HEMOGLOBINOPATHIES:
THE INHERITED DISEASES OF HEMOGLOBIN

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Readings: Your genetics text uses Hemoglobinopathies to illustrate "Principles of Molecular Disease". Thompson and Thompson: Genetics in Medicine-Nussbaum, McInnes and Willard, 7th Edition pp. 323-343. – Good Summary.

Your Hematology Textbook: *Chapter 6, Genetic Disorders of Haemoglobin, 71-90.*

Learning Objectives – After studying this material you **should** be **able** to: (1) State the three categories of hemoglobinopathies, (2) State the chains in fetal and adult Hb, (3) State the 5 kinds of structural variants and their major (three) clinical consequences, (4) Discuss every aspect of sickle cell anemia, (5) State the consequences of HbS, HbSC and HbS Thal diseases, (6) explain the basic molecular problem of unstable Hbs and the clinical consequence, (7) based on your *understanding* of each Hb variant with abnormal O_2-affinity given in class, interpret any variant based on the facts given, (8) State the relevance of polycythemia and cyanosis to Hb-variants, (9) State what a methemoglobinemia is, (10) Define thalassemia and the consequences of having an excess of a normal chain, (11) State the basic defect in most α-thalassemias, (12) List the major causes (mutations) found in β-thalassemias, (13) State the defect in HPFH (Hereditary Persistance of Fetal Hemoglobinemia), and how patients compensate for the most common type of HPFH. **(14) State how HbF expression is regulated and how new therapies for SCA and Beta-Thalassemia major may be developed based on recent discoveries about how HbF expression is regulated.**

HEMOGLOBINOPATHIES: SIGNIFICANCE

- Hbopathies are the *inherited disorders* of Hb.
- Most common single-gene disorders in world population.
- Includes *common* inherited diseases:

  Sickle Cell Anemia
  β-thalassemias

- Disease the Great Educator
  - Hbopathies illustrate profound generalities about **ALL** inherited diseases.
  - Proves/confirms hypotheses and concepts about how Hb works.
  - Hbopathies clarify what is **MAGNIFICENT** about Hb.

    Answer: **EVERYTHING** it does is essential for *survival*!

HEMOGLOBINOPATHIES: CLASSIFICATION

THREE CATAGORIES (By type of molecular defect)

Structural Variants (~750 known)

Abnormal *Protein Structure*

Thalassemias

Decreased synthesis of otherwise normal α or β chains.

α°, α^{+} - Thalassemias

β°, β^{+} - Thalassemias

Defective Developmental Switching

Hereditary Persistance of Fetal Hemoglobinemia

Read

CATEGORIES OF INHERITED DISEASES NOT ILLUSTRATED BY HEMOGLOBINOPATHIES

(Just for the record)

Inherited diseases of collagen illustrate some additional types of inherited diseases.

1. Protein Processing Defects

   (e.g., Ehlers Danlos Syndromes)

2. Protein Synthesis Defects resulting in OVERPRODUCTION of normal chains

   (e.g., Keloid, Idiopathic fibrosis)

3. Synthesis of specific Protein **ISOFORM** in the wrong cell type.

   (e.g., Some types of osteogenesis imperfecta)

4. Autosomal dominant (Hbopthies usually recessive)

FETAL vs. ADULT Hb

Parental Genes

$2\alpha, 1\beta, 1\delta, 2\gamma$ $2\alpha, 1\beta, 1\delta, 2\gamma$

Offspring Genes

$4\alpha, 2\beta, 2\delta, 4\gamma$

(4α and 4 γ are clinically relevant)

HEMOGLOBIN PROTEIN

Fetal	Adult
α_2, γ_2	α_2, β_2 ($HbA_1 = 98\%$) α_2, δ_2 ($HbA_2 = 2\%$)
	α 141 amino acids β 146 amino acids

Note:

- α and β chains are homologous
- γ and δ chains are strongly homolgous to β chains and substitute for β chains in adult Hb A_2 (δ) and fetal Hb (γ). All of these genes of the β-globin family arose from gene duplication and are on the same chromosome.

FYI-There are also three early embryonic (~first 6 weeks) forms of Hb which contain "α-like" and/or "β-like" chains (Hemoglobins Gower 1 and 2, and Hemoglobin Portland.

FFT: Hb is ALWAYS a tetramer containing 2 α-like and 2 β-like chains each of which folds into a subunit that binds one O_2 at the Fe^{2+} of the heme prosthetic group.

NB: We will see that even abnormal forms of Hb found in thalassemias are tetramers $\alpha_4, \beta_4, \gamma_4$ that form when the amounts of one normal chain, e.g., α or β are low. These forms cannot release O_2!

HEMOGLOBIN THE MAGNIFICNENT

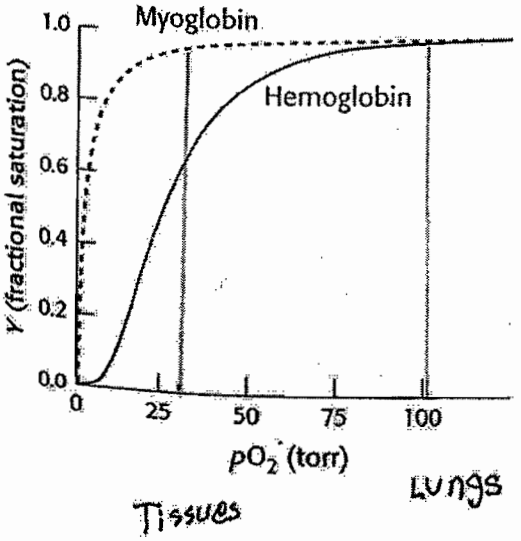


Figure 7.7 Oxygen binding by hemoglobin. This curve, obtained for hemoglobin in red blood cells, is shaped somewhat like an "S," indicating that distinct, but interacting, oxygen-binding sites are present in each hemoglobin molecule. For comparison, the binding curve for myoglobin is shown as a dashed black curve.

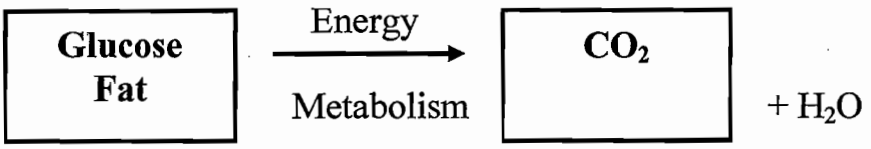
- Myoglobin (Mb) is homologous to Hb, but is monomeric – illustrates what Hb would be like without its 4°
- Both Mb could (if it were there in blood instead of in muscle) and Hb can nearly saturate with O_2 in the **LUNGS**.
- ONLY Hb has the ability to SUPPLY O_2 to tissues.
- **FFT**: Hb binds O_2 "well" (saturates in lungs). Hb does NOT bind O_2 "too well" (releases O_2 at tissues)

Q How??

A: Allosteric properties:

Positive Homotropic Effect
Negative heterotropic effects
H^+, CO_2-Bohr Effect
BPG (BPG-2, 3 Bisphosphoglycerate)

THE BOHR EFFECT, TOTAL BODY ACID-BASE BALANCE AND THE ALLOSTERIC PROPERTIES OF Hb

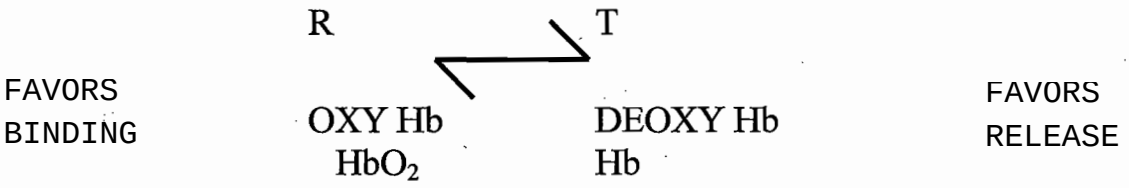


$$H_2O + \mathbf{CO_2} \leftrightarrow \text{“}H_2CO_3\text{”} \leftrightarrow \mathbf{H^+} + \mathbf{HCO^-_3}$$

Hb binds about 10% of all the CO_2 produced per-day

AND

Hb binds H^+ *equivalent* to about 30% of all the CO_2 produced per day. (30% of CO_2 converted to H^+ and HCO^-_3 in RBC)



FFT:

BINDING OF H^+ AND CO_2

1. Takes care of a lot of the CO_2 (acid) produced every day by energy metabolism.

   AND

2. HELPS Release O_2 from hemoglobin!!!!

   AND

3. BPG has the strongest allosteric effect that helps O_2 release from Hb

   AND

**Positive homotropic effect also helps release of O_2. As HbO_2 leaves the lungs and goes to the tissues, O_2 is released and the R-state (high affinity) is converted to the T-state (lower affinity); favoring the release of O_2.

Rhetorical Question: How important (essential) is each of these allosteric effects?

Hbopathies: THE STRUCTURAL VARIANTS

Clinical Classification of Structural Variants

A. The *Sickle Syndromes*

- Sickle Cell Anemia (and trait)
- All patients with at least one β^S gene
  - HbSC disease
  - HbS/β-thalassemia disease

B. The *Unstable Hemoglobins* (more than 100 known)

- Congenital Heinz Body (Hemolytic) Anemia (CHBA)
  Hb precipitation → Heinz bodies in RBC

* About ½ of all structural variants that show clinical symptoms.

C. With Abnormal Oxygen Affinity (more than 70 known)

- High affinity Hbs
  - More common (detected clinically) than low affinity Hbs
  - More serious than low affinity Hbs
- Low Affinity Hbs
  - Probably detected less because fewer clinical symptoms

Hb bind O_2 too well = ✗

D. The Methemoglobins (at least 6 variants known)

- Defect near heme-Fe^{2+} binding site on α or β subunits. Fe^{3+} instead Fe^{2+}, only Fe^{2+} reversibly binds O_2

E. Structural Variants with Thalassemia Phenotype

- Like thalassemias (have less α or β-chains but **NOT** due to decreased synthesis of otherwise *normal* chains.
- Due to synthesis of abnormal chains.
  - Nonsense (Termination Mutants) - shorter chains
  - Hemoglobin Lepore-fusion of β and δ genes.
  - Elongated (e.g., mutation in termination codon). - longer chains

Important Page

IMPORTANT

Practical Classification of Hb Variants by Clinical Consequences

A. *Polymorphic Variants* (high frequency variants)

Polymorphism – at least 1% of a population has the variant – major public health problem in those populations.

Hbs: S, C, and E are polymorphic traits-note α and β thalassemias which are not structural variants are also polymorphic traits – high frequencies in specific populations.

B. *Oxygen Carrying Variants*

High O_2 – affinity (poor O_2 release) / compensates for defect

- Secondary **POLYCYTHEMIA** (erythrocytosis) – increased RBC made to compensate for defect

Low O_2 – affinity

- Cyanosis, but no compensation is required.

Hereditary Methemoglobinemias – Fe^{3+} - **CYANOSIS** ? problem

C. Unstable Variants

HEMOLYTIC ANEMIA

Big Picture For Hb Variant Types A, B, & C.

Type A. High incidence – much higher than typical inherited diseases

Type B. Polycythemia or cyanosis - But fine-ish

Type C. Hemolytic anemia (also in Thalassemias)

Dun memorize, heed/read

HEMOGLOBIN STRUCTURAL VARIANTS: GENERAL

- β-chain mutations – MOST COMMON DETECTED.
- Fewer α-chain than β-chain mutations detected because have 4 α genes. Less likely to detect clinical manifestations.
- γ-chain (fetal Hb) – difficult to detect because fetal and have 4 γ chains – (about 35 known)
- δ chain (few mutations detected – NO known function) – (about 15 detected) – (Increase in δ-chains, HbA_2 does help in diagnosis of β-Thalassemia minor)

one letter of one codon

Far and away **MOST** Hb-variants are due to **POINT** mutations (substitution of one letter of codon) and **SINGLE** *amino acid substitutions*.

Two mutations in same gene resulting in two amino acid substitutions in same chain – rare, but found.

Termination, insertion, deletion, frame-shift mutations have all been detected.

FFT: Hbopathies **ARE** representative of other inherited diseases of globular proteins for the types of mutations observed and their effects on functions! Genetics: *Autosomal Recessive* and heterozygotes are usually healthy.

mutations are random

Moreover, have already detected about 45% of probable single base substitutions that lead to disease - that's amazing and suggests that everything that can happen, does happen.

SICKLE CELL ANEMIA

- $\beta 6 glu^- \rightarrow$ Val (diagnosis-symptoms and electrophoresis)
- Before aggregated-binds and releases O_2 normally
- Deoxy-HbS "wants" (ΔG) to polymerize to form a specific, fibrous polymer, but $\Delta G\ddagger$ high – **RATE SLOW**
- Usually blood passes through capillaries ***FASTER*** than polymerization can occur – for this reason, sickle cell crises are episodic – do not occur continuously. **THANKS**
- Vaso-occlusive crisis **(episodic)**
  - Deoxy HbS polymerizes, gels
  - RBC sickle – rigid, inflexible
  - Occlude capillaries which in turn blocks entry of more RBC with HbO_2
  - O_2 delivery decreases, ~~deoxyHb~~ deoxy Hb S increases and polymerizes and sickling increase to further occlude capillary
  - Infarction: anoxia – severe, agonizing pain – with or without cell death
- Sickling that occurs in venous circulation is largely reversible upon oxygenation in the lungs
- Sickle-shaped RBC are fragile-shortened life-time which gives rise to the chronic hemolytic **ANEMIA** of sickle cell anemia. (destroyed in spleen) (disease)
- Changes in RBC membrane and at surface of endothelial cells lining capillaries contribute to the vaso-oclusive crisis.

10 A

ADDITIONAL CONSEQUENCES OF SCD

- Vision loss due to multiple Vaso-occlusive Crises in eyes.
- Chronic Kidney Disease (not just the medulla).
- Bone Crises leading to avascular necrosis (very painful and possible bone death)
- Spleen atrophy
- Cardiac hypertrophy due to chronic edema.

SICKLE CELL DISEASE-Continued.

***TREATMENTS : NEW NATIONAL GUIDELINES (JAMA, 9/10/14)**

- ***Analgesics : (potent, morphine family) IV during vaso-occlusive crises**
- **Hydration (prevent polymerization, compensate for damage to collecting ducts in renal medulla due to repeated vaso-occlsive crises) leads to impaired H_2O reabsorbtion by countercurrent mechanism (normal mechanism for concentrating urine is impaired.)**
- ***NEW recommendation: Begin HYDROXYUREA at age 9 months even if symptom free ! Yikes/WOW !**
- ***Transfusions for Acute Chest Syndrome , acute stroke. Not priapism**

CRISES PREVENTION

- **HYDRATION**
- **HYDROXYUREA**
- **PROPHYLACTIC ANTIBIOTICS (Penicillin)-prevent invasive pneumoccal infection until age 5(longer if spleenectomy) or major poor spleen function.**
- **Transfusions after stroke (Lifetime). !!!!!!!!!**
- **Bone marrow transplant of allogenic hematopoietic stem cells !**

FFT These new guidelines strongly recommend Hydroxyurea and Transfusions . These are the most widely available disease modifying therapies, and are being underused. Stem cell therapies are making progress and may also being under used.

11 A

SCD GENETICS

- **Autosomal Recessive.**
- **People with HbS-trait ;i.e. heterozygotes make $\beta^S\beta^A$ chains. Co-dominant gene expression (both genes expressed).**
- **Almost ALWAYS symptom free. Clinically silent. No anemia.**
- **Selective advantage against malaria which accounts for the high incidence of homo-and heterozygotes for β^S.**
- **Even heterozygote infants are less susceptible to CNS damage (stroke) because RBC cannot support the reproductive cycle of malaria parasite (Cells lyse before life cycle is completed)**
- **HbS gene is polymorphic (high wherever malaria is high). Africa, India, Parts of middle east (less severe due to higher fetal Hb than other populations). HbF inhibits aggregation of deoxy-β chains.**

HEMOGLOBIN C AND HbSC DISEASE

- HbC β_6 glu → Lys (West Africa, 1-2% African Americans)
  Are carriers
- MILD hemolytic anemia, splenomegaly

*FFT much less serious to substitute a positively charged Lys for negatively charged Glu than the Val substitution! (three types of amino acids, at least when amino acid side chains are on the surface)

- HbSC disease is more serious than HbC disease.
- Similar to sickle cell anemia!, but less severe.
- Unexplained tendancy for repeated vaso-occlusive crises in eye-can lead to blindness!! Also SCD, but later in life.
- Mild anemia, splenomegaly, possible aseptic necrosis fermoral or humoral heads, possible chest syndrome (pulmonary vaso-occlusion)

SICKLE CELL - β Thalassemia Disease

(β-thalassemia-no chains (β^0) or less (β^+) chains made)

- Coinherit β^S β^{Thal} Genes.
- $\beta^S\beta^0$ Thal or $\beta^S\beta^+$ Thal
- $\beta^S\beta^0$ patients only make β^S chains and only have sickle cell Hb. Disease is similar to sickle cell disease.
- $\beta^S\beta^+$ Thal patients who have mild β^+ may make 30% of normal β^+ - 30% HbA – clinically like sickle cell trait or β^+ Thal trait – fine-few if any symptoms.

"Two diseases are better than one" in this case, $\beta^S\beta^+$ better than $\beta^S\beta^S$ or $\beta^+\beta^+$.

FFT - β^S functions fine provided it does **NOT** aggregate.

HbE (FYI)

- Most common Hb variant (Thailand, Laos, Burma, Bangladesh).
- β_{26} Glu → Lys (3D structure change and decreased synthesis – splice error with this mutation). **Mutation in exon near splice site.**
- Homozygotes asymptomatic, slight anemia.
- Hb β^{Thal} β^E can be serious because HbE gene also decreases amount β-chains.

UNSTABLE HEMOGLOBINS

- **Single-amino acid substitutions that disrupt normal FOLDING (more than 100 known).**

- **Poorly folded hemoglobins are insoluble and precipitate in RBC to form inclusion bodies in RBC-Heintz Bodies-RBC (less flexible, fragile in spleen)**

- **Congenital Heintz Body Anemia (a hemolytic anemia RBC-destroyed) (CHBA)**

- **ALL globin chains have 7-8 helical regions (labeled A through H) – no β-sheets-75% α-helix and the rest unordered where chains bend and turn.**

*** Protein sequences that form α-helices usually have a central, sequence rich in hydrophobic amino acids.**

13 Unstable Hb Variants

Hydrophobic Amino Acid (Often Leu)	⟶	**PRO Forces a bend Breaks Helix**

FFT – Normal 2° structure REQUIRES a continuous stretch of amino acids that favor that structure. Pro hates center of ∝-helix bc uniquely/automatically causes a chain to bend.

FFT→ 2° helps determine 3°!

"INTERESTING" UNSTABLE Hb VARIANTS

- Hb Hammersmith

Dun have to memorize

β_{42} Phe → Ser

Change of type-aromatic to polar uncharged

Sounds Bad, right?

BUT where is that Phe in 3°?

In the hydrophobic pocket that binds the hydrophobic prosthetic group (heme-protophorphyrin 1X ring)

→ Δ3° - HEME NOT BIND WELL

HOW SURVIVE?

ONLY HETEROZYGOTES KNOWN!!!!!

Note: Hb-Hammersmith is AD. because have some symptoms.

FYI: You are **NOT** required to memorize what each Hbopathy is, as in: "what is Hb-Hammersmith?", but you are required to **UNDERSTAND** each example and its relevance.

HEMOGLOBINS WITH ABNORMAL OXYGEN AFFINITY

A. Hbs with abnormally **HIGH** affinity for O_2.

1. Hb-McKees Rock (near Pittsburg)
   β_{145} Tyr $\rightarrow$ termination
   (β-chain has 146 residues-this is penultimate Tyr)

YIKES! This is the Tyr that pops out of a hydrophobic pocket when O_2 binds to normal Hb! That leads to breaking of salt links that converts T to R state as part of the positive homotropic effect. **NOTE**: ***NEED*** the buried Tyr to form the salt links that stabilize the T-state which favors O_2 release in the tissues.

NOTE: When delete last two residues, no stable T-state can form (only have R-state) because need that Tyr. If no stable T-state, no positive homotropic effect occurs because already locked in R-state. (no shift from T to R)

So McKees Rock **ALWAYS** in R-relaxed state (High affinity because cannot form T-state). McKees Rock Hb is a **HIGH OXYGEN AFFINITY** variant because it cannot form T-state!

HOW LIVE/SURVIVE?
compensates w/

ONLY heterozygote AND with secondary **POLYCYTHEMIA** (erythrocytosis) - increased RBC production!

FFT- Formation of the T-state, as Hb travels from the lungs to the tissues is **ESSENTIAL** for **O_2-RELEASE** from Hb!

NOTE: The physiologic compensation of overproducing RBC works for abnormal Hbs that can bind but not release O_2 well. Just make more cells (with more Hb) to try to keep the **TOTAL** O_2 released normal.

This does not occur for Hbs with a *decreased* affinity for O_2. In general heterozygotes for those Hbopathies are cyanotic (bluish) but otherwise clinically OK because can release O_2, and do not need any compensation.

HIGH AFFINITY Hbs

2. Hb-Hiroshima

β_{146} His → Asp

YIKES! This is THE **HIS** which becomes a *stronger* weak base in deoxy Hb when O_2 is released. Responsible for the H^+ part of the Bohr effect!
(An Asp moves near this His in T-state to help bind H+ better)

Lose negative heterotropic effect of H^+, O_2 - release decreased, O_2-affinity increased. (Binding curve shifts LEFT-towards lower O_2 to bind, higher affinity)

Survival → Heterozygotes only; secondary polycythemia.

3. Hb-Syracuse

β_{143} His → Pro

That His helps bind BPG at domain between β-subunits to stabilize T-state-facilitate release of O_2

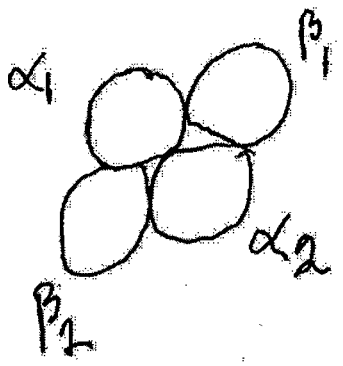
Less BPG binds, less T-state, more R-state O_2-affinity increased.

Yikes! Survival?? Heterozygotes only and secondary polycythemia.

→ FYI!

Hb Functions as 2 α·β Dimers

Bisphosphoglycerate



← Salt Links in T-State
"Taut" conformation

- Subunits move a lot during positive homotropic effect.
- α·β dimers move relative to each other when salt links break between α_1 β_2 and β_1 α_2
- Changes at α_1 β_2 inteface are especially important in positive homotropic effect.

Tissue - Want R→T

Lung - Want T→R

IMPORTANT

4. Hb-Kempsey (β_{99} Asn → Asp)

- Recall that a major part of the allosteric effects in Hb is **LARGE** changes in 4° - large movements of subunits relative to each other.

Hb-Kempsey mutations affect subunit interactions. "Locks" Hb in 4° of R-state!!

Q: Are the 4° changes of T/R equilibrium essential for Hb-function and survival?

A: ONLY heterozygotes. Secondary Polycythemia.

NOTE: $\alpha_1\beta_1$ dimers normally move relative to the $\alpha_2\beta_2$ dimers because of changes at the $\alpha_1\beta_2$ interface!

Most of the high affinity Hbs are due to a changes at the interface of the $\alpha_1\beta_2$ subunits like Hb-Kempsey or the BPG binding site.

FFT: Disease the Great Educator *proves* the importance of changes in quaternary structure to release of O_2 from Hb. Hbopathies also prove that the positive homotropic effect, Bohr Effect (H^+/CO_2 binding) and BPG binding are ALL **ESSENTIAL** for O_2 release.

HEMOGLOBIN WITH ABNORMAL OXYGEN AFFINITY

B. Hbs with abnormally **LOW** affinity for O_2

- Fewer Low affinity Hb–variants are known than high affinity variants
  - Probably because less serious clinically, fewer low affinity Hbs detected.
- Hb Kansas β_{102} Asn → Thr

At $\alpha_1\beta_2$ interface, near β_{99} Asn → Asp –Hb Kempsey mutation which has the *opposite* effect!

VERY SENSITIVE AREA

R-state 4° **UNSTABLE**

Locked in T-state (Deoxy Hb)

Hb Kansas binds worse, releases better.

Cyanosis – Probably due more to increased release of O_2 at tissues than decreased saturation in the lungs.

No compensation required

One heterozygote for Hb Kansas purported to be excellent tennis player!?

Increased O_2 delivery?

FFT: Major "Problem" in evolution was to evolve a Hb that could release O_2. **WOW**: What a selective advantage: The ability to saturate with O_2 in the lungs and release O_2 at tissues by allosteric affects.

FFT: Subunit interfaces are functionally vunerable to mutations-critical for normal O_2 affinity and release though far from Fe-O_2 binding site!

THE METHEMOGLOBINS

- There are 2 His on either side of the flat heme prosthetic group. One His binds to the Fe^{2+} atom and moves when O_2 binds to begin the chain of events that leads to the positive homotropic effect. The other His is near, but unbound to the Fe in normal Hb.

- 4 of 6 known HbM-mutations are at these His sites on α or β chains.

His	→	Tyr
CAU	→	UAU
CAC	→	UAC

- Tyr-OH probably binds to Fe
  $Fe^{2+} \rightarrow Fe^{3+}$
  Ferrous to ferric
- Patients: Cyanosis, but otherwise asymptomatic!

  Cyanosis – "bluish appearance" is due to decreased amounts of oxy – Fe^{2+} - Hb – (which gives red color of blood).

Q: How is that possible??? A: Heterozygotes

★ **FFT**: Only Fe^{2+} reversibly binds O_2 in Hb. RBC has whole metabolism to keep Fe^{2+} in reduced state.

Need ferrous iron

Look out of interest

FYI

A. Structual Variants with Thalassemic Phenotype

Hb Lepore

- N-terminal half of normal δ - chain fused with C-terminal half of normal β-chain.
  - New β fusion, "β-like" chain.
  - Decreased normal β-chains

- Fusion gene mutations occur during crossing over, homologous recombination during meiosis-formation of germ cells.
- Symptoms like moderate to severe β-thalassemia.
- Recall Philadelphia chromosome of chronic myelogenous leukemia.
  - Chromosomal translocation.
  - Fusion protein formed with ABL proto-oncogene (tyrosine kinase) and BCR gene.
  - ABL increased tyrosine kinase activity.
  - CML.
  - New "miracle drug" (Gleevac) -designed.

IMPORTANT

THALASSEMIAS

- Decreased synthesis of otherwise normal α or β chains.
- Both α and β thalassemias are polymorphic traits/disorders – high incidence in specific populations. α^{Thal} (China), β^{Thal} Mediterranean, India. US – high incidence (Like sickle cell anemia, β thalassemias (even heterozygotes) have selective advantage against malaria. Thal. populations follow distribution of mosquito distribution in hot, humid climates)
- α-Thalassemias usually due to gene deletion.
- β-Thassemias: over ~~170~~ **200** different mutations known. **point**
- Most ~~affect~~ **== most effects leading to Beta - Thal**

  mRNA **- AMOUNT**
  - Transcription-amount **(Promoter mutation)**
  - Processing, e.g., splicing errors-common
  - Translation (initiation, nonsense, frameshift mutations **(Not as common)**
  - **5′** - Capping or **3′** - Poly A tail **(processing defects)**

- Clinical Hallmark: α or β-Thalassemia results in an **IMBALANCE** in the amounts of α or β chains

  - Excess of normal chain-precipitates-RBC fragile – hemolytic anemia
  - Also, hypochromic, microcytic anemia due to less than normal amount Hb–like Fe-deficiency anemia.

α-THALASSEMIAS

- Recall, unlike β-genes, inherit 2 copies of α-genes from each parent (total 4).
- Unlike β Thal, α Thal can affect both fetal and adult Hb.
- Hydrops fetalis (South East Asia-China).
  - No α chains, all 4 α-genes deleted.
  - γ_4 homotetramers form in fetus-Hb-Bart's.
  - Severe intrauterine hypoxia.
  - Fatal, H_2O accumulation in brain and other organs. (Usually fatal *in utero*, some newborns live for days)
- α-Thal after birth
  - β_4 homotetramers-HbH-forms (HbH disease – when 3 α genes deleted)
  - Precipitate as RBC inclusions similar to Heinz bodies.
  - RBC fragile → anemia **(hemolytic)**
  - Severity of anemia depends on number α genes deleted (NOTE: ≈ 15% Afro-Americans are silent carriers of α Thal – 1 α gene deleted). **NO EFFECT.**

FFT neither γ_4 nor β_4 homotetramers **RELEASE** O_2!!

Only $\alpha_2\gamma_2$ in fetus or $\alpha_2\beta_2$ (and $\alpha_2\delta_2$) in adults have the ability to **RELEASE** O_2 (Binding is not the evolutionary problem-release is)

Note: Deletions of α-genes are easy to understand as errors that occur during unequal homologous recombination is meiosis. (Homologous gene sequences on different chromosomes can pair and recombine during meiosis)

TABLE 11–3
Clinical States of Alpha-Thalassemia Genotypes

Clinical Condition	Number of Functional α Genes	Genotype	α Chain Production
Normal	4	αα/αα	100%
Silent carrier	3	αα/α–	75%
α-thalassemia trait (mild anemia, microcytosis)	2	α–/α– or αα/– –	50%
Hb H (β4) disease (moderately severe hemolytic anemia)	1	α–/– –	25%
Hydrops fetalis or homozygous α-thalassemia (Hb Barts: γ4)	0	– –/– –	0%

* **REALLY IMPORTANT** *

β-THALASSEMIAS

- Decreased β-chains.
- α_4 tetramers form and precipitate. Hemolytic anemia; hypochromic microcytic anemia – less normal HbA_1 α_2 β_2.
- **ONLY** effects are after birth when β genes are expressed.
- HbA_2 levels increase in β-Thal heterozygotes. **Beta-Thal Minor**
- Single base-pair substitutions **- Beta-Thal Major**
- *** "HOMOZYGOTES" – 2 β Thal alleles (often not the same)-Thalassemia major-severe anemia-chronically ill.
  - β°-Thal, no β-chains, No HbA_1 — **Cooley Anemia Sythesis defects**
  - β^+-Thal, some β-chains, some HbA_1
  - Decreased life expectancy
  - Treatment: Repeated Transfusions (Fe-chelator for Fe-overload), bone marrow transplant **(See Ramifications)**

"compound heterozygote"

Heterozygotes ($\beta^A\beta^{Thal}$)

- Thalassemia minor **(Beta)**
  - Slight anemia, clinically OK
  - Electrophoresis, less HbA_1, **MORE** HbA_2-diagnostic

--- **Dangerous if give Fe for microcytic hypochromic anemia**

*** **Compound Heterozygotes - See next page**

good summary

β- THALASSEMIAS **REFINED: MEDICAL CLARIFICATIONS**

Homozygotes for β-thalassemia are classified as β- thalassemia major or intermedia based on the genetic abnormalities and clinical consequences.

β-Thalassemia major patients are either homozygotes or compound heterozygotes(carry 2 different abnormal genes for β-globin) for severe β-globin gene mutations.

β-thalassemia intermedia patients are compound heterozygotes in which at least one of the mutations has a mild phenotype.

Severe anemia in β-thalassemia major is due to both increased apoptosis of developing erythroblasts (markedly decreased survival) and shorter half-life of mature RBC due to destruction in the spleen; i.e. the hemolytic anemia of β-thalassemia.

check ferritin levels !

β-Thalassemia major patients require repeated transfusions for survival beginning in infancy, and are at high risk for severe iron-overload.(Note: the body has no effective means for excreting excess iron.) **Treat with Fe-chelators**

β-thalassemia intermedia patients have a mild to moderate anemia and either do not require transfusions or can begin transfusions later than β-Thalassemia major patients depending on the severity of the anemia which is variable depending on the exact mutations involved.

most

IRON METABOLISM , OVERLOAD,AND CHELATORS

- Most of total body iron is in Hb. (not cytochromes or Fe-enzymes).
- Fe- released from RBC when degraded in spleen is recycled.
- Fe- levels are regulated by absorption from the GI-tract.
- The body has no mechanisms for excreting excess Fe. (very unusual for a normally required nutrient; we have mechanisms for excreting Cu and Zn).
- Many SCA and β° - Thal Major patients require transfusions on a regular basis. This leads to Fe-overload from the RBC-Hb that is being transfused into the patient.
- If untreated, this will lead to a toxic, fatal Fe-toxicity (liver and heart toxicity).
- Fe-levels are measured by total serum iron, or Ferritin levels. It is also possible to monitor Liver and Heart levels by MRI methods.
- Fe-chelators bind Fe , and the complex is excreted from the kidney.
- Desferoxamine (Desferal) was the classic Fe-Chelator used with SCA and β°-Thal Major patients receiving repeated transfusions. Its major disadvantage is that it must be delivered subcutaneously and slowly for 8-12 hrs.
- Deferasirox is an effective Fe-Chelator that is given ORALLY, ONCE DAILY.
- SCA and β°- Thal Major patients can now receive regularly scheduled transfusions and Fe-chelation therapy and achieve a more normal life-expectancy while monitoring Fe-levels.
- Let's not forget that these therapies with testing are not available globally to large populations of patients living in other countries.

TYPES OF β-THALASSEMIA MUTATIONS

FYI

- β-gene Deletion-β^0-India
- Decreased synthesis of normal mRNA – most β-(Thal-β^0 or β^+)
  - RNA splicing defects-the most common!
    - (Splice junction, intron, and exon mutations that affect splicing-activate cryptic splice site)
  - Promoter mutants-common
  - Abnormal 5^1–cap site-common
  - 3^1–PolyA-signal defects-common
- Decreased functional mRNA-less common
  - Initiation mutations-β°
  - Nonsense mutations-early in gene--β°-thal, e.g., codon 39
  - Frameshift mutations-early in gene--β^0-thal

LEARN

BIG PICTURE

Most β-thal mutations decrease the AMOUNT of β-globin mRNA

- Promoter mutants
- RNA-splicing mutants (most common)
- RNA capping or polyA addition mutants

Problem-excess α_4 chains precipitate, RBC-fragile-**Hemolytic** anemia. Wide range of severity depending on how much β-chain is made.

HEREDITARY PERSISTANCE OF FETAL HEMOGLOBINEMIA (HPFH)

(A DEVELOPMENTAL DEFECT)

- Normal switch from γ to β-gene expression
  - γ-chain synthesis begins to decrease and β–chain synthesis begins to increase at ~ 36 weeks gestation.
  - By 12 weeks postnatal-~95% β, 5% γ and by ~ 1 year, 0.5%γ (98%β, 2%δ) (δ starts to increase at ~ 6 weeks postnatal after β>γ for first time).
- HPFH
  - Perinatal switch does not occur. **Keep making HbF (Gama-chains)**
  - **HIGH** levels of γ-chains and therefore high HbF ($\alpha_2\gamma_2$) continues postnatally.
  - Clinically not serious: Homozygotes – 100% HbF – most commonly due to deletion of δβ gene cluster. OK because compensate for *higher* O_2 affinity of fetal Hb by overproducing ~~Hb~~ **RBC (~~Polycythemia~~)**
  - HPFH is also caused by HPFH promoter mutations
  - Heterozygotes of HPFH make about 17-35% HbF – not serious.
  - $\delta\beta^0$ Thalassemia is a disease related to HPFH – also due to gene deletion but generally more serious than HPFH even in heterozygotes because less HbF is made (reason unknown).

FYI

(different mutation)

FFT: Medical Research significance: Development of a drug that would turn on HbF to levels seen in HPFH would be fantastic for treating sickle cell anemia and β-Thalassemia. Would block aggregation of HbS and provide "β-like" gene for serious β-thalassemia.

(See ramifications)

HBF → good & treat sickle cell/β-thalass

γ γ δ β β Gene cluster

Fetal Infant

BETA –zero THALASSEMIAS VERSUS HPFH

YIKES !!!!!!!!!!!!

BETA-ZERO THAL

SPLICING DEFECT
NO β- CHAINS
β – GENE IS **INTACT** , A SINGLE BASE CHANGE
INFANT: REPEATED TRANSFUSIONS,Fe-CHELATOR,
DECREASED LIFE EXPECTANCY

HPFH

DELETE WHOLE δ β- CLUSTER
NO β- GENE
MILD DISEASE

WHAT ? HOW ?

CONTINUE TO MAKE HIGH AMOUNTS OF FETAL Hb

Quirky –specific deletion (subtle unknown mechanism)

AMAZING and TRAGIC !!!!!!!!!!

RAMIFICATIONS : HEMOGLOBINOPATHIES (updated-2012)

The first published description of Sickle Cell Anemia appeared 100 years ago (1910). As pointed out in a recent Science article (Science (2010) 329, 291-292) this anniversary " is a sober reminder that we have far to go to meet the ***global challenges*** posed by this disorder." After 30 years of intensive analysis of globin genes, consistently effective therapy remains elusive.

- Many of the more than 200,000 SCA babies born annually in Africa will die before age 5 yrs (World Health Organization Estimate).
- Incidence in USA : about 50,000 people have SCA. 70,000 - 100,000 now.
- "The ***global*** need to develop uniformly effective, (easily administered), and inexpensive therapy is enormous and growing " (Science 2010).
- Research continues for possible gene, stem cell, RNAi, and bone marrow transplant therapies, but progress is slow, and may not succeed globally.

Questions : What drug is Cora, the sickle cell patient we met last semester taking to help prevent her sickle cell crises , and how does it work ?

Think!!!!!!!!!!!!!!!!!!!!!!

Hydroxyurea which increases levels of HbF (fetal hemoglobin) ,which in turn inhibits aggregation of HbS, the first step leading to a crisis. However, the increase in HbF is relatively small. Estimated need about 15% HbF to prevent crisis.

Difficult question : What has prevented/limited development of more effective, new drugs to increase HbF levels which would be ***much more effective*** for SCA and β-Thalassemia Major patients than any present treatment ?

Think.....

Answer: Practically nothing was known about how HBF gene expression is normally turned off during normal development !!!!!!!! (One way" school stinks" is that we do not talk much about what is UNKNOWN.)

NEWS FLASH (AMAZING BREAKTHROUGH) : a big success story of SNP-Genome Wide Association Studies. Elucidation of a major transcription factor that normally regulates the silencing of γ (fetal) globin gene expression during normal development . Named : BCL 11A. (This discovery was made by comparing a large number of SCA & β-Thalassemia subjects who had more HbF and milder forms of these diseases in a large population (Sardinia) that also had severe SCA & β-Thal major patients.)

High HbF is associated with reduced BCL 11A, abundant expression of BCL 11A is only found in adult erythroid cells, and down regulation of BCL 11A expression leads to robust HbF expression.

Yippee !!!!!!!!!!!!!!!!!!!!!!!!

Obviously, BCL 11A is a promising new target for small, low molecular weight drug therapy. Inhibiting the activity of BCL11A (and/or other proteins that work with BCL11A in transcription complexes) should increase HbF levels significantly.

" Reinvigorating research for effective and hopefully low-cost,(easy to administer) therapy for SCA (and β-Thalassemia major) have never been more pressing (and perhaps more promising)." (Science,2010 article).

This is an exciting new discovery !!!!!!!!!!!!!!!!!!!!!!!!

NEWS Flash # 2 (2012)

Wow!!!!!!!!! This subject is developing rapidly.

Recent Publication : Demonstrates in principle that only inhibiting the BCL11A protein itself or its expression in this complex system would be sufficient to raise the levels of HbF enough to PREVENT ALL sickle cell crises. ALSO SUFFICIENT to treat β Thal major without transfusions.

PROGRESS : TREATMENTS FOR SCA AND β-THAL MAJOR

- Enhancer that specifically regulates expression of BCL11A in developing red blood cells was recently discovered.
- Disruption of that enhancer would specifically lead to depletion of BCL11A in RBC.
- That in turn, would lead to large increases in HbF expression in mature RBC.
- Possible promising treatments for SCD and β-Thal major.
- Science (2013) 342, 253-257
- Simple summary /perspective : Science (2013) 342,206-207.

101 MEDICAL GENETICS

HEALTH AND DISEASE

Hbopathies – Types Abnormalities/Defects

Developmental, synthesis, folding (2°, 3°);, HbS

4°, Heme binding, Fe^{2+}, Allosteric Properties

+ homo, H^+, CO_2, BPG, 4°-movements

SEEMS THAT ANYTHING THAT CAN GO WRONG, DOES!

Overall incidence Hbopathies is high because polymorphic traits,

Sickle, Hb-C, E, β-Thal, α-Thal

4.83% of world population are carriers of an ~~1/150~~ abnormal Hb gene

Hb is representative of other genes/proteins - types defects

BUT

→ **HEALTH USUALLY PREVAILS**

- **Other inherited diseases – lower frequency. Not polymorphic (e.g. MD)**
- **HETEROZYGOTES – symptom free or tolerable in autosomal recessive diseases. A rule of thumb for Globular Proteins in general.**
- **ISOFORMS (HPFH) Biology Principle**
- **Compensation (polycythemia)**