Hemoglobinopathies

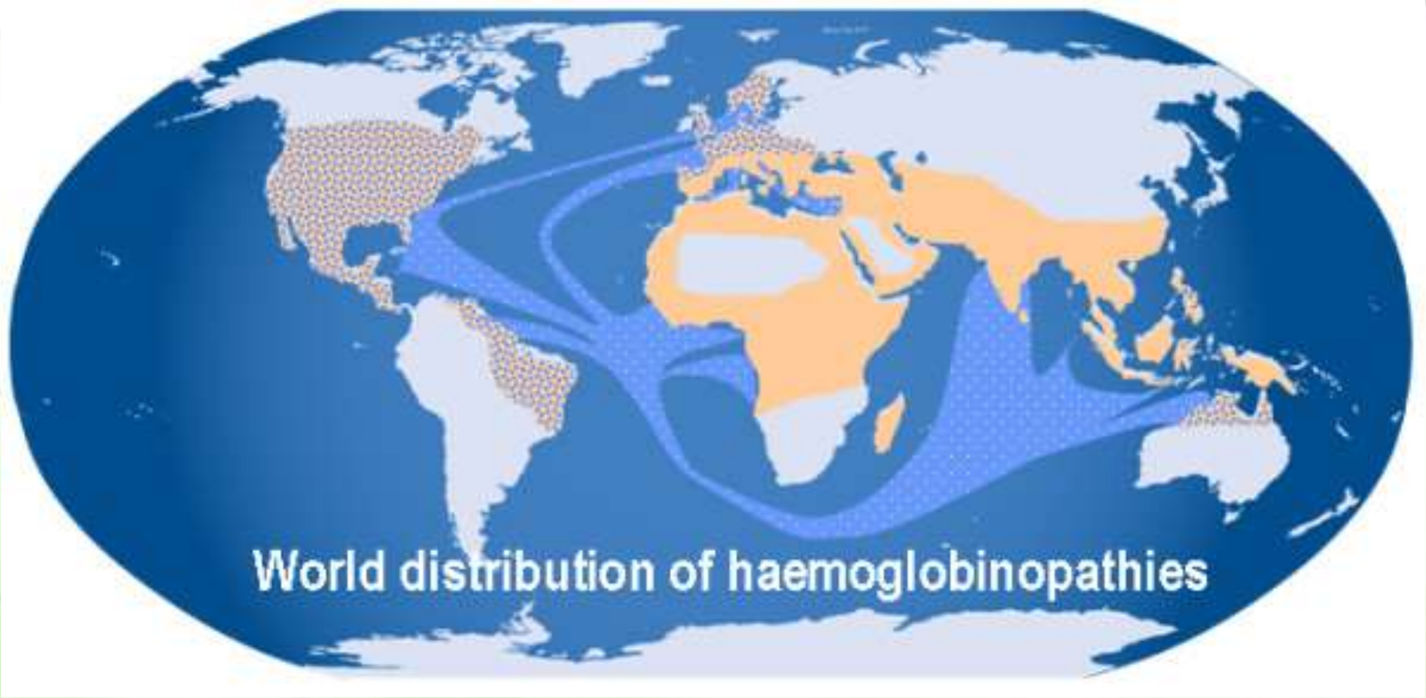
Prof. Mamoun Ahram
Hematopoietic-lymphatic system

Resources

- This lecture
- Mark’s Basic Medical Biochemistry, Ch. 44

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).
- Hemoglobin disorders account for 3.4% of deaths in children < 5 years.



Hereditary hemoglobins disorders

- Quantitative abnormalities are abnormalities in the relative amounts of α and β subunits (thalassemias).
- Qualitative abnormalities: mutations resulting in structural variants.
  - Over 800 variants have been identified.
- Hereditary persistence of fetal hemoglobin (HPFH): impairment of the perinatal switch from γ to β globin.

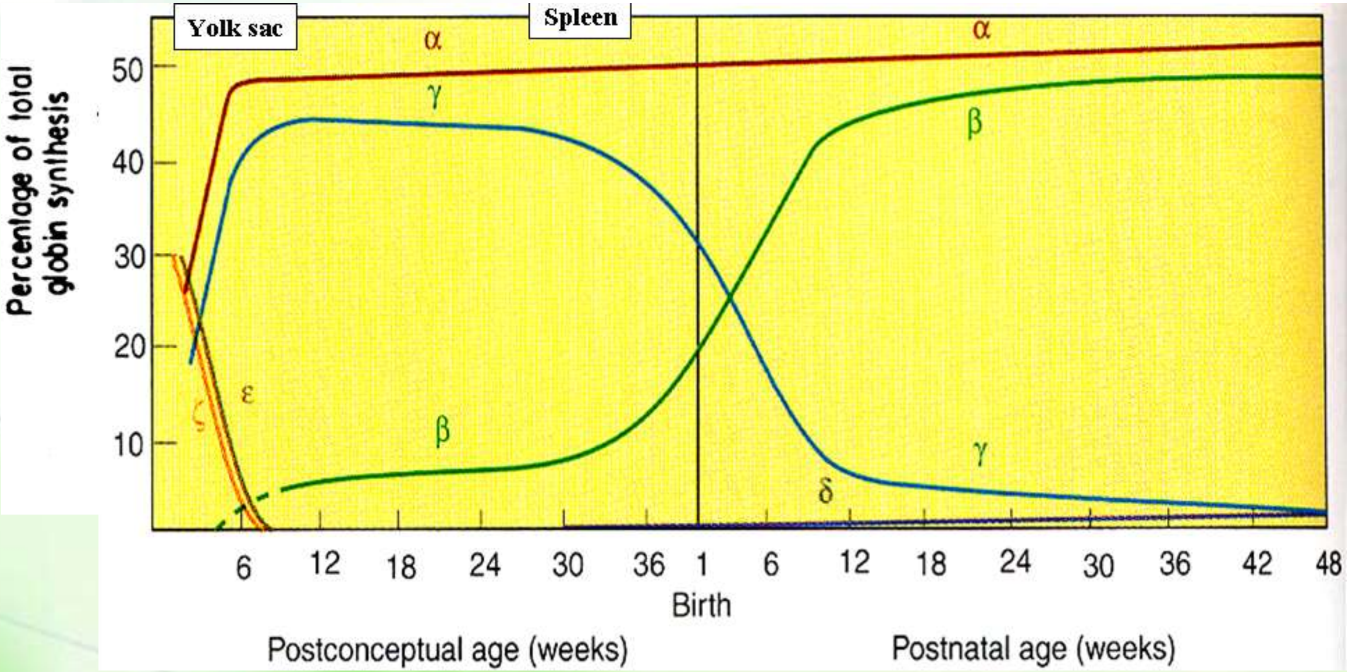
Quantitative abnormalities (thalassemias)

Thalassemias

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

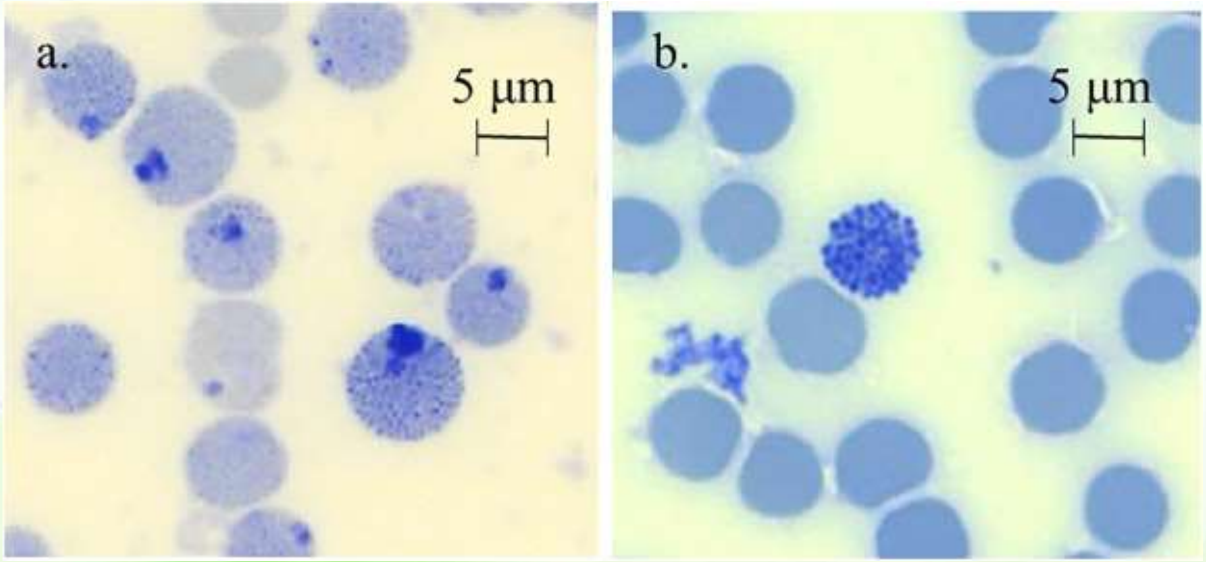
The Alpha-Thalassemias

- Alpha-thalassemia: underproduction of the α-globin chains.
- HbA ($\alpha2\beta2$), HbF ($\alpha2\gamma2$), and HbA2 ($\alpha2\delta2$) are all affected in α-thalassemia.



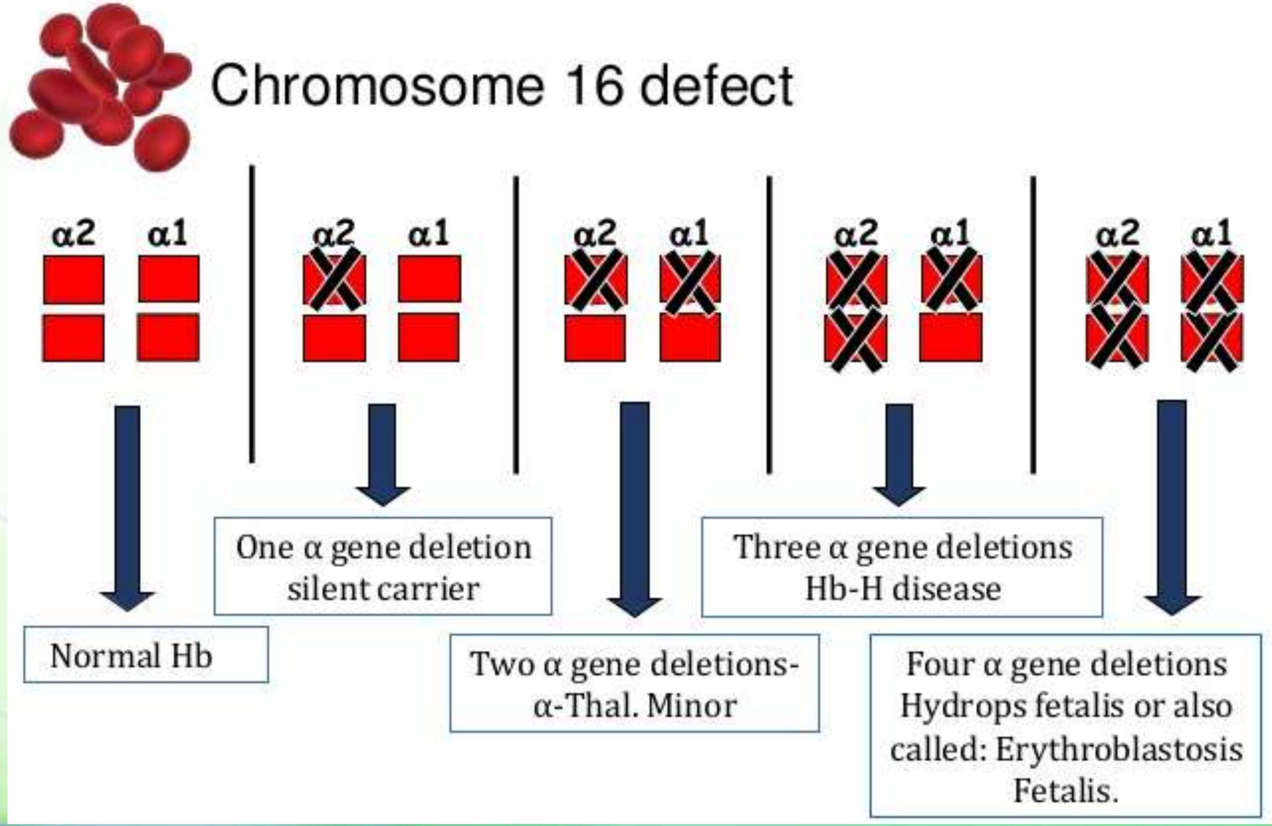
HbH

- With reduction of α chain production, and β-chain production is established, homotetramers of β ($\beta 4$ or HbH) are formed.
- The HbH tetramers have a markedly reduced oxygen carrying capacity.
- Main type of mutation is deletion (rarely point mutations)



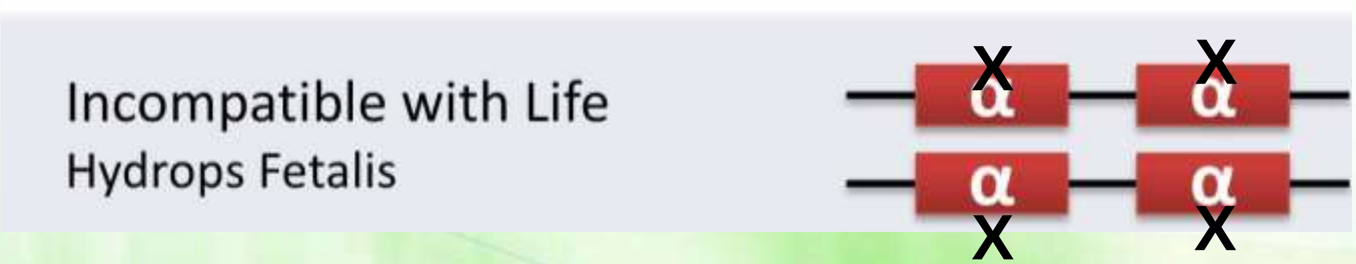
Variable severity

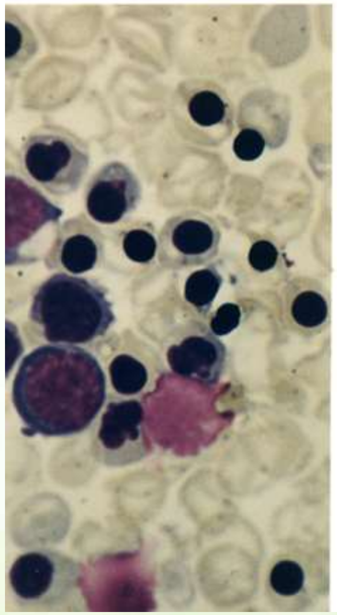
- With α-thalassemias, the level of α-globin production can range from none to very nearly normal levels.
- This is due in part to the fact that each individual has 4 genes.



Hydrops fetalis

- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a tetramer of γ-chains.
- γ4 or Hb Bart: a homotetramer of γ.
- Hb Bart has no oxygen carrying capacity resulting in oxygen starvation in the fetal tissues.
- This situation is called hydrops fetalis.
- Stillbirth or death shortly after birth occurs.

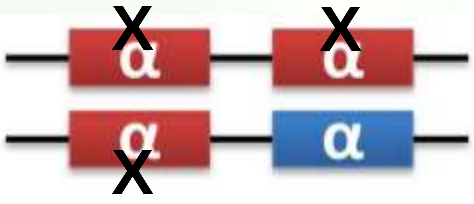


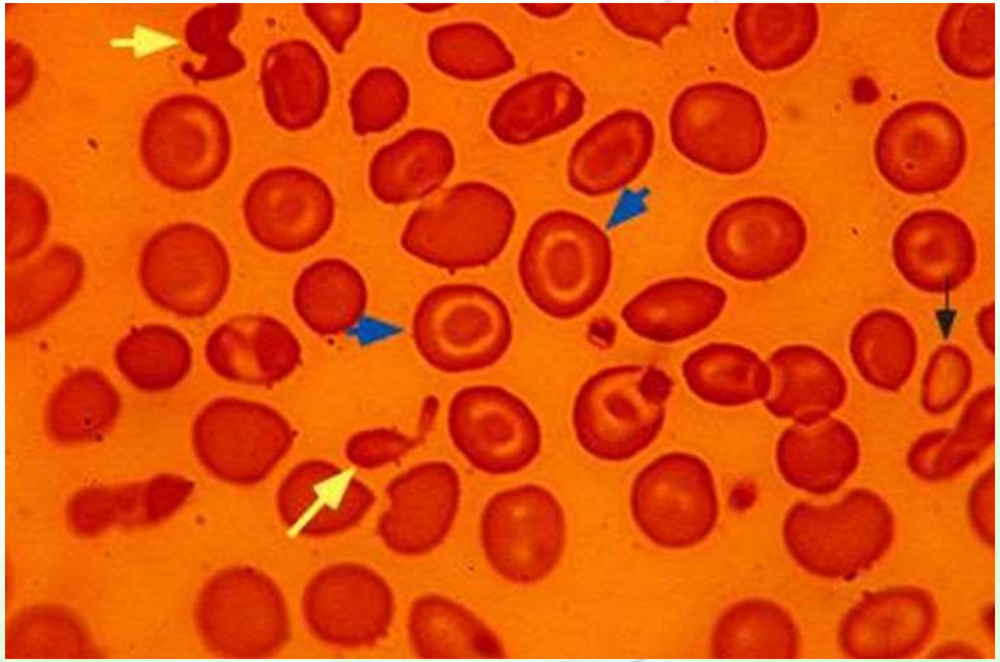


Hemoglobin H disease

- 3 of 4 genes deleted.
- Mild to moderate hemolytic anemia in adults.
- A high level of β4 tetramer is present.
- Clinically, it is known as hemoglobin H disease.
- The disease is not fatal.

Hb H Disease: Symptomatic
Hemolytic and Microcytic anemia
Splenomegaly

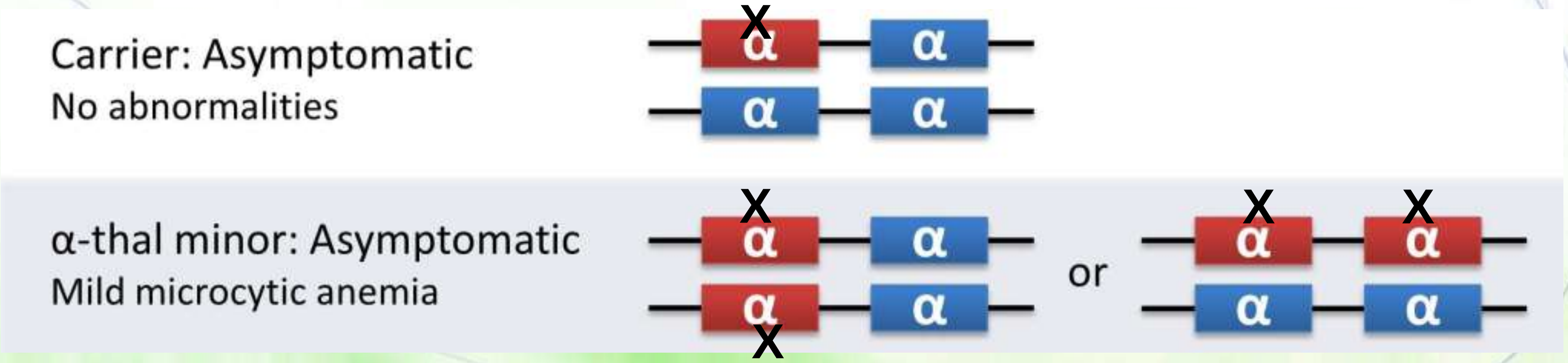




Minor α-thalassemia and silent carrier

- α-Thalassemia trait: If 2 of the 4 genes are inactivated.
  - The individuals are generally asymptomatic.

- 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

The beta-thalassemias

- β-globins are deficient and the α-globins are in excess and will form α-globin homotetramers.
- Main type of mutation is point mutations, mutations within the promoter or LCR, 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.

β-Thalassemia major and minor

- A complete lack of HbA is denoted as β^0-thalassemia or β-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.
- Individuals heterozygous for β-thalassemia is termed β-thalassemia minor.
- Affected individuals carry one normal β-globin gene and a mutated gene.
- Individuals with beta-thalassemia minor are generally asymptomatic.

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: HbE

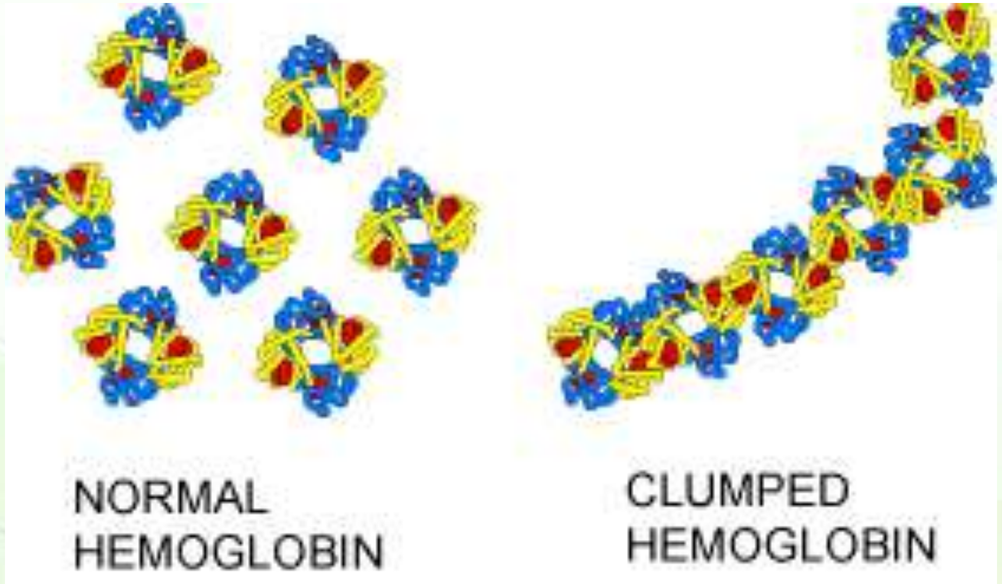
Qualitative abnormalities

Classification of molecular mutations

- Mutations in surface residues
  - Usually asymptomtic (e.g. HbE); an exception is HbS
- Mutations in internal residues
  - Often producing unstable hemoglobin, Heinz bodies and causing hemolytic anemia (e.g. Hb Hammersmith, Hb Constant Spring (Hb CS))
- Mutations stabilizing methemoglobin
  - Stabilizing heme-Fe^{+3}; resulting in cyanosis
- Mutations at α1-β2 contacts
  - Altered oxygen affinity (mainly higher; a condition known as polycythemia)

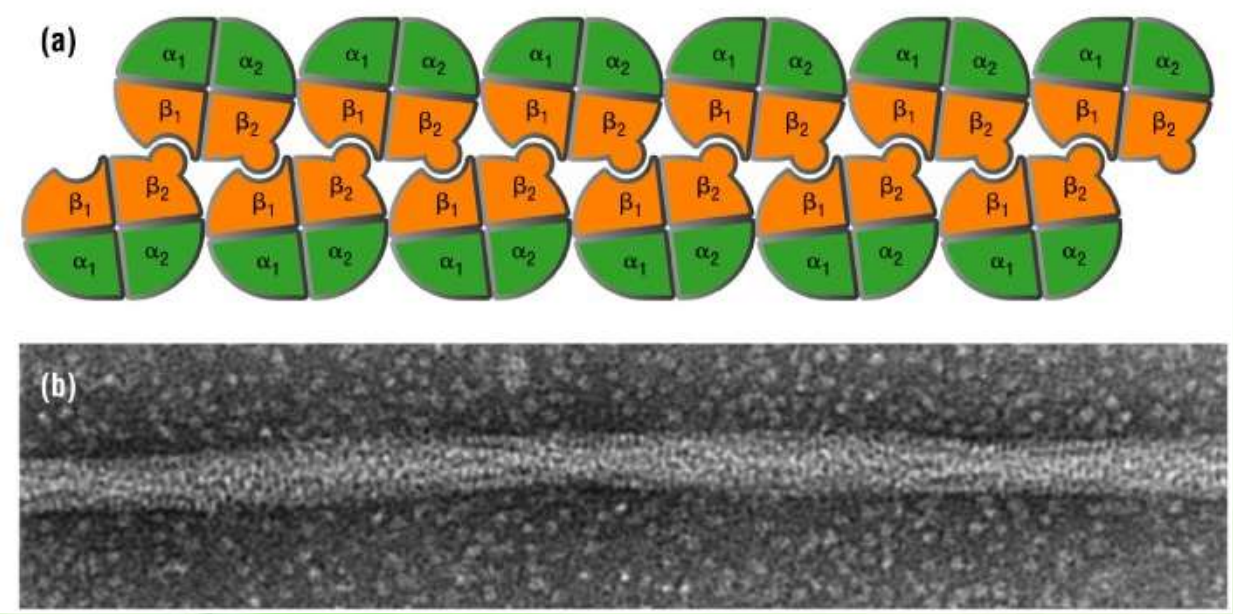
Sickle cell hemoglobin (HbS)

- It is caused by a change of amino acids in the 6th position of β globin (Glu to Val).
- The hemoglobin is designated $\alpha 2\beta s2$ or HbS.
- The hemoglobin tetramers aggregate into arrays upon deoxygenation in the tissues.
- 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).



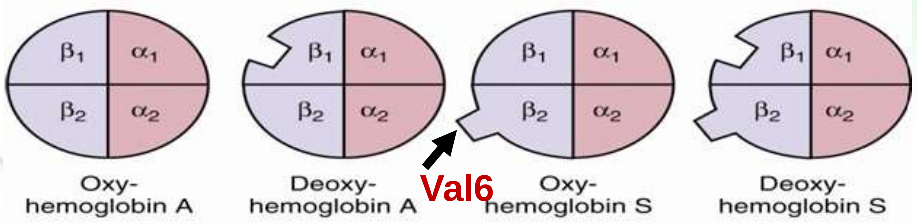
Cellular effect on system

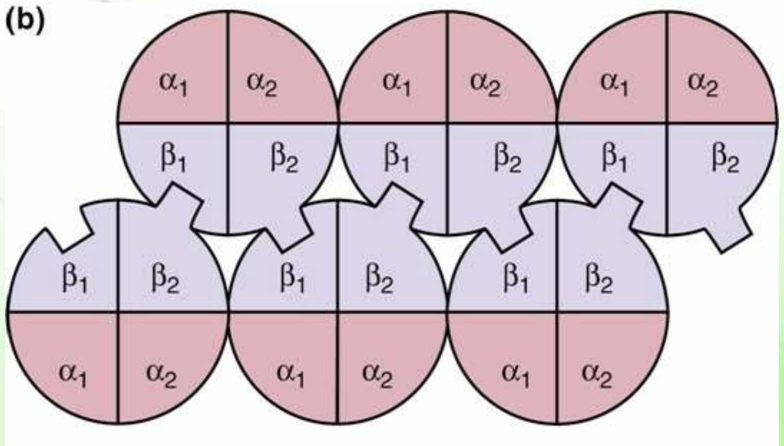
- Repeated cycles of oxygenation and deoxygenation lead to irreversible sickling.
- Cells cannot squeeze though capillaries in a single file and therefore block blood flow causing local hypoxia.
- Long-term recurrent clogging of the capillary beds leads to damage to the internal organs, in particular the kidneys, heart and lungs.



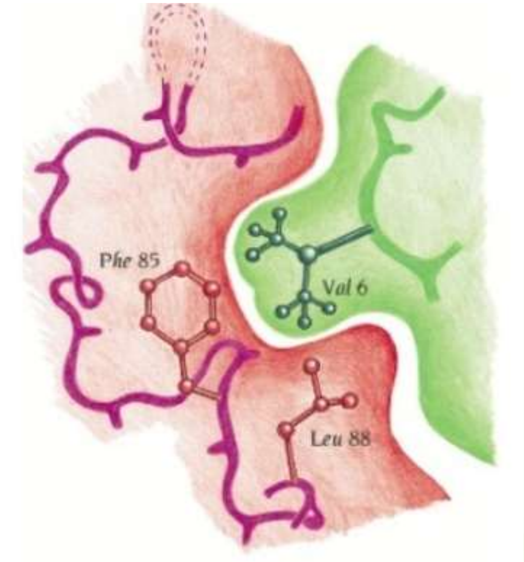
How does the fiber form?

- Fiber formation only occurs in the deoxy or T-state.
- The mutated valine of β2 chain is protruded and inserts itself into a hydrophobic pocket on the surface of β1 chain.





Deoxyhemoglobin S polymerizes into filaments



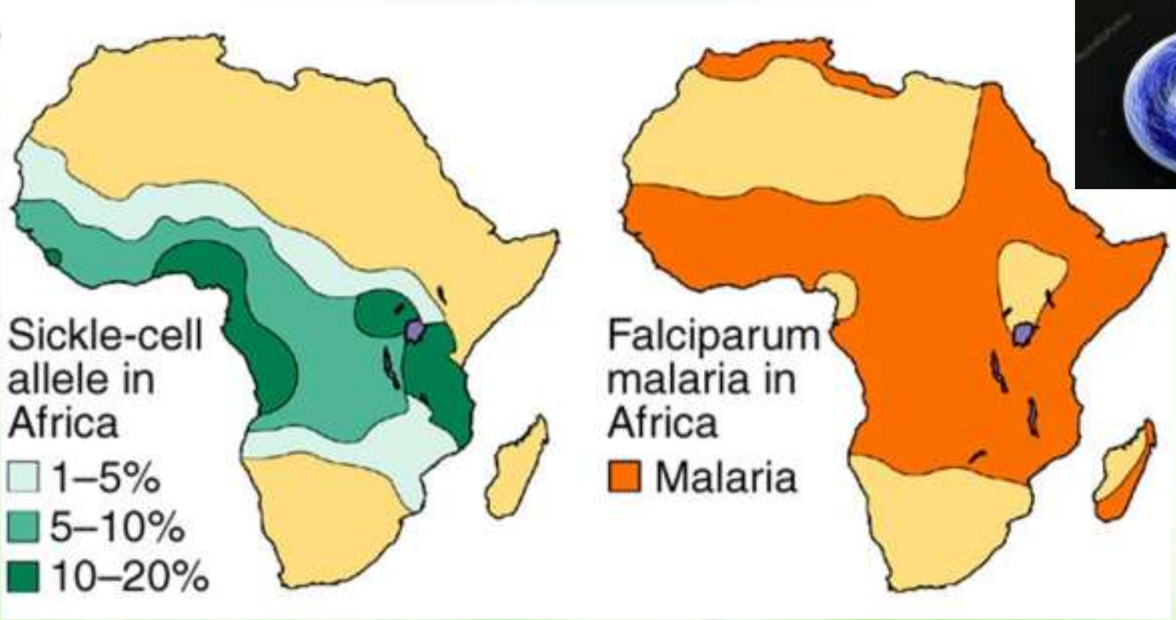
<u>Variables that increase sickling</u>

- **Decreased oxygen pressure (high altitudes)**
- **Increased pCO_2**
- **Decreased pH**
- **Increased 2,3-BPG**
- **Dehydration (why?)**

Sickle cell trait

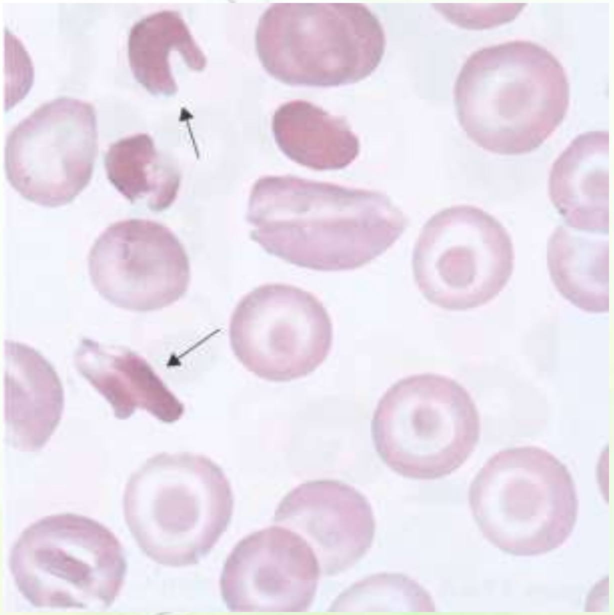
- It occurs in heterozygotes (individuals with both HbA and HbS), who are clinically normal, but their cells sickle when subjected to low oxygen.

- Advantage: selective advantage from plasmodium falciparum that causes malaria. Why?



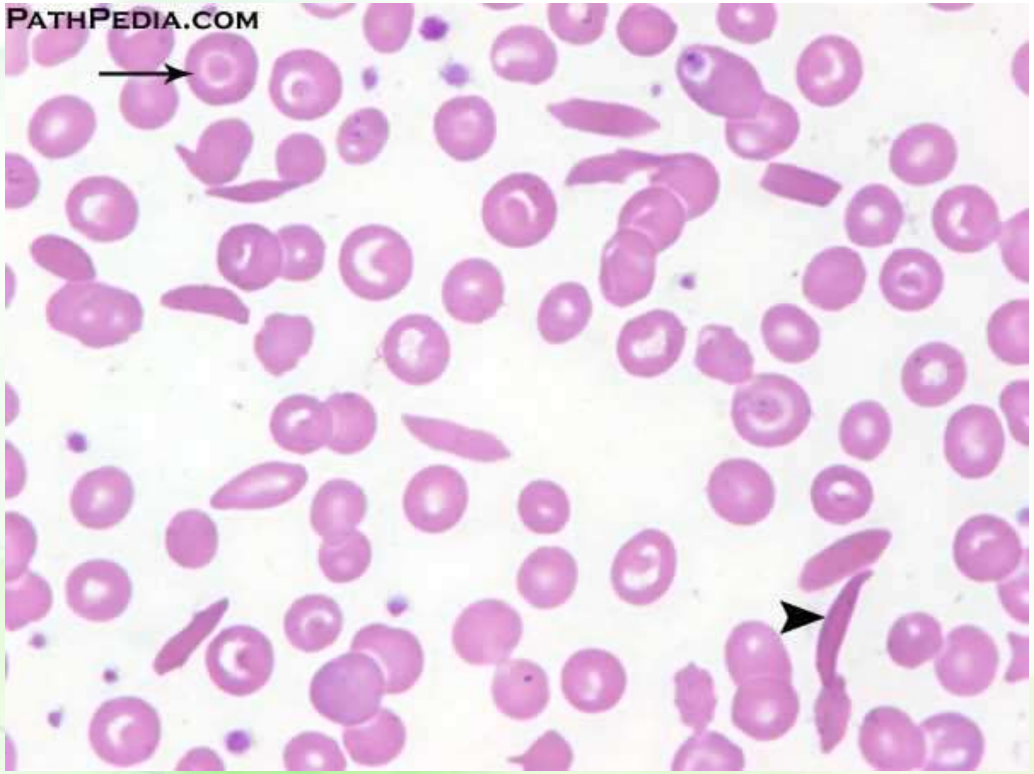
Hemoglobin C (HbC)

- (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 problem causes only a minor hemolytic disorder.

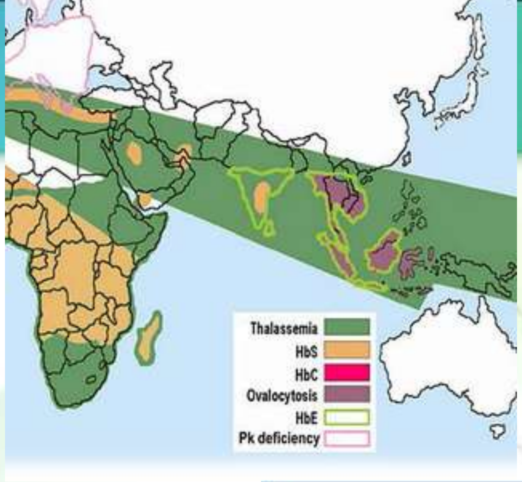


HbSC disease

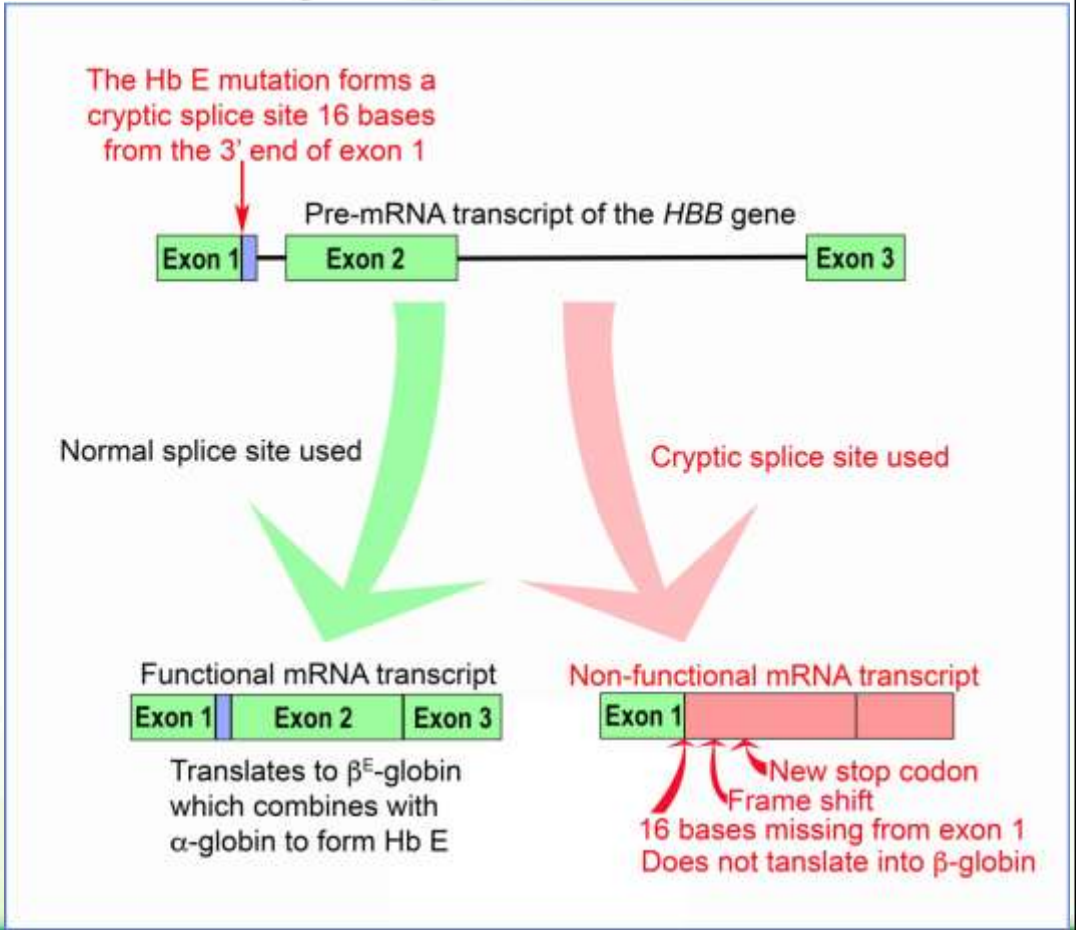
- Individuals with both βc and βs mutations have HbSC disease, a mild hemolytic disorder which may have no clinical consequences, but it is clinically variable.



Hemoglobin E

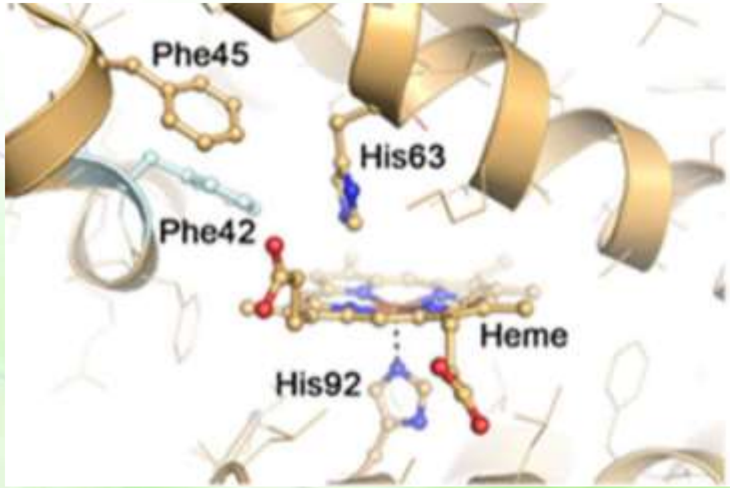


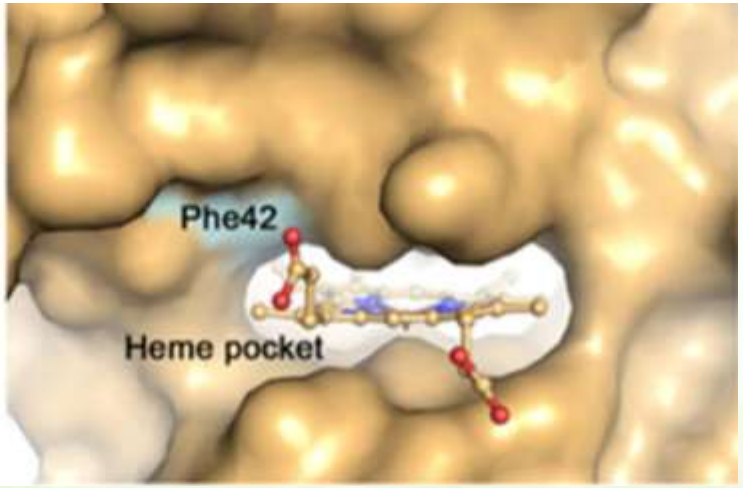
- It is common in Southeast Asia
- It has both quantitative and qualitative characteristics.
- It is caused by a point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating an alternative RNA splice site and a defective protein.
- Individuals with this mutation make only around 60% of the normal amount of β-globin protein.



Hb Hammersmith

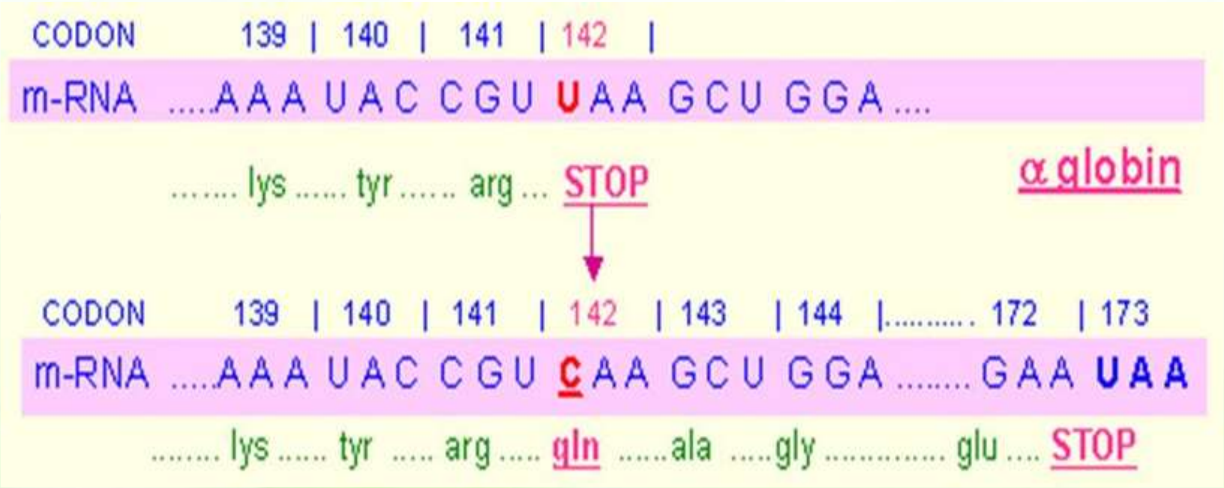
- Hb Hammersmith results from a point mutation that leads to formation of unstable hemoglobin and denaturation of the globin protein.
- The most common point mutation of Hb Hammersmith substitutes an internal phenylalanine with a serine within the β globin, reducing the hydrophobicity of the heme-binding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





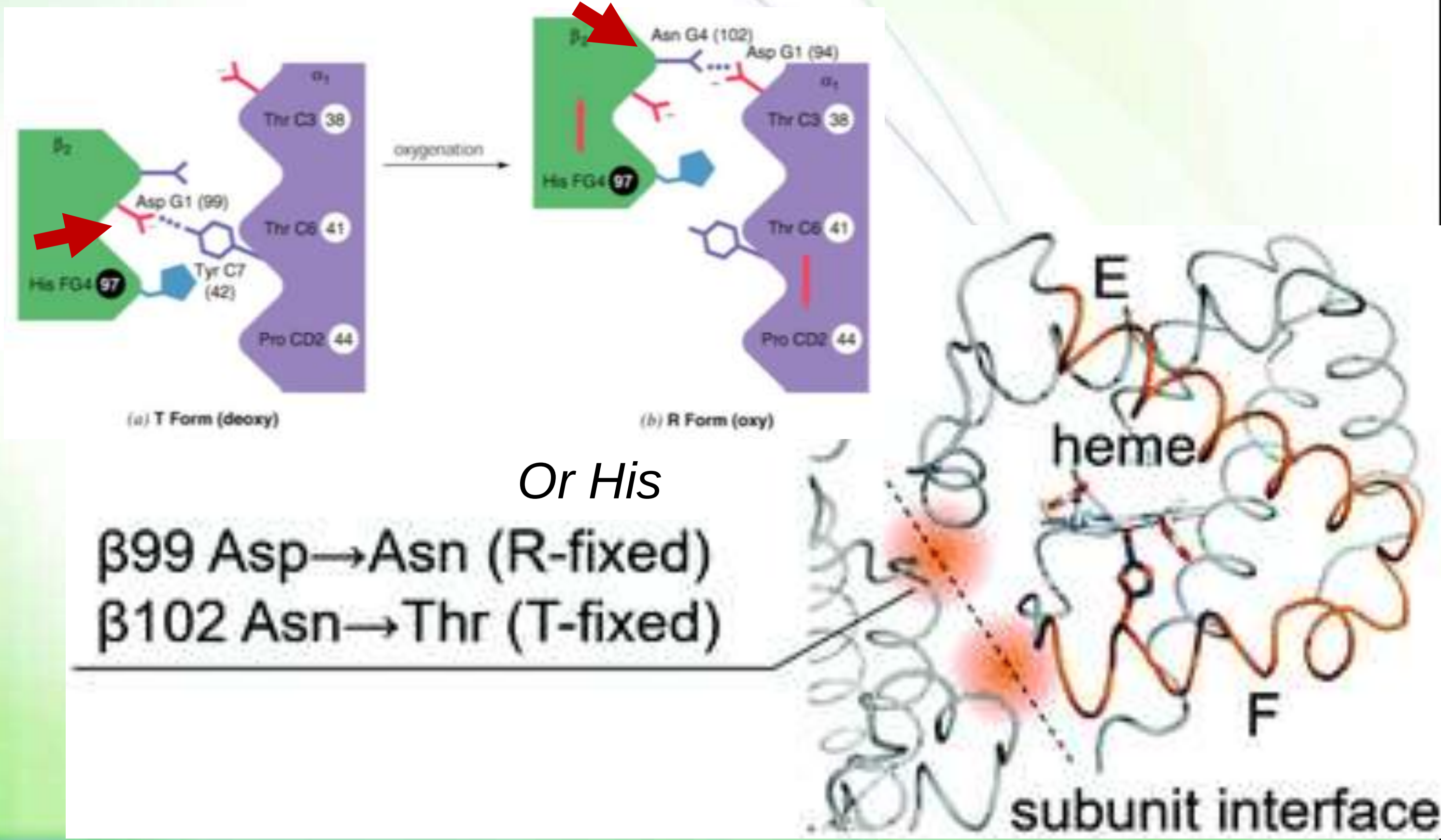
Hb Constant Spring (Hb CS)

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



Mutations at α1-β2 contacts

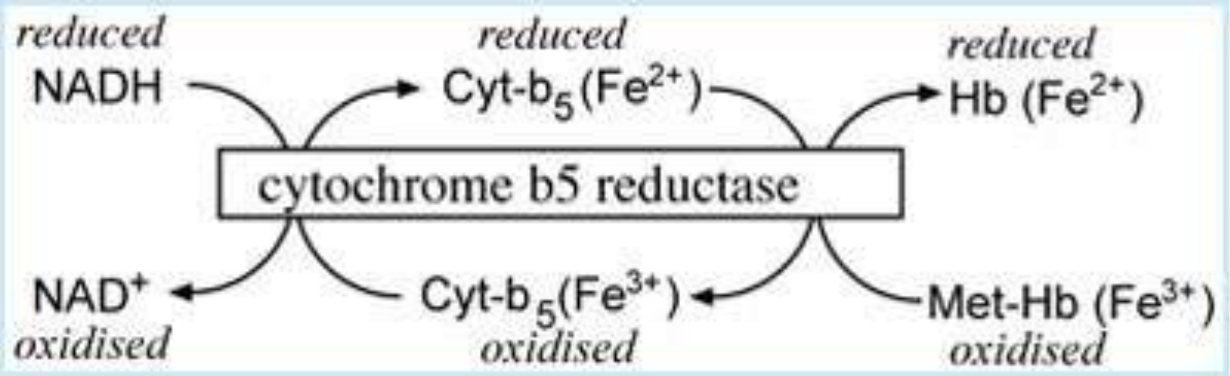
- Hb Cowtown: Substitution of His146 (responsible for the Bohr Effect) to Leucine produces more hemoglobin in the R state (increased affinity).
- Elimination of hydrogen bonds between the chains can also alter the quaternary structure:
  - Hb Yakima: stabilization of the R state (Asp G1 (99) to His).
  - Hb Kansas: stabilization of the T state (Asn G4 (102) to thr); decreased cooperativity.



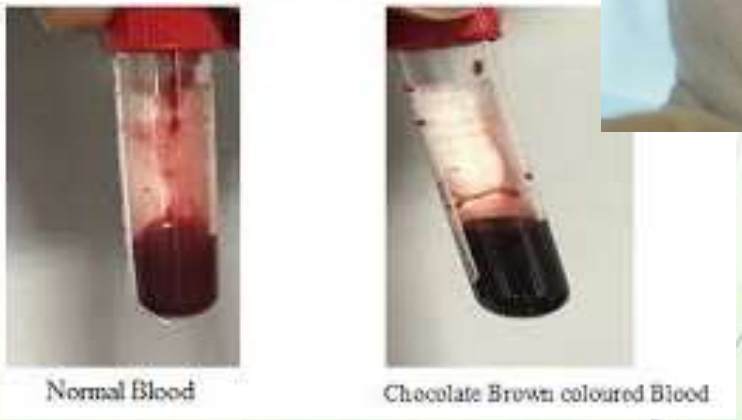
Altered Oxygen Transport

Methemoglobin (HbM)

- Oxyhemoglobin can undergo reversible oxygenation because its heme iron is in the reduced (ferrous, Fe^{+2}) state.
- During oxygen release from heme, Fe^{+2} is oxidized to Fe^{+3}, forming methemoglobin (HbM), except that the enzyme methemoglobin reductase reduces iron back.
- If not, a condition known as methemoglobinemia develops.

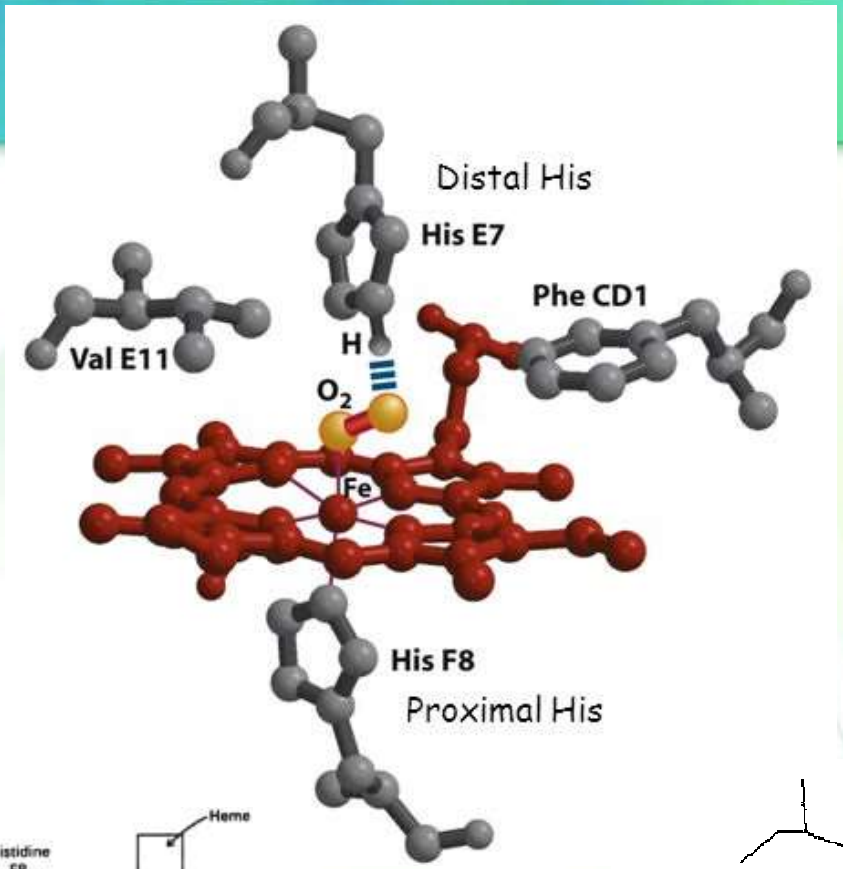


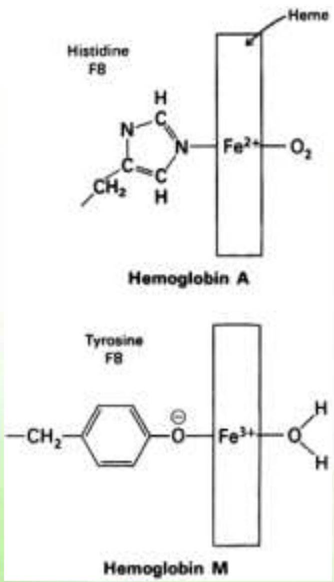
Methemoglobin reductase AKA
NADH-Cytochrome b5 reductase

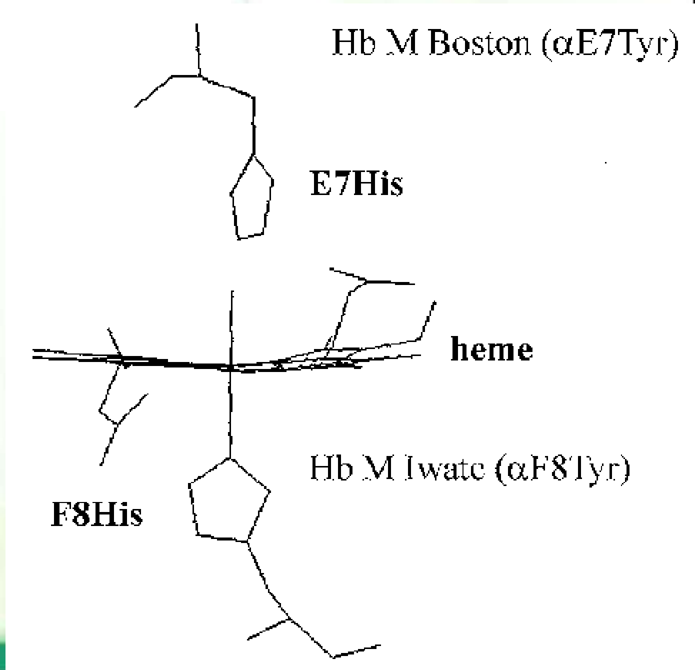


Why HbM?

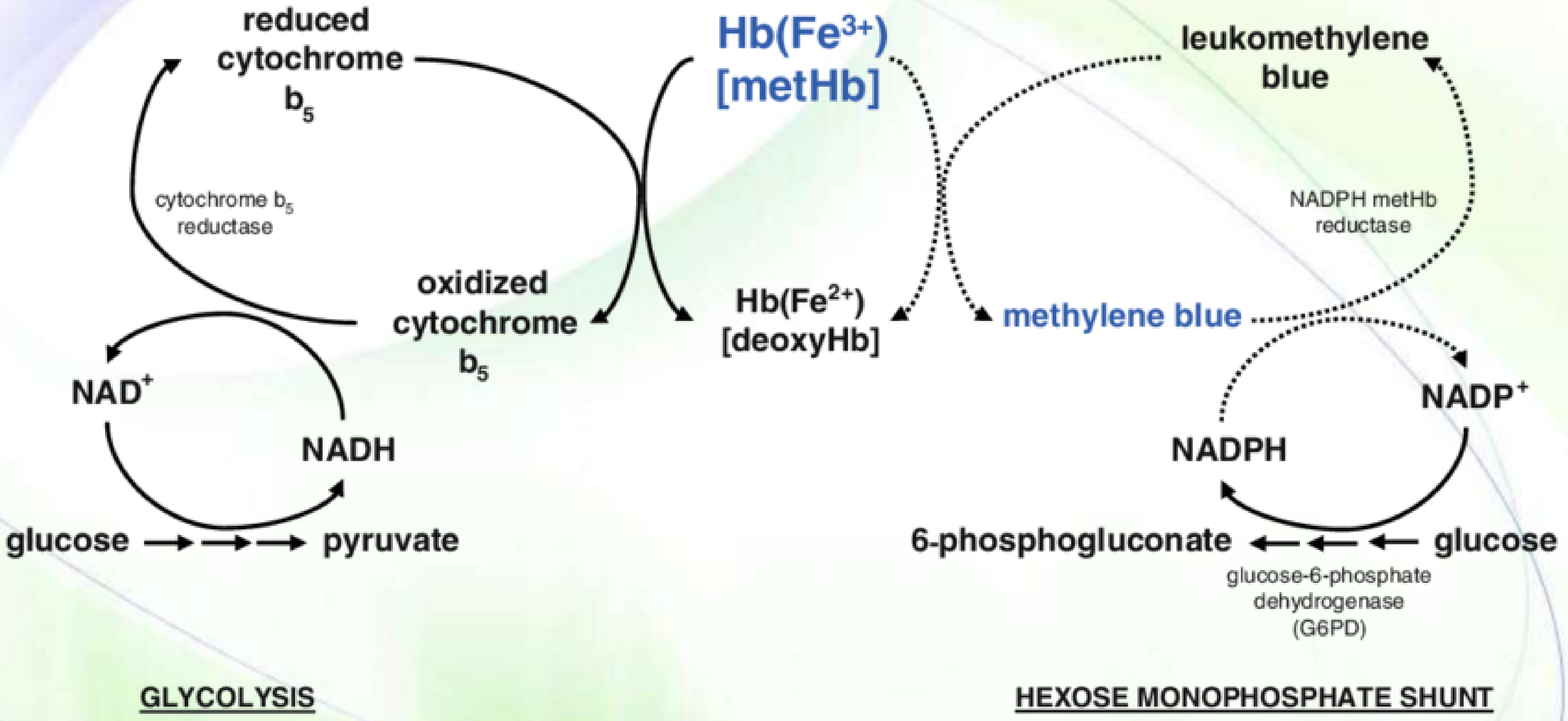
- Some mutant globins (α and β) bond with heme in such a way as to resist the reductase.
  - Hb Boston: distal histidine is mutated into a tyrosine resulting in oxidation of ferrous iron by tyrosine's oxygen.
  - HbM Iwate: proximal histidine is replaced by a tyrosine.
- A deficiency of the reductase enzyme.
- 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.

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.

Article | Published: 12 October 2022

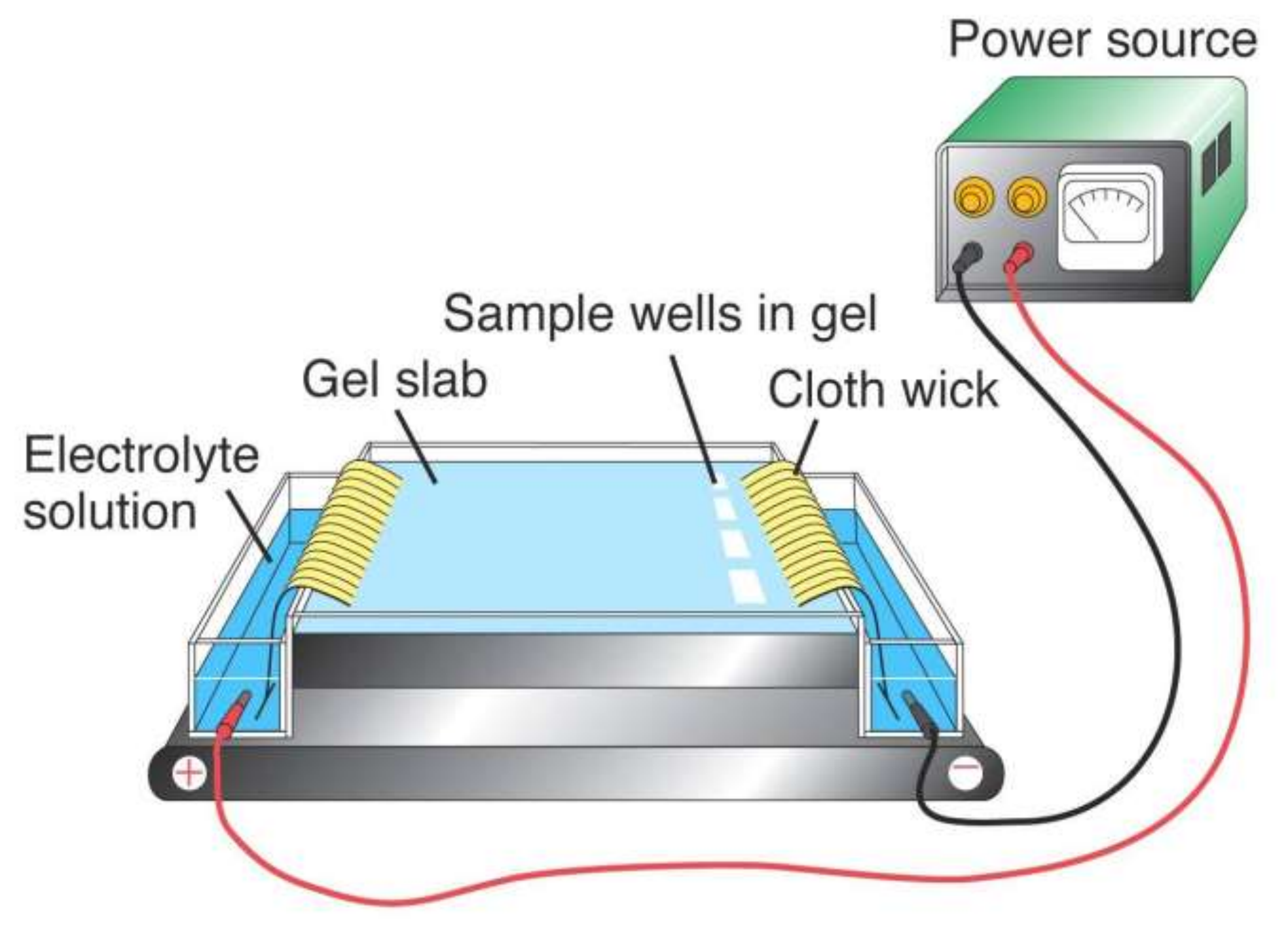
Activation of γ-globin expression by hypoxia-inducible factor 1α

Ruopeng Feng, Thiyagaraj Mayuranathan, Peng Huang, Phillip A. Doerfler, Yichao Li, Yu Yao, Jingjing Zhang, Lance E. Palmer, Kalin Mayberry, Georgios E. Christakopoulos, Peng Xu, Chunliang Li, Yong Cheng, Gerd A. Blobel, M. Celeste Simon & Mitchell J. Weiss

Abstract

Around birth, globin expression in human red blood cells (RBCs) shifts from γ-globin to β-globin, which results in fetal haemoglobin (HbF, $\alpha_2\gamma_2$) being gradually replaced by adult haemoglobin (HbA, $\alpha_2\beta_2$)[1]. This process has motivated the development of innovative approaches to treat sickle cell disease and β-thalassaemia by increasing HbF levels in postnatal RBCs[2]. Here we provide therapeutically relevant insights into globin gene switching obtained through a CRISPR–Cas9 screen for ubiquitin–proteasome components that regulate HbF expression. In RBC precursors, depletion of the von Hippel–Lindau (VHL) E3 ubiquitin ligase stabilized its ubiquitination target, hypoxia-inducible factor 1α (HIF1α)[3,4], to induce γ-globin gene transcription. Mechanistically, HIF1α–HIF1β heterodimers bound cognate DNA elements in *BGLT3*, a long noncoding RNA gene located 2.7 kb downstream of the tandem γ-globin genes *HBG1* and *HBG2*. This was followed by the recruitment of transcriptional activators, chromatin opening and increased long-range interactions between the γ-globin genes and their upstream enhancer. Similar induction of HbF occurred with hypoxia or with inhibition of prolyl hydroxylase domain enzymes that target HIF1α for ubiquitination by the VHL E3 ubiquitin ligase. Our findings link globin gene regulation with canonical hypoxia adaptation, provide a mechanism for HbF induction during stress erythropoiesis and suggest a new therapeutic approach for β-haemoglobinopathies.

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