Hemoglobinopathies

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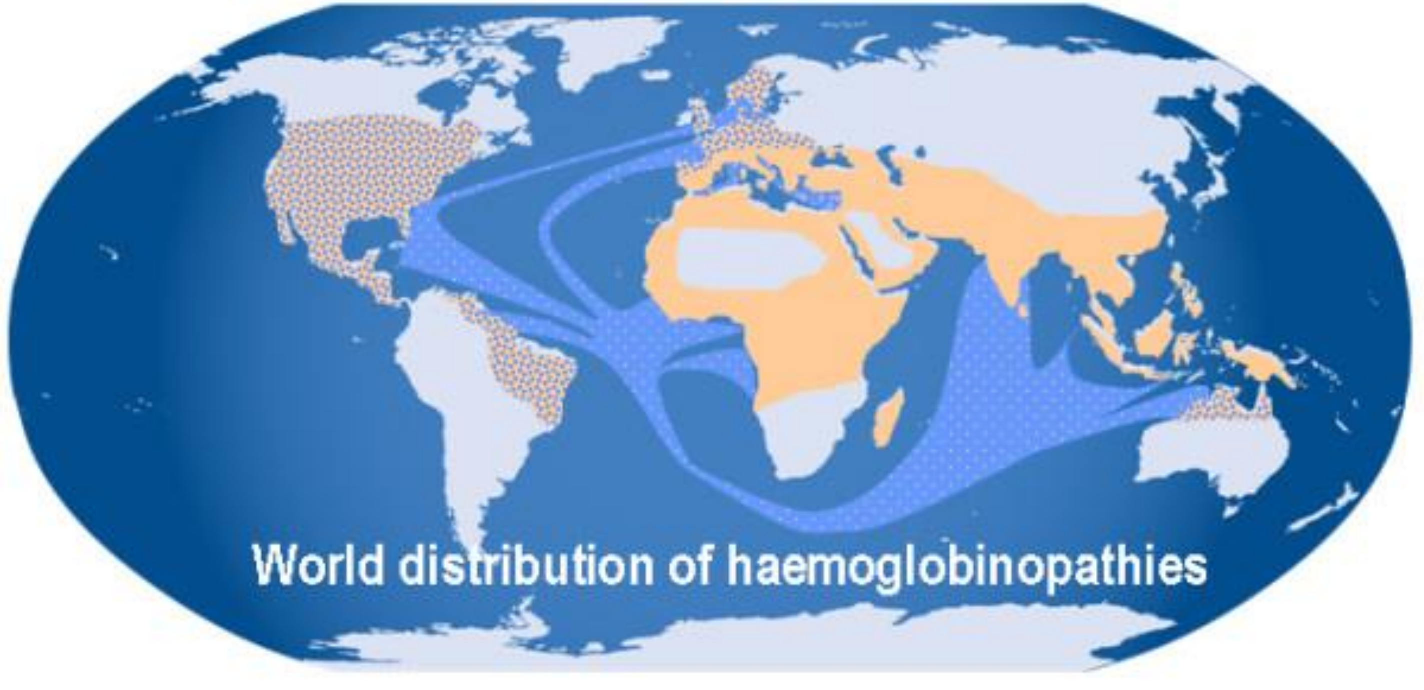
Hematopoietic-lymphatic system

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.


World distribution of haemoglobinopathies

Hereditary hemoglobins disorders

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

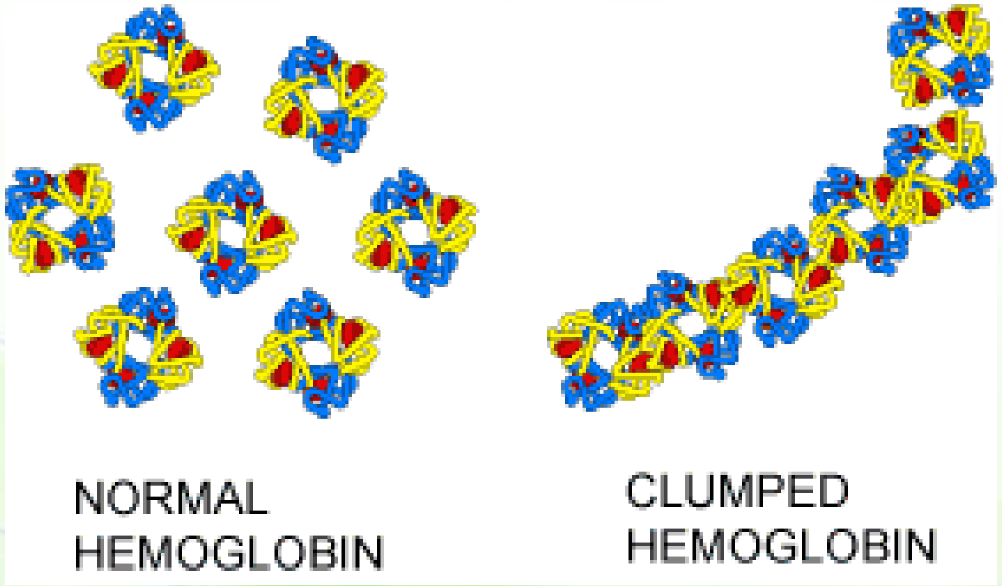
Qualitative abnormalities

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)
- 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)

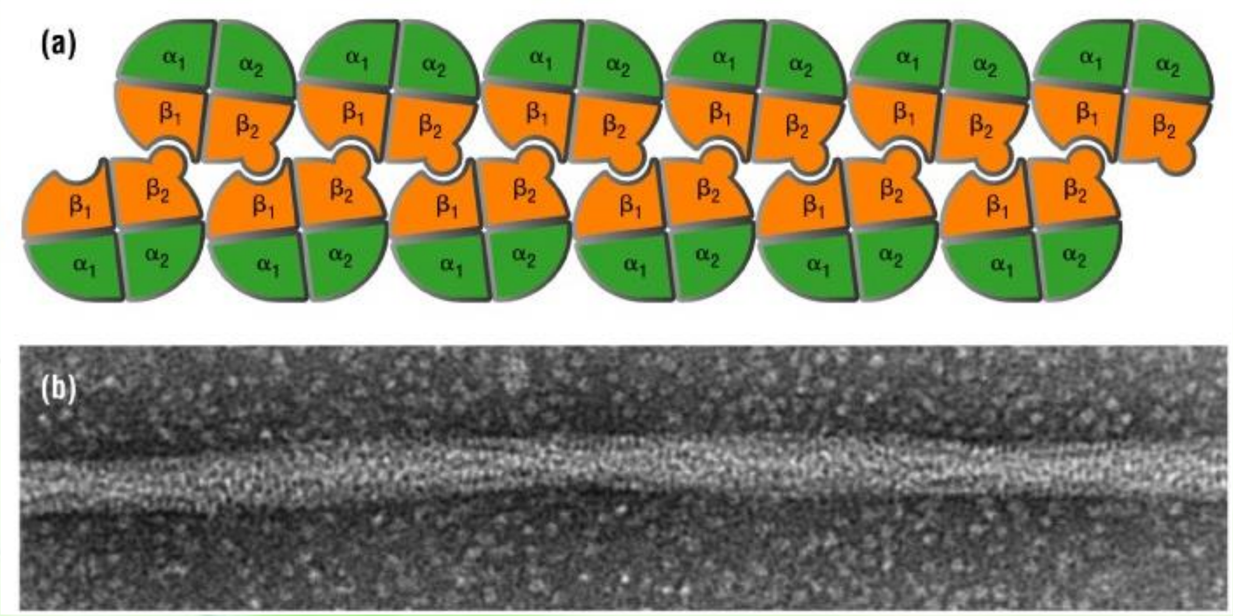
Sickle cell hemoglobin (HbS)

- 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).



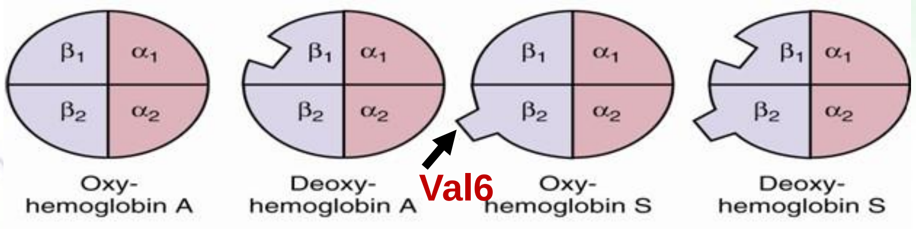
Cellular effect on system

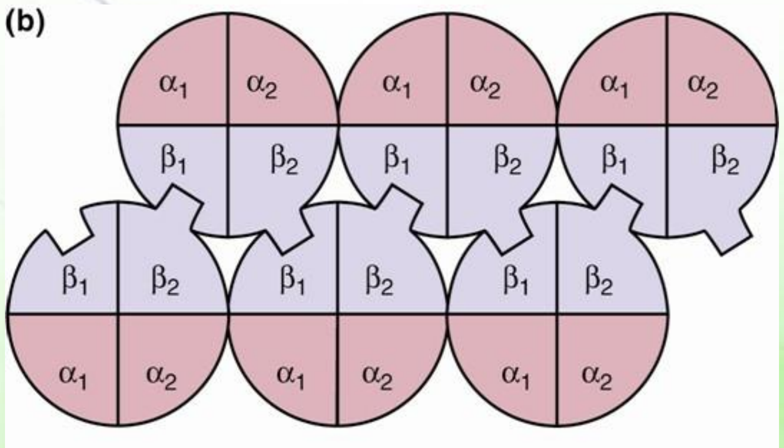
- Repeated cycles of oxygenation and deoxygenation lead to irreversible sickling.
- Cells cannot squeeze though capillaries in a single file and therefore block blood flow causing local hypoxia.
- Long-term recurrent clogging of the capillary beds leads to damage to the internal organs, in particular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



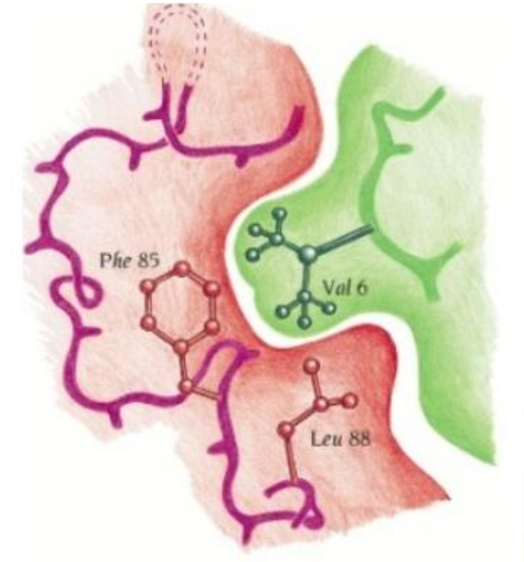
How does the fiber form?

- 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



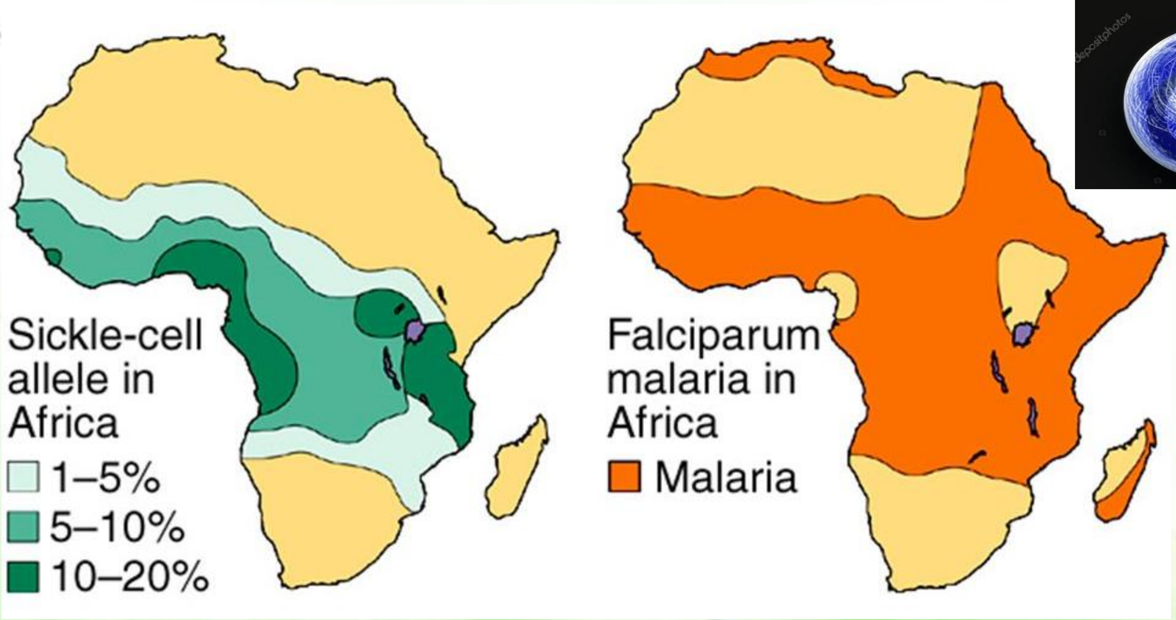
<u>Variables that increase sickling</u>

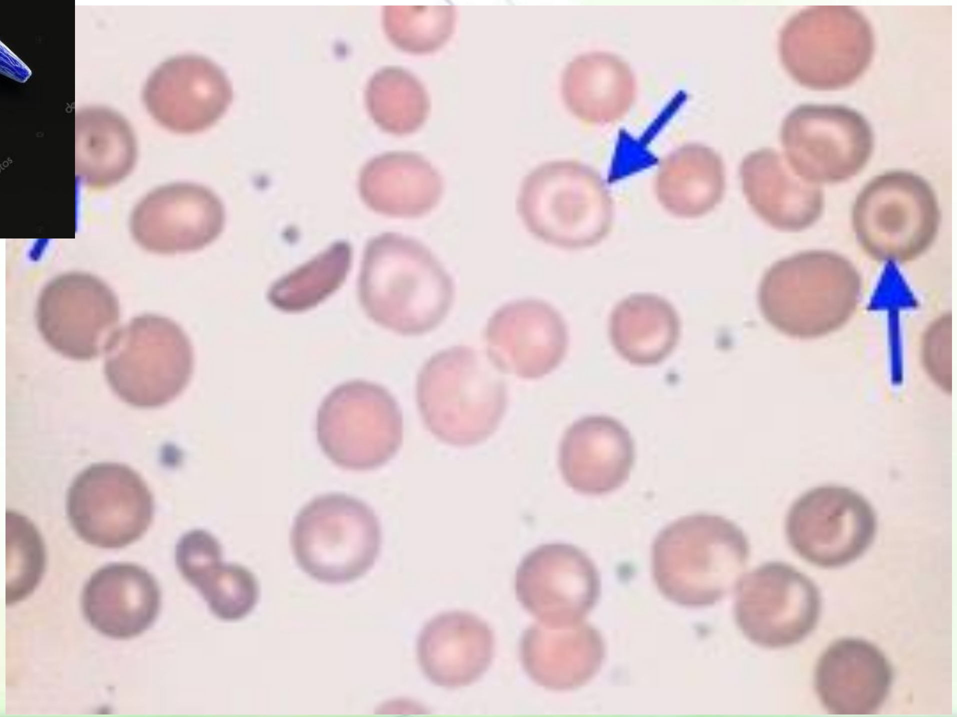
- **Decreased oxygen pressure (high altitudes)**
- **Increased pCO_2**
- **Decreased pH**
- **Increased 2,3-BPG**
- **Dehydration (why?)**

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.

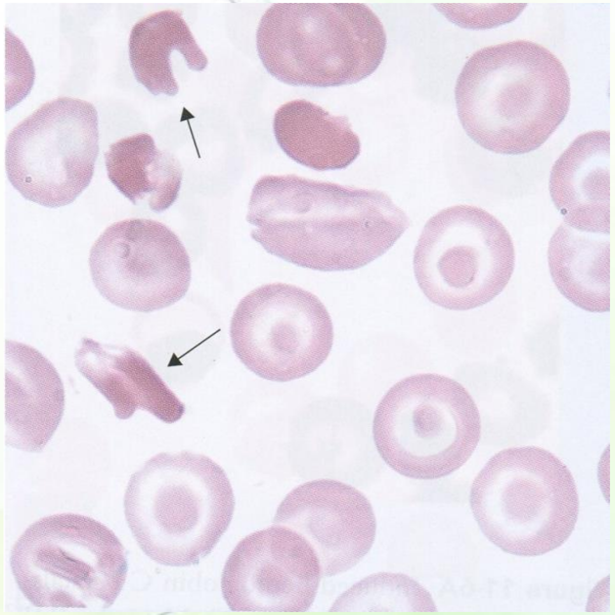
- Advantage: selective advantage from plasmodium falciparum that causes malaria. Why?





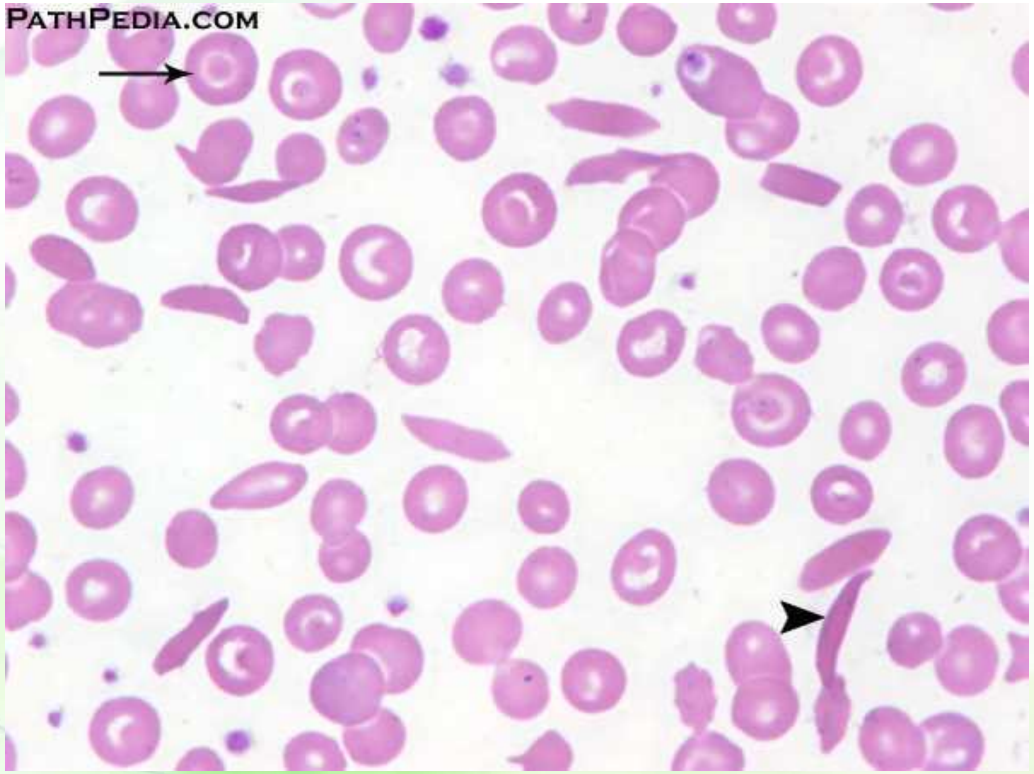
Hemoglobin C (HbC)

- (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

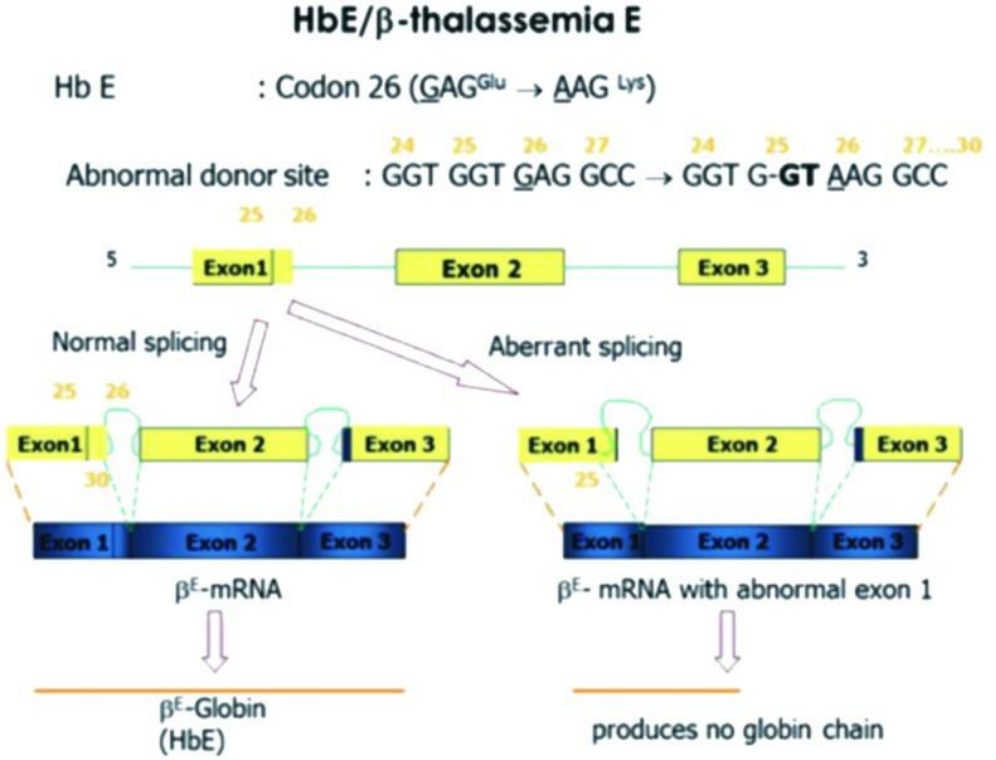
- Individuals with both βc and βs mutations have HbSC disease, a mild hemolytic disorder which may have no clinical consequences, but it is clinically variable.



Hemoglobin E

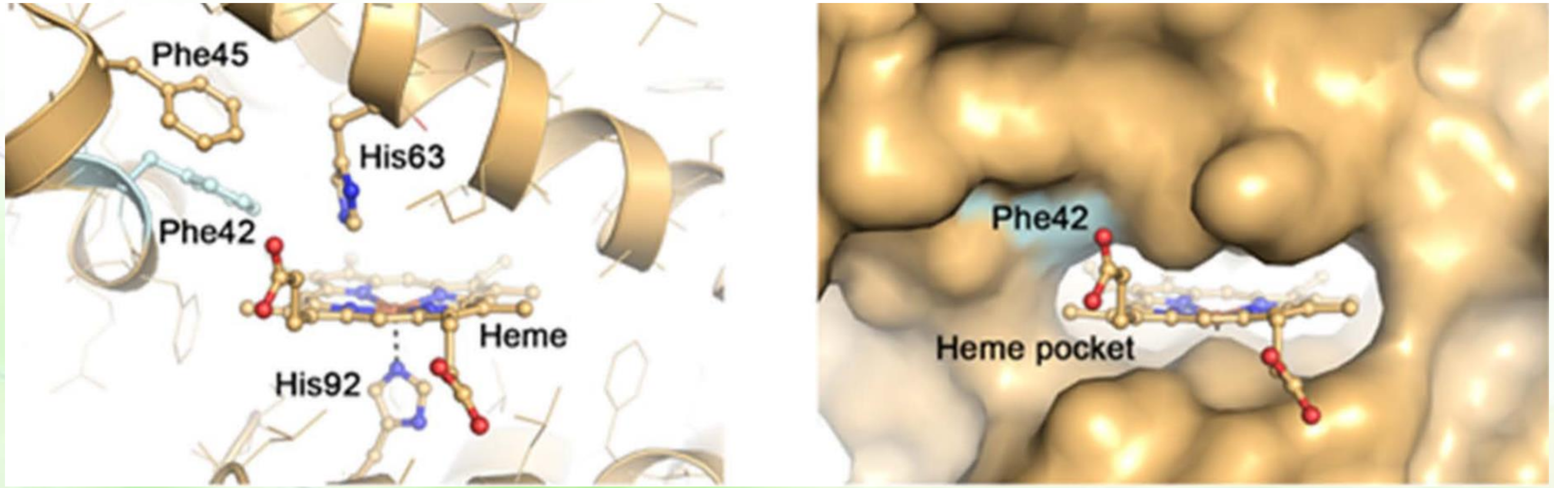
- It is common in Southeast Asia
- It has both quantitative and qualitative characteristics.
- It is caused by a point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG) creating an alternative RNA splice site and a defective protein.

- Individuals with this mutation make only around 60% of the normal amount of β-globin protein.



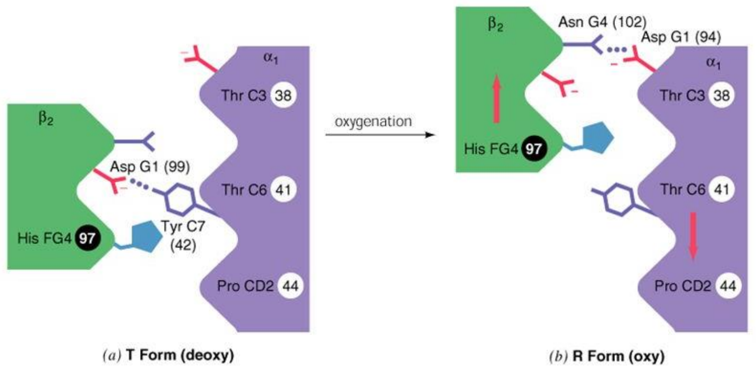
Hb Hammersmith

- 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.



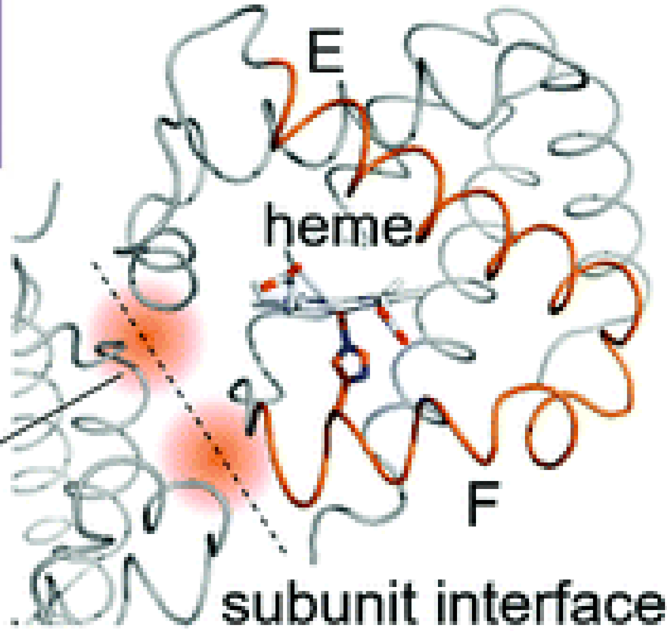
Mutations at α1-β2 contacts

- 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 Kansas: stabilization of the T state (Asn G4 (102) to thr); decreased cooperativity.
  - Hb Yakima: stabilization of the R state (Asp G1 (99) to His).



Or His

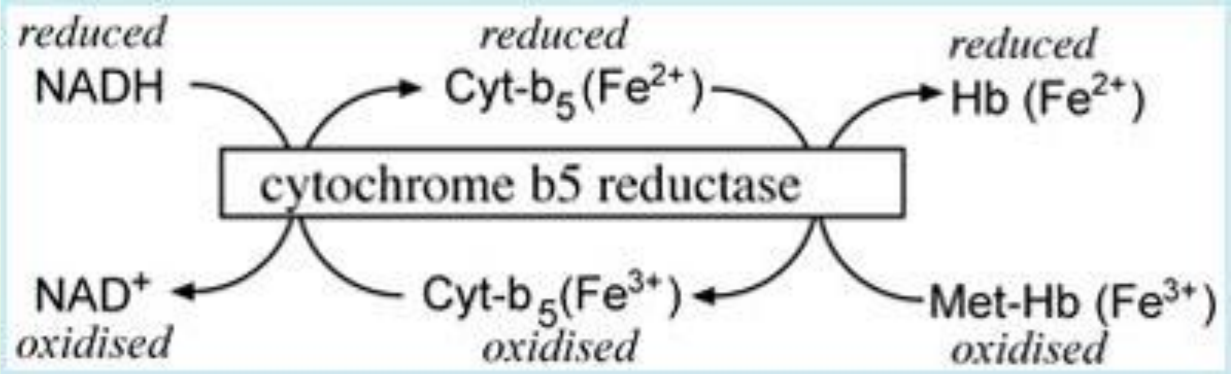
β99 Asp→Asn (R-fixed)
β102 Asn→Thr (T-fixed)



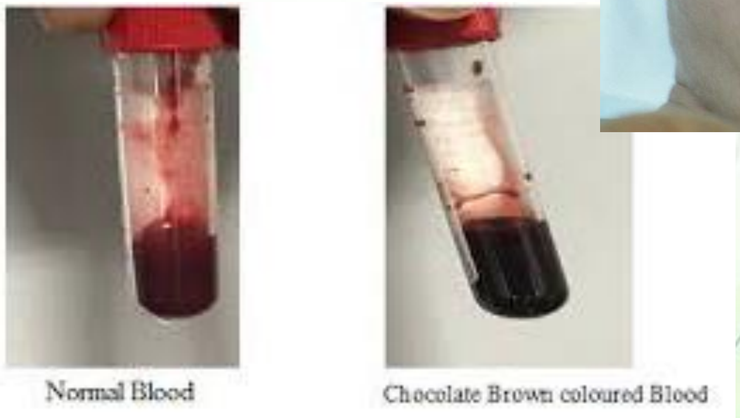
Altered Oxygen Transport

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.



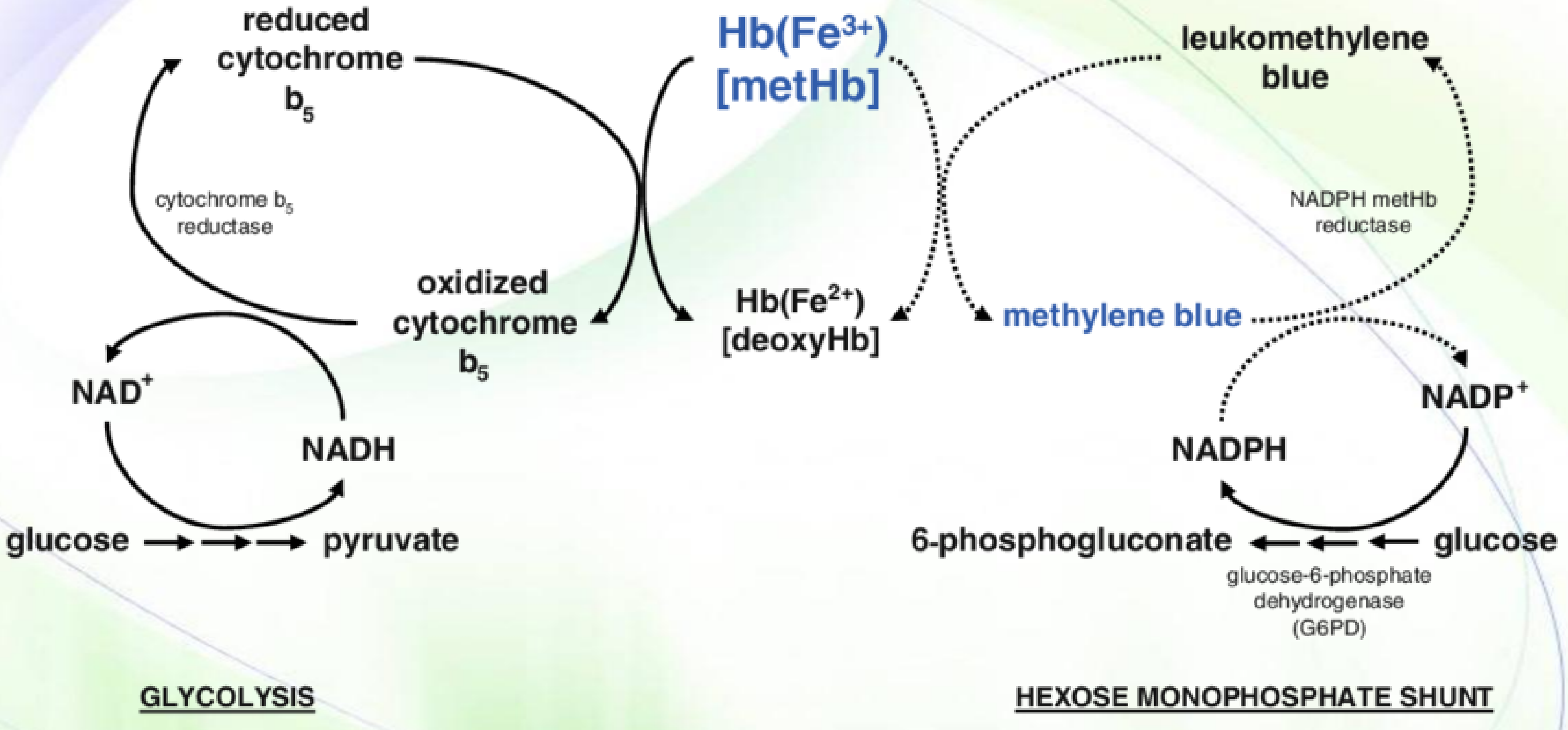
Methemoglobin reductase AKA NADH-Cytochrome b5 reductase



Why HbM?

- Some mutant globins (α and β) bond 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. It also attracts H2O into the pocket.
  - 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.

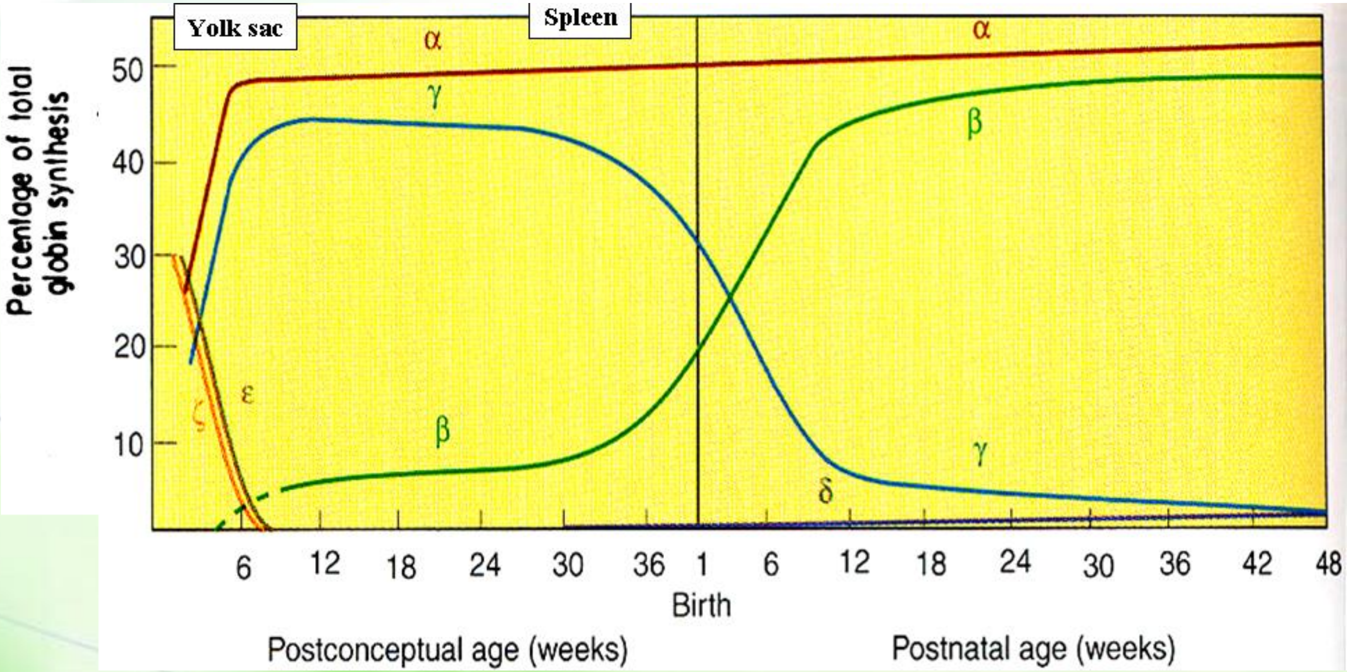
Quantitative abnormalities (thalassemias)

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.

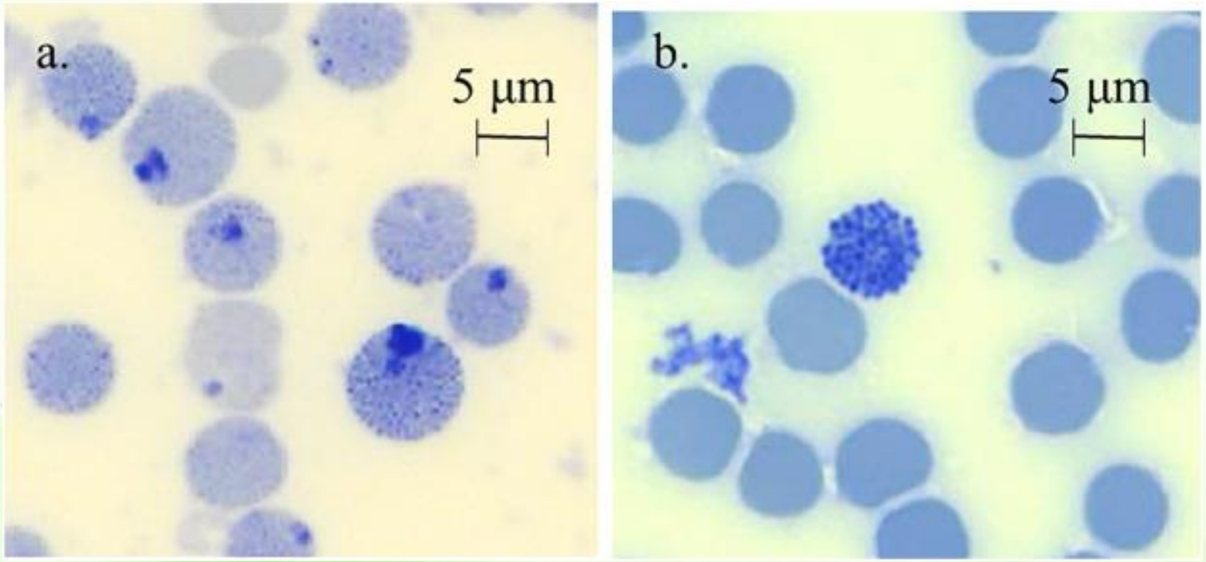
The Alpha-Thalassemias

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



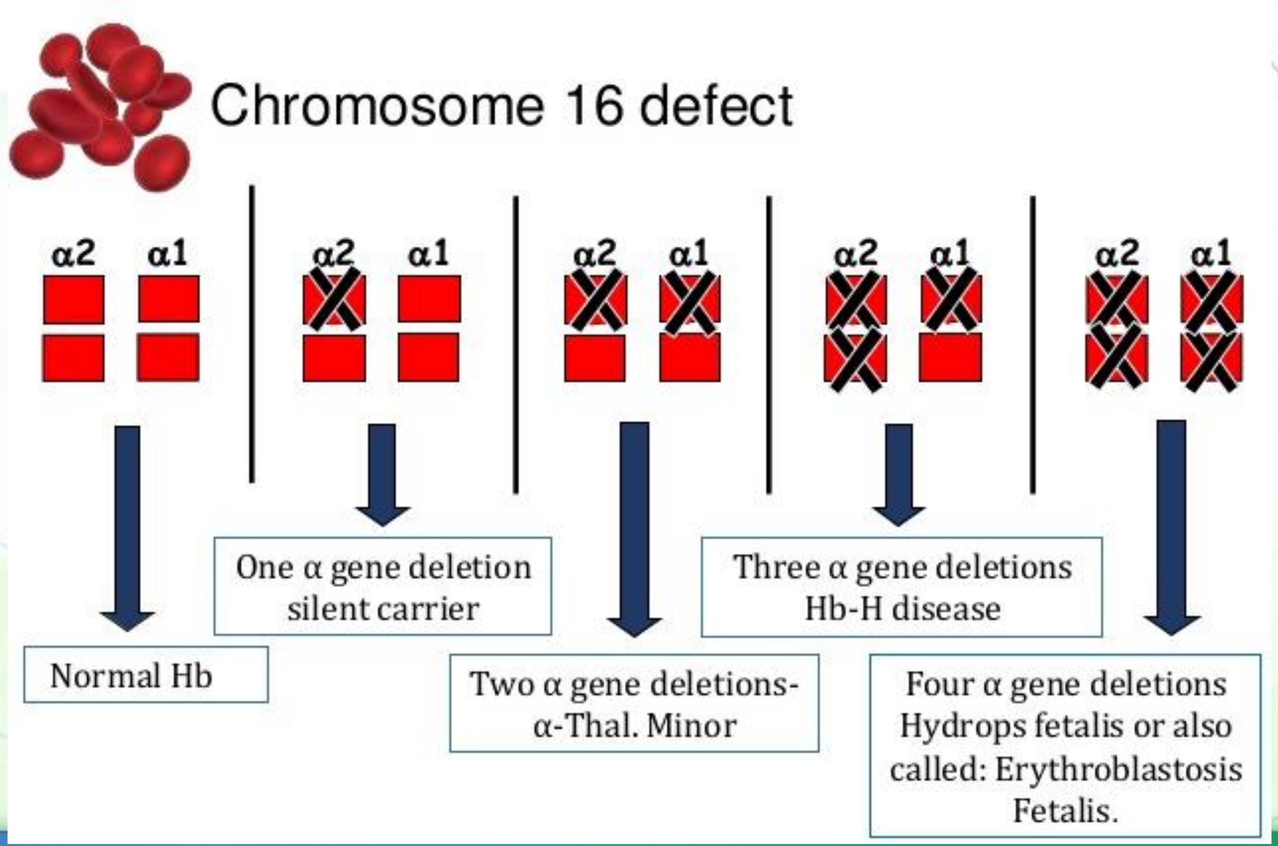
HbH

- 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)



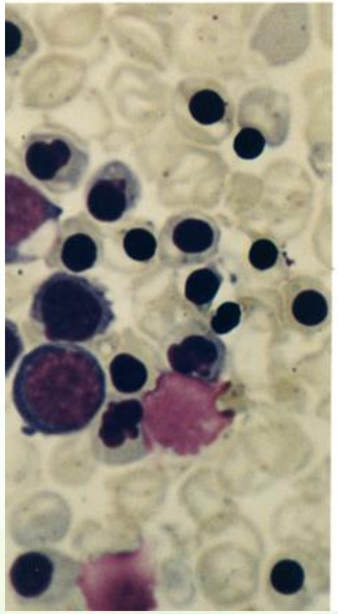
Variable severity

- With the 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.



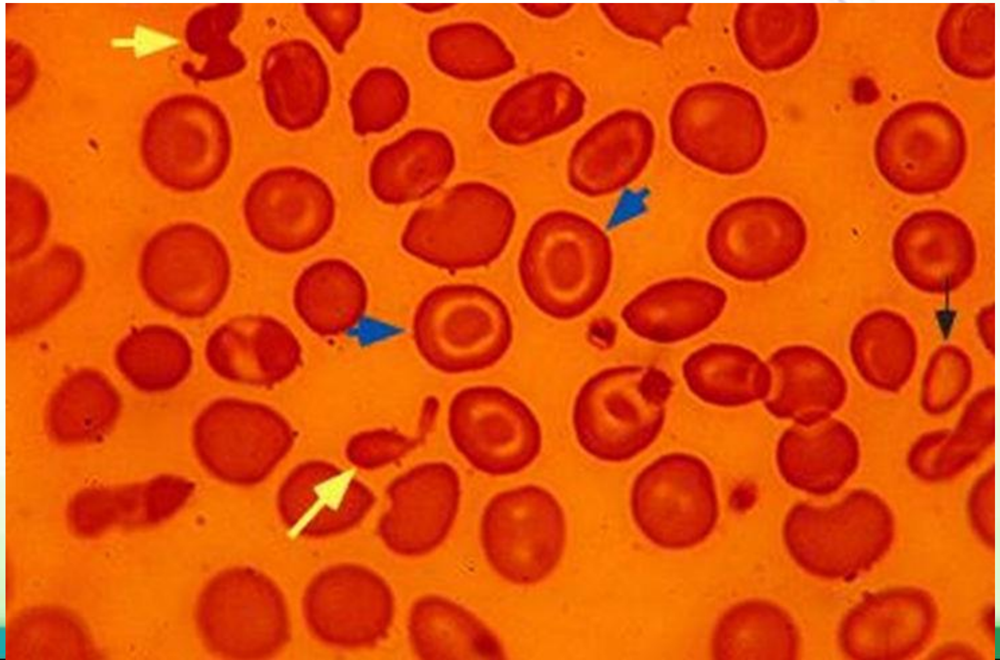
Hydrops fetalis

- 4 of 4 genes are deleted.
- The predominant fetal hemoglobin is a tetramer of γ-chains.
- $\gamma 4$ 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.



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, 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

- A complete lack of HbA is denoted as β0-thalassemia or β-thalassemia major.
- Afflicted 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.

β -Thalassemia minor

- Individuals heterozygous for β-thalassemia is termed β- thalassemia minor.
- Afflicted individuals carry one normal β-globin gene and a mutated gene.
- Thalassemia minor individuals 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

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

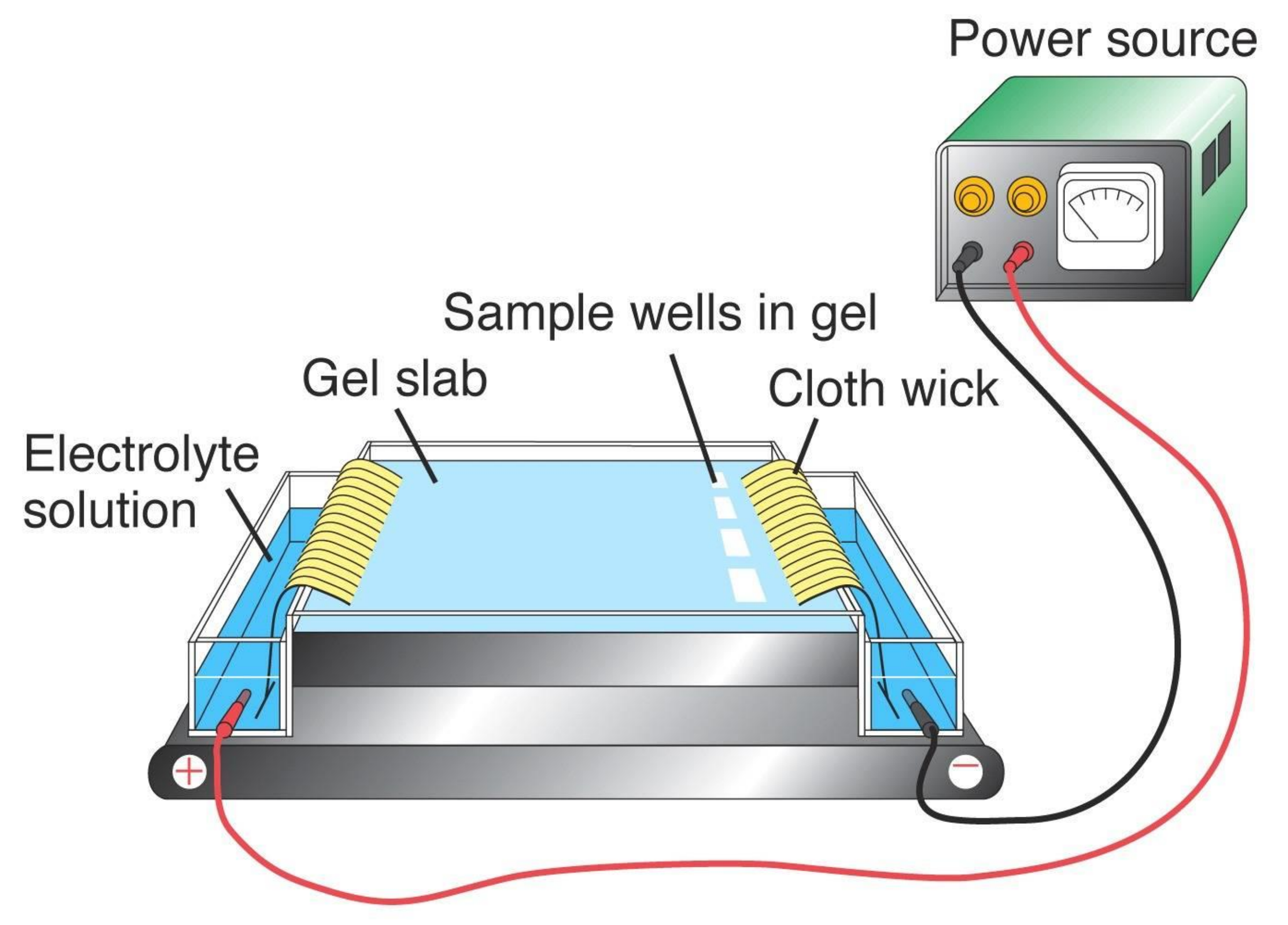
Switching from fetal to adult hemoglobin

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 are hemoglobin standards
- Lane 2 is a normal adult
- Lane 3 is a normal neonate
- Lane 4 is a homozygous HbS individual
- Lanes 6 and 8 are heterozygous sickle individuals
- Lane 7 is a SC disease individual