansas

Mutations at α1-β2 contacts

- Eliminating hydrogen bonds between the chains can also alter the quaternary structure and affects cooperativity.
- Hb Cowtown: Substitution of His146 (responsible for the Bohr Effect) to leucine produces more hemoglobin in the R state (increased affinity) reducing O_2 supply to the tissue.
- Hb Yakima: stabilization of the R state (Asp G1 (99) to His) reducing O_2 supply to the tissue.
- Hb Kansas: stabilization of the T state (Asn G4 (102) to Thr) less binding of O_2 in the lungs.

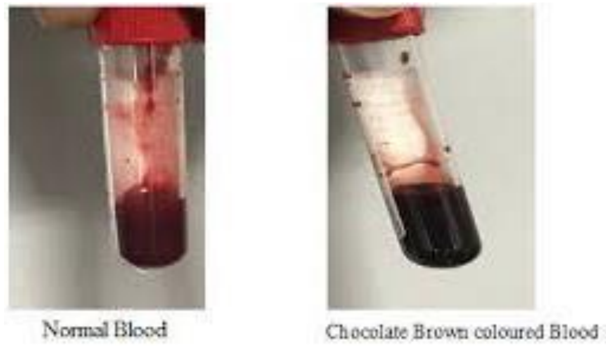


D- Mutations stabilizing methemoglobin

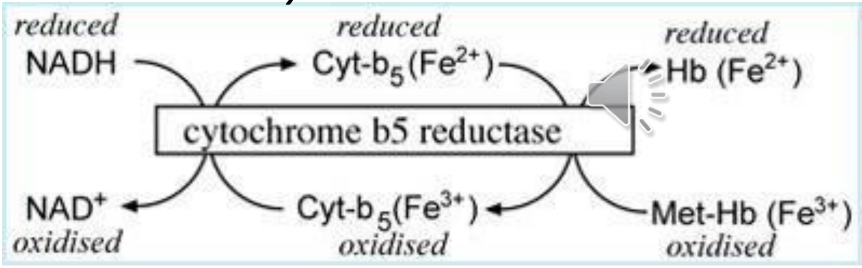
- Reversible: Reagents
- Nonreversible: Boston and Iwate

Methemoglobin (HbM)

- Hemoglobin can be reversibly oxygenated because iron remains in the reduced (ferrous, Fe^{+2}) state.
- Oxygen binding to Fe^{+2} may cause the oxidation of Fe^{+2} to Fe^{+3}, forming methemoglobin (HbM), except that the enzyme methemoglobin reductase reduces iron back.
- Symptoms are related to the degree of tissue hypoxia, and include anxiety, headache, and dyspnea. In rare cases, coma and death can occur. Treatment is with methylene blue, which is oxidized as Fe+3 is reduced
- The erythrocytes of newborns have approximately half the capacity of those of adults to reduce methemoglobin.

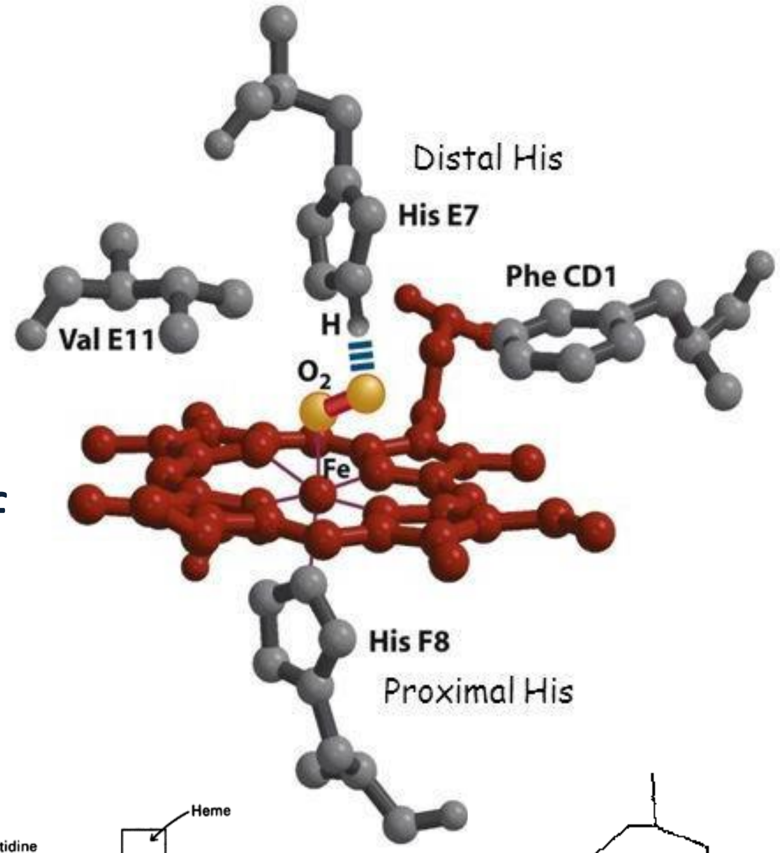


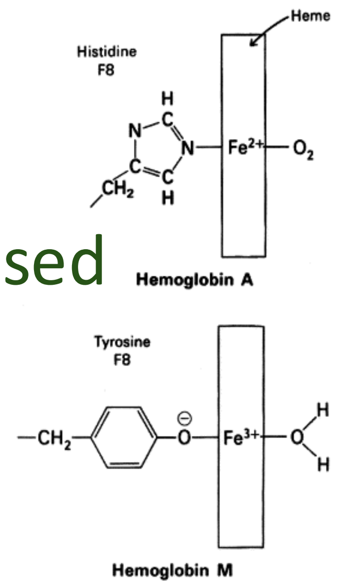
Methemoglobin reductase AKA NADH-Cytochrome b5 reductase

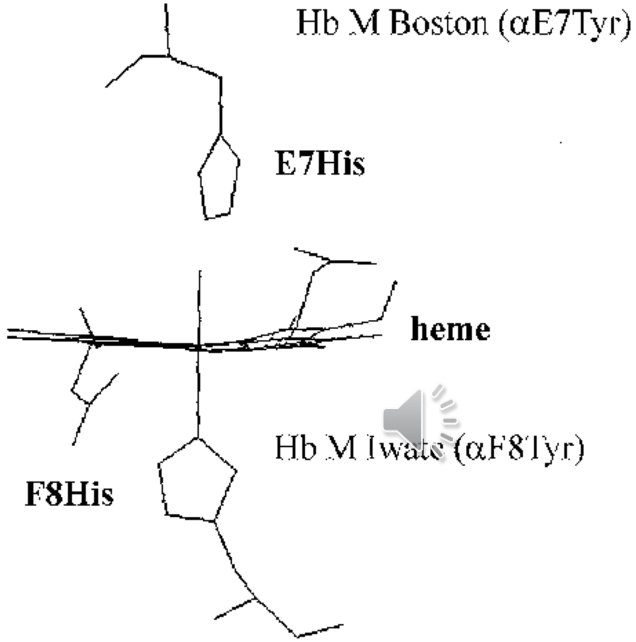


Why HbM?

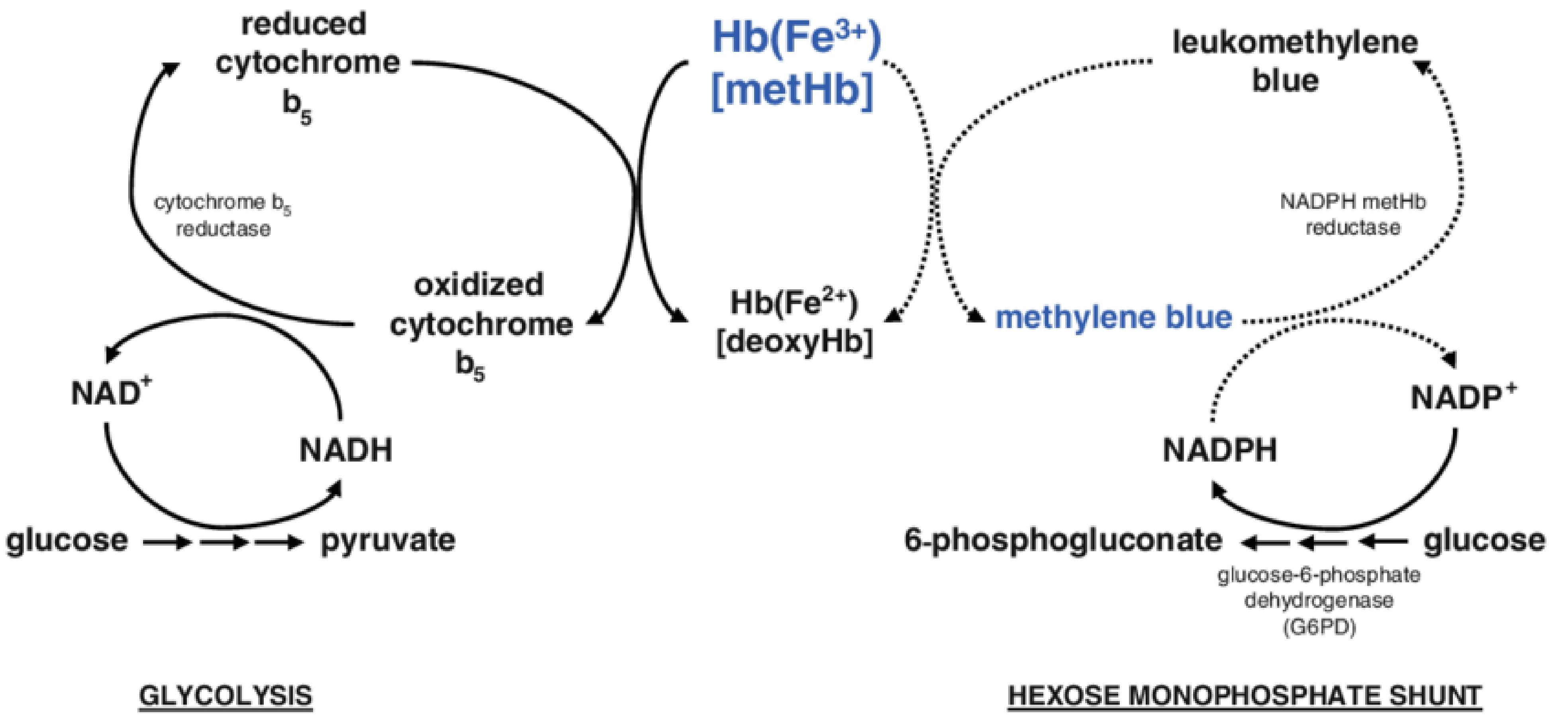
- Some mutant globins (α and β) bond with heme in such a way as to resist the reductase, **(non-reversible):**
  A. Hb Boston: **distal histidine** is mutated into a tyrosine resulting in oxidation of ferrous iron by tyrosine's oxygen.
  B. HbM Iwate: **proximal histidine** is replaced by a tyrosine.
  C. A deficiency of the reductase enzyme.
- Others are **reversible** such as those caused by: Certain drugs or drinking water containing nitrates.







Treatment (methylene blue)



Solid arrows (→) represent normal physiology. Dotted arrows (····▸)indicate pathway only active in presence of methylene blue.

NADPH reduces methylene blues to leukomethylene blue which reduced Fe+3 to Fe+2, NADPH comes from the Hexose monophosphate shunt

What we are covering in this lecture

3. Hereditary persistence of fetal hemoglobin

Hereditary persistence of fetal hemoglobin (HPFH)

- Persons with HPFH continue to make HbF as adults.
- Because the syndrome is benign most individuals do not even know they carry a hemoglobin abnormality.
- Many HPFH individuals harbor large deletions of the δ- and β-coding region of the cluster.
- There is no deletion of the fetal globin genes.
- Think: treatment for β-thalassemia!!!!

GENE REGULATION

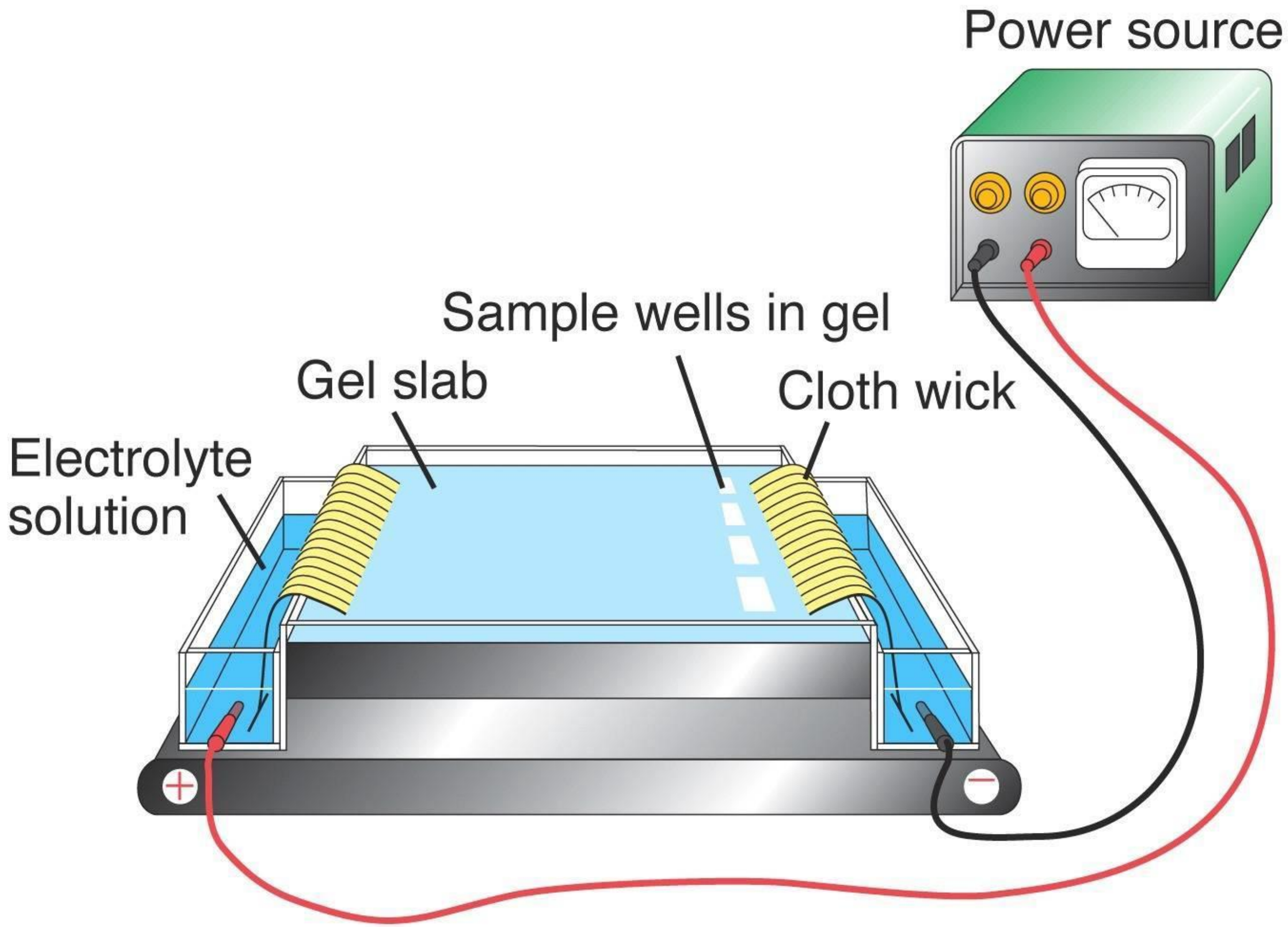
Switching from fetal to adult hemoglobin

Xunde Wang & Swee Lay Thein

Nature Genetics **50**, 478–480(2018) | Cite this article

The switch from fetal to adult hemoglobin relies on repression or silencing of the upstream γ-globin gene, but identification of the transcriptional repressors that bind to the sites at which a cluster of naturally occurring variants associated with HPFH (hereditary persistence of fetal hemoglobin) are found has been elusive. A new study provides mechanistic evidence for the direct binding of BCL11A and ZBTB7A, two previously identified γ-globin gene repressors.

Hemoglobin Electrophoresis



Mutation and migration

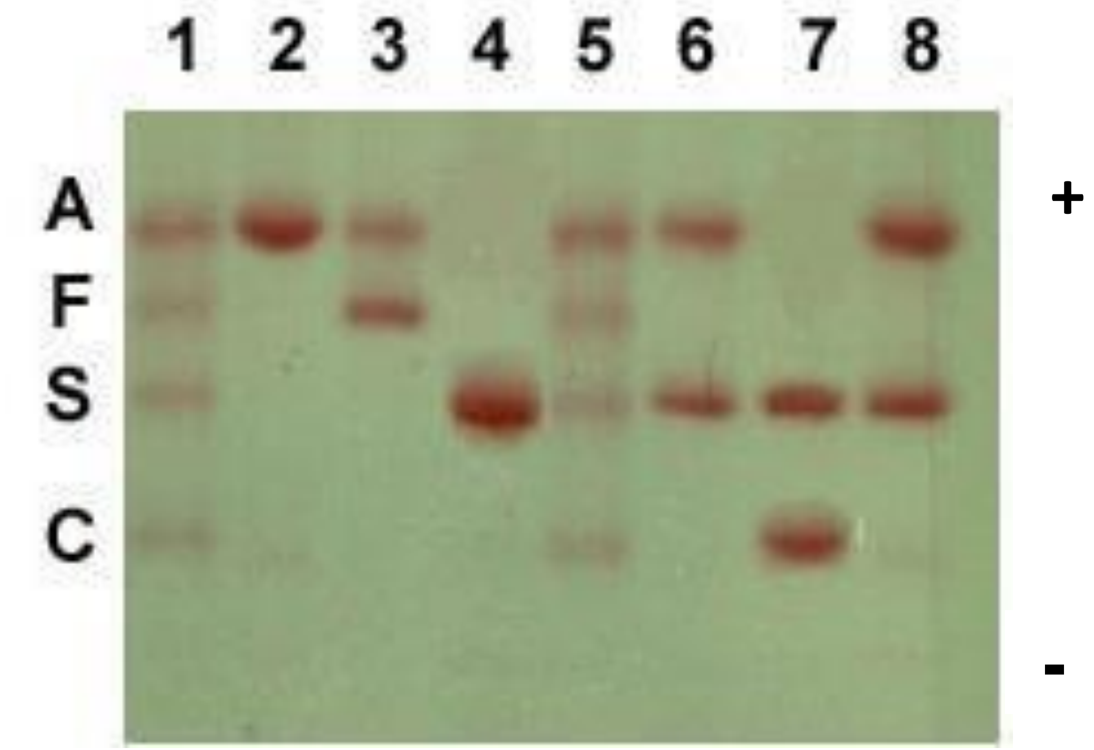
- Amino acid substitution in abnormal Hbs results in an overall change in the charge of the molecule.
- Therefore, Hb migration in a voltage gradient is altered.
- Electrophoresis of hemoglobin proteins from individuals is an effective diagnostic tool in determining if an individual has a defective hemoglobin and the relative ratios of the patient's hemoglobin pattern.

Examples

- In Sickle Cell hemoglobin, replacement of a negatively-charged glu in the standard HbA by a neutral val in HbS results in a protein with a slightly reduced negative charge.
- In homozygous individuals, the HbA tetramer electrophoreses as a single band, and the HbS tetramer as another single band.
- Hemoglobin from a heterozygous individual (with both alleles) appears as two bands.
- Since HbC contains a lysine instead of the normal glutamate, HbC will travel even faster to the cathode.

Results

- Lanes **1** and **5**: **Hb standards**
- Lane 2: normal adult
- Lane 3: normal neonate
- Lane 4: homozygous HbS
- Lanes 6 and 8: Sickle cell trait
- Lane 7: HbSC disease



Biochemistry Quiz 3

For any feedback, scan the code or click on

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide #29, 3rd point Slide #31, 6th line	Qualitative Serine (Positively Charged)	Quantitative Serine
V1 → V2			