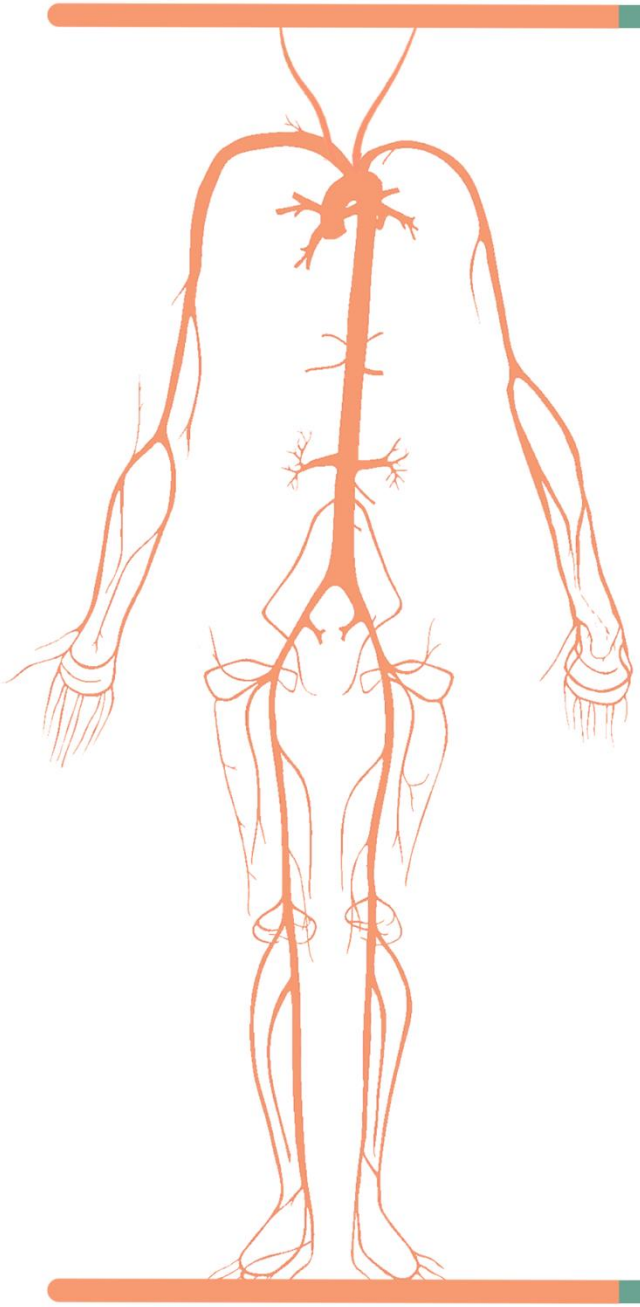
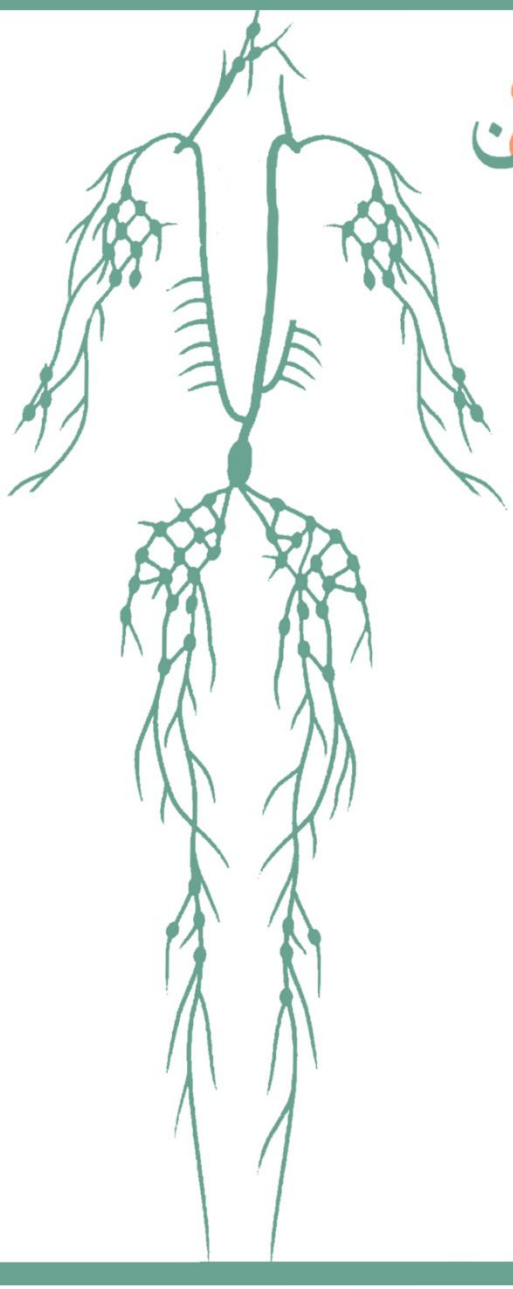
Biochemistry

HematoLymphatic





Title: Sheet 1

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In this sheet we will have a recap of what we took in Biochemistry 1, with some extra details to make things interesting. Have fun

- Proteins having non-protein groups attached to them are known as **holoproteins**.
- **Apo-protein**: a holoprotein without its non-protein group.

- Example of holoprotein:

Hemoproteins → Protein + non protein group(**heme)**

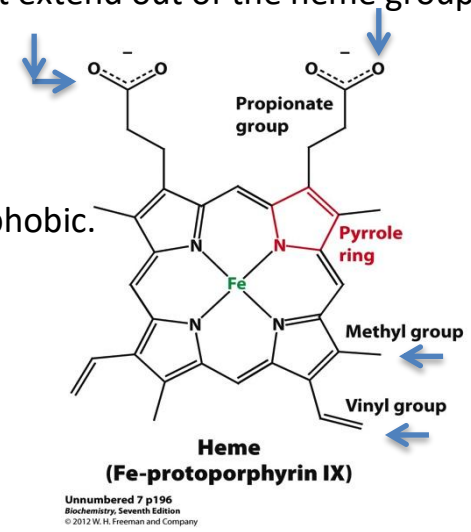
Examples of heme containing proteins:

Heme- containing proteins	Function
1. Myoglobin, hemoglobin	Binds oxygen –> to transfer or to store O2
2. Cytochrome p450	Detoxification of xenobiotics (toxins) in liver.
3.cyt c, cytb5	Electron transport chain
4- heme containing sensor proteins	a. heme sensor b. gas sensor (O2,CO,NO)

What is heme?

- Heme is an organic molecule composed of four pyrrole rings.
- Pyrrol ring: it is a cyclic molecule it has branches that extend out of the heme group such as:

a. methyl group
b. vinyl group
c. charged propionate group (hydrophilic). Other than the 2 propionate groups, the whole molecule is hydrophobic.



Heme's structure

- heme = Protoporphyrin 9 + an iron atom

In the center of the heme molecule we have an iron atom, which has six coordinates (form six covalent bonds), four of them with the four pyrrol rings of the heme itself, one with oxygen and the last one with proximal histidine of hemoglobin)

HEMOGLOBLIN

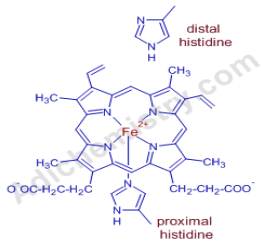
- Hemogloblin is heterotetramer globular protein made up of 4 chains.
- 2 alpha + 2 beta chains.
- Each chain binds to 1 heme molecule.
- Alpha chains are composed of 141 amino acids.
- Beta chains are composed of 146 amino acids, its last amino acid is histidine at the carboxylic end.

Hemogloblin : Alpha 1/heme, beta 1/heme
+
Alpha 2/heme , beta 2 /heme

- Four heme per hemogloblin, each heme binds to 1 oxygen.
  1 hemoglobin → 4 heme → 4 oxygen

Characteristics of hemoglobin:
It is a globular protein, so it is charactarized by having:
1. hydrophilic and charged amino acids on the surface
2. hydrophobic, nonpolars amino acids in the inside.

Special amino acids of hemoglobin:
In the core of hemoglobin we have TWO POSITIVELY charged HISTIDINES.
these molecules play special roles in the function of hemoglobin.
1. Proximal histidine
2. Distal histidine



Histidine's function :

1. proximal histidine: covalently linked to the iron atom of the heme.
2. distal histidine: stabilizing or regulating the entry of oxygen.

Positive co-operativity

- binding of one oxygen makes it easier for the rest of oxygens to bind.
- oxygen binds to the heme's iron, this binding will change the structure of hemo-globin. The changed structure of the hemoglobin will facilitate the binding of the second oxygen, and so on.
  Side note : As more oxygen binds, the affinity between oxygen and hemoglobin increases.

We said hemoglobin is a heterotetramer, as dr. Mamoun calls it dimer of dimer

Dimer A	Dimer B
Alpha 1 + beta 1	Alpha2 + Beta2
Hydrophobic interaction between $\alpha 1+\beta 1$	Hydrophobic interaction between $\alpha 2+\beta 2$
There is an electrostatic interaction between Dimer A and Dimer B	

- Hemo-globlin can work in two states/ two structures and they are (R and T) states.
- The mechanism that helps with changing the structures is the interactions mentioned in the table.

Difference between the structures *T and R* of hemogloblin:

1. They differ in the orientation or 3D position of amino acids.
   - and this is due to the movement of polypeptides as a result of the oxygen binding to heme.

2. Different binding affinity to oxygen:

- T form → having low affinity to oxygen.
- R form → having high afiinity to oxygen.

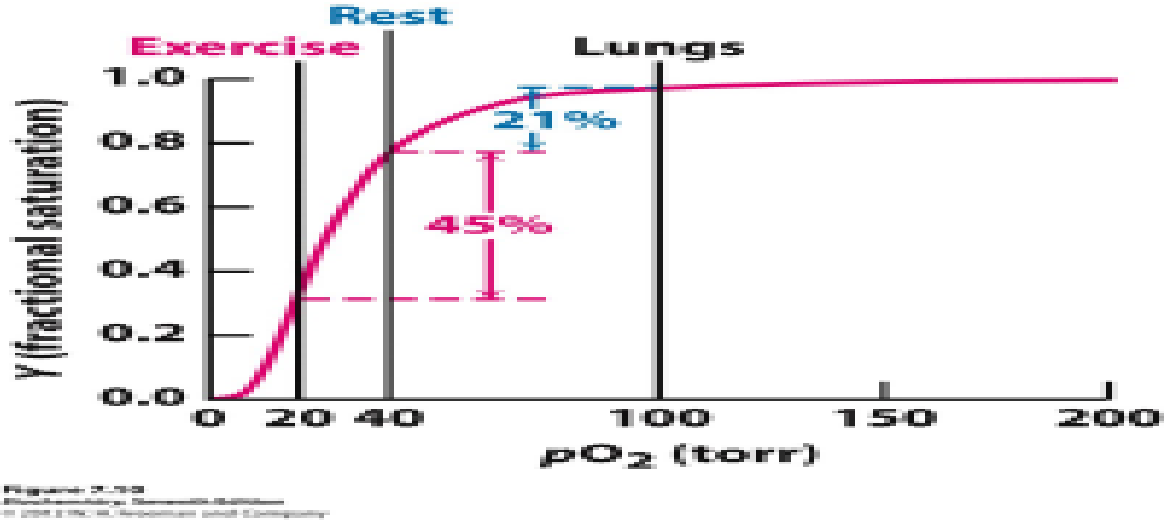
Note:

**High affinity → Strong bond between oxygen and hemoglobin → Harder for oxygen to leave hemoglobin (This state is preferred when no oxygen supply is needed)*

**Low affinity → weak bond between oxygen and hemoglobin → oxygen easily leaves the hemoglobin (this state is preferred when oxygen supply is low)*

Oxygen saturation curve

- Please notice in the next page how the pink curve has a SIGMOIDAL (S) shape.



- <u>As any other curve we have x axis an y axis</u>

  x axis :
  represents the different levels of oxygen pressure PO2(torr).
  ***different amounts or availability of oxygen** *

  y axis :
  represents the saturation of oxygen.
  by satuation we mean the amount of oxygen bound to hemoglobin

  - ➢ pO2 α Saturation of hemoglobin with O2.
  - Notice how as (PO2) increases, the (saturation) binding of oxygen to hemoglobin also increases. → DIRECTLY PROPORTIONAL.

  - ➢ Saturation and PO2 α affinity
  - Directly proportional.

- By Affinity we mean the strength between oxygen and hemoglobin.
- As the oxygen pressure falls, oxygen is released to the cells.
- During exercise about 40% of hemoglobin is saturated
- In lungs about 100% of hemoglobin is saturated, when PO2 = 100
  NOTICE: how the curve is SIGMODIAL.
  - this indicates that hemoglobin is an ALLOSTERIC PROTEIN.

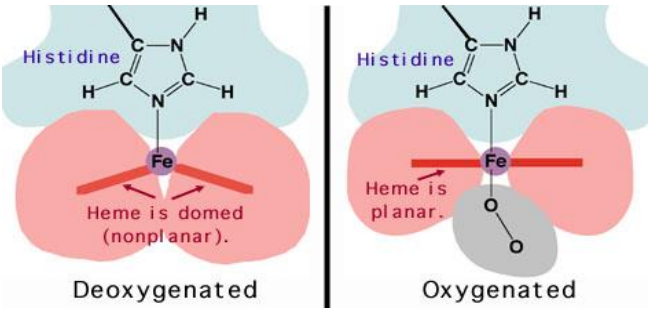
Proteins having more than 1 shape → allosteric Proteins.
Alloesteric P → sigmodial curve

- Why does hemoglobin have different structures (R,T)?
  - As oxygen binds to the heme of hemogloblin, the quaternary structure of hemoglobin changes.

❖ **Recall: Proteins have four level of structures:**

- Primary structure: sequences of amino acids.
- Secondary structure: sequences of the amino acids are linked by H- bond forming alpha helix and beta sheet.
- Teritary structure: 3D structure of a polypeptide.
- Quarternary structure: the overall structure of the protein and it's made of more than 1 polypeptide.

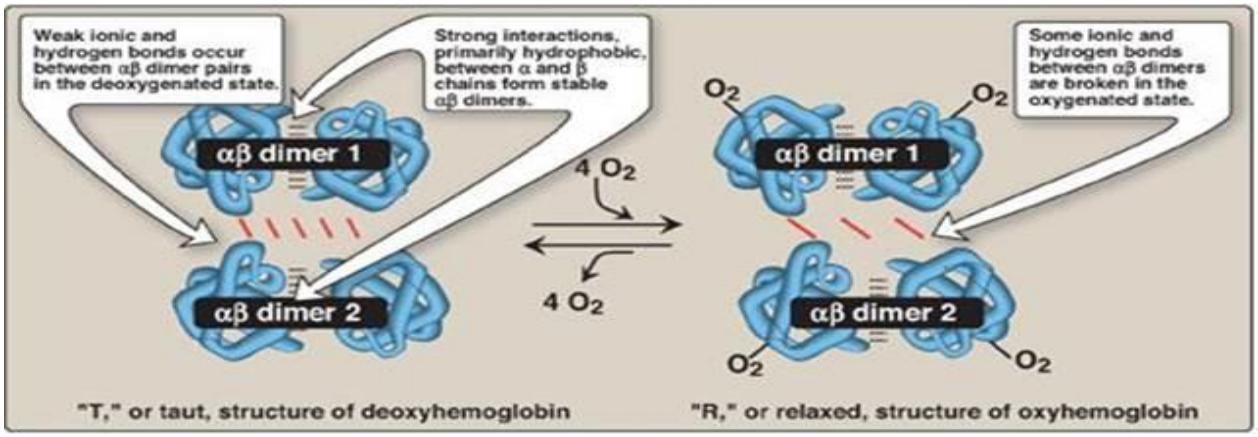
- How does binding of oxygen to heme change the quaternary structure of hemogloblin?
  - Deoxy hemoglobin: iron of heme is bound to proximal histidine of the hemoglobulin→ repulsion force occurs between the hydrophobic heme and the proximal histidine→ bends the heme giving it a dome shape.
  - oxygenated hemoglobin: iron of heme is bound to proximal histidine and oxygen→ the repulsion between the heme and the proximal histidine is reduced → the heme will go back to it's plannar shape→ distal heme will stabilize the oxygen interaction.



❖ This movement changes the structure of heme molecule & alpha helix.

As the heme is pulled upward to its planar position, it also pulls the proximal histidene → which is part of the alpha helix of the hemoglobulin.

- The change of alpha helix → changes the tertiary structure of the oxygen bound polypeptide→ this will lead to the breakage of the electrostatic interaction, between the dimers (dimer A , dimer B). → converts the hemoglobin from T state to R state→ changes the hemoglobin's affinity.



What happens at the level of the amino acids?

- beta chain → 146 amino acids.
- Alpha chain→ 141 amino acids.

Last amino acid on beta chain is histidine known as histidine 146 HC3→ meaning it's present at the carboxy terminus and its histidine is number 3.

In the **T state** → the C- terminal his146 amino acid of the **B- globulin** forms two ionic pairs:

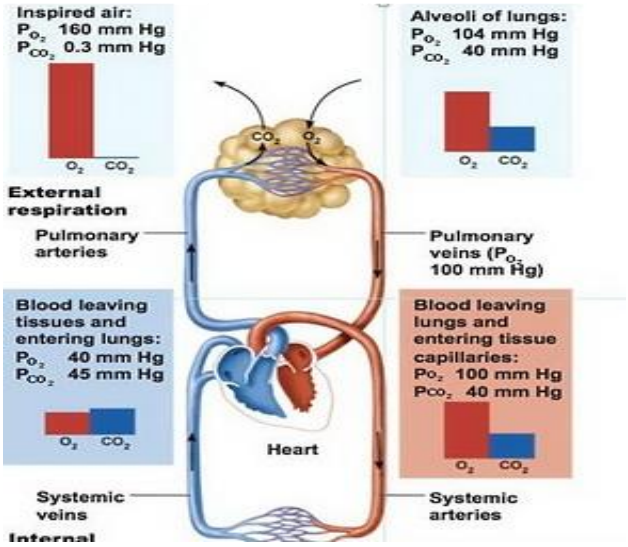
1. the C- terminal carboxylate group ion-pairs to Lys C5 of the opposite α-globulin, β2 to α1 or β1 to α2.
2. its side chain ion pairs to the ASP FG1 of the same β-globin.

- When O2 binds, the alpha1- beta1 globin rotates 15º relative to axis of deoxy alpha2-beta2.
- The alpha1 surface slides and **his146 relocates and loses contact** with **LYS C5 and Asp FG1.**
- As alpha 1 relocates, a hydrogen bond is formed, between Asp of alpha chain and Asn of beta chain → stabilizing the R state.

  - Having these two structures make huge signifcance in the function of hemoglobin.
    - it's important for hemoglobin to bind to oxygen with a high affinity in the lungs, And low affinity at the tissues.

Oxygen distribution in blood

This is an illustration that shows the level of oxygen in different conditions or different circumstances:



First: Inspired air has the highest amount of oxygen relative to carbon dioxide.

Second: in alveoli of lungs the level of oxygen still high about 104torr and CO2 = 40torr.

Third: As blood leaves the lungs the level of PO2 =100torr.

is

Hemoglobin's route, oxygen distribution:

The oxy- hemoglobin reaches the tissue → the tissues are very low in O2 and high in CO2, (because metabolism). → A shift in HB's structure from (R-state to T-state) → leads to weak interaction between hemoglobin and oxygen → Hemoglobin releases the oxygen into the tissue → as hemoglobin leaves the tissue the O2=40torr is almost equal to CO2=45torr → hemoglobin returns back to lung → hemoglobin gets saturated again.

Altitude and oxygen levels:

- PO2, saturation, and affinity are all affected by difference in altitudes
- at sea level arterial oxygen is about 100 torr.
- At high altitudes maximum arterial oxygen is 60 torr.
- At high altitude the Atmospheric pressure is reduced → leading to a reduced arterial oxygen
- There is 40% reduction in the amount of oxygen that leaves the lungs.
- Altitude→ inversely propotional to atmospheric pressure.
- Atmospheric pressure → directly proportional to arterial and venous oxygen level.

The change between R and T happens, will it happen gradually or directly?

We have two models that explain how hemoglobin changes structure:

1. MWC, The concerted model (**More accurate)**

2. The sequential, induced fit or KNF model (**less accurate)**

MWC: states that the protein exists in two states:

Either the T (taut, tense) state with low affinity OR the R (relaxed) state with high affinity. T or R and nothing in between

Increasing ligand concentration drives the equilibrium between the R and T toward the R state (+ve cooperativity).

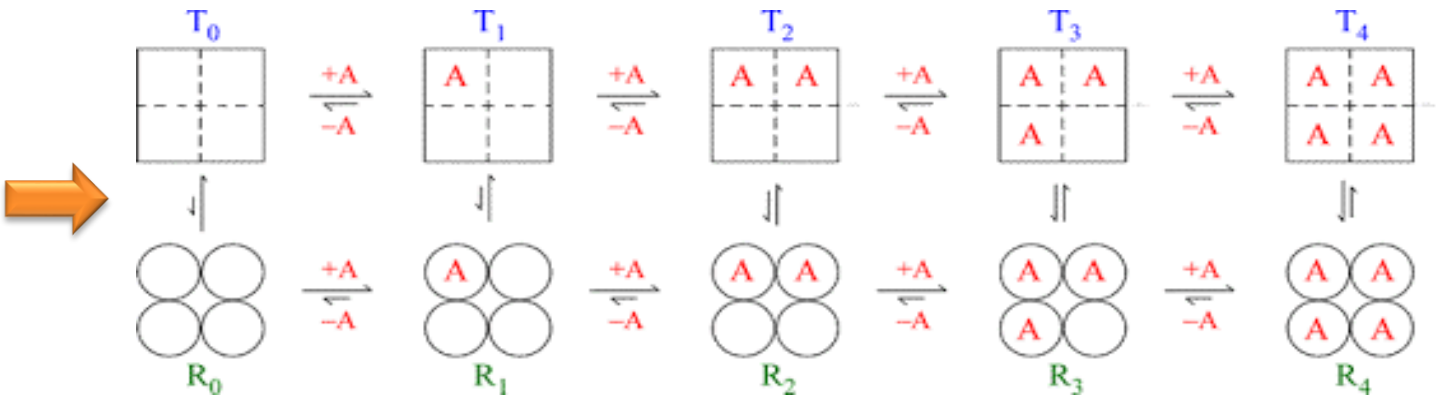
- Homotropic effect:

The effect of ligand concentration on the conformational equilibrium. Ex:(oxygen).

- Heterotropic effector:

Other effector molecules that bind at sites distinct from the ligand binding site and (To be discussed).

THE MWC MODEL



please notice the arrows between T and R state.

- T and R are in equilibrium so T would change to R, or vice versa.
- In the absence of oxygen hemoglobin exists more in the T state than the R.
- Notice the direction of the arrows between them, yes even in the absence of oxygen hemoglobin can be found in the R state, but it's very little compared to T state.

- As we increase oxygen the equilibrium changes a little bit so now you have a greater proportion of R state but still less than the T state.

REMEMBER :

- As we add oxygen the equilibrium shifts from T state to R state.
- As we remove oxygen the equilibrium shifts from R state to T state.
- MWC only suggest positive cooperativity
- In MWC hemoglobin exists in two states only
- R and T are in equilibrium

2- THE SEQUENTIAL, INDUCED FIT, KNF MODEL:

- This model claims that it's not just either T or R state, there are actually other structures in between. We have intermediates.
- Less accurate.
- This model suggests both positive and negative cooperativity
- The conformational changes take place independently, but help with other subunits to undergo their conformational changes with less energy.
- When one oxygen moelcule binds to one polypeptide it changes this polypeptide from T → R. Then, this changed polypeptide changes the structure of the neighboring polypeptides to something in between. And the cycle goes on, and this gradual change will also affect the affinity of hemoglobin toward oxygen.

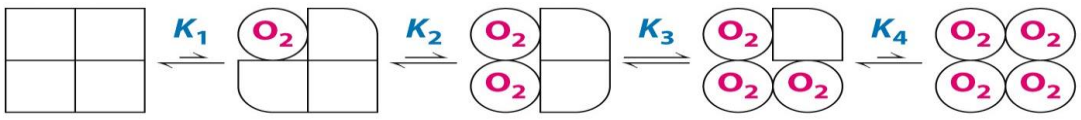


Figure 7.14
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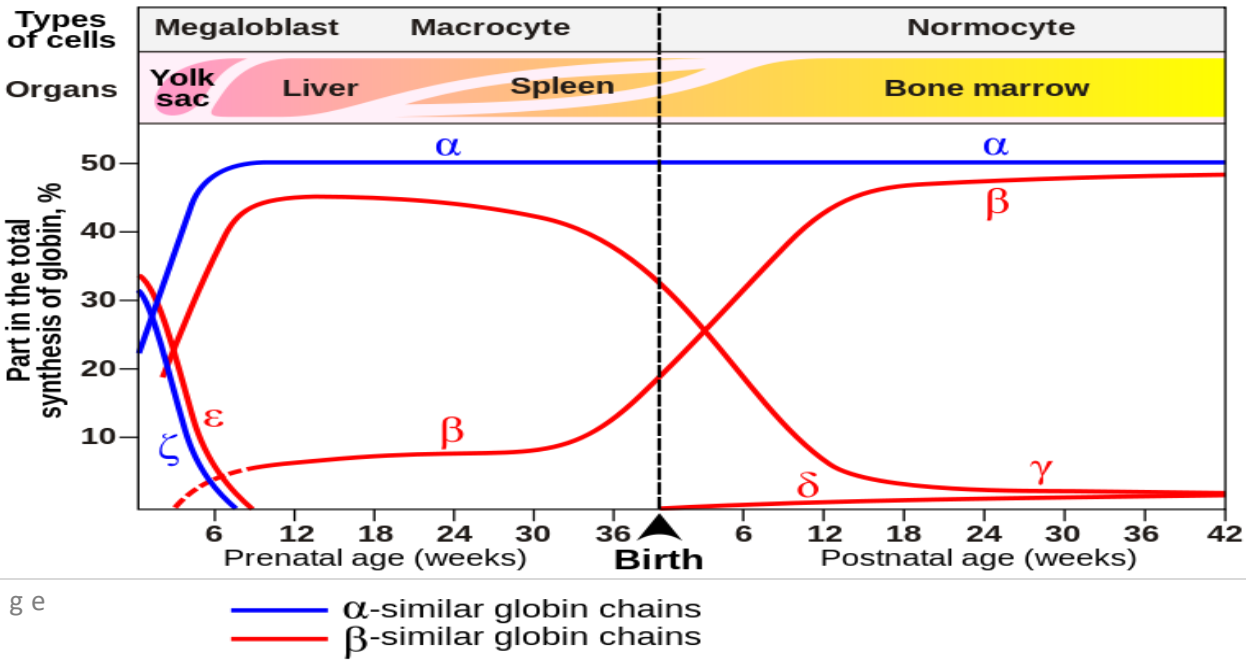
Different forms of hemoglobin throughout development



HUMAN DEVELOPMENT	EMBRYONIC STAGE	BEGINNING OF FETAL STAGE	Beginning of adult stage
Beginning of its synthesis	First few weeks of embryonic development	By 6-8 week of gestation	Shortly before birth
Location of the synthesis	Within the yolk sac	Starts from the liver	
Major HB	HBE GOWER1 = (2ZETA+2EPSILON)	HBF =2(alpha2+gamma2)	HBA 1 = 2(alpha+beta)
Minor forms of HB	1. HBE GOWER 2 = 2(ALPHA2+EPSILON2) 2.HBE PORTLAND1 = (ZETA2+GAMMA2) 3.HBE PORTLAND2= (ZETA2+BETA2)		HBA2 = 2(alpha +delta)

IMPORTANT NOTES

- The Alpha polypeptide remains throughout life.
- Shortly before birth, the gamma polypeptide gradually switches to beta globulin.
- At birth synthesis of both gamma and beta chains occurs in the bone marrow.
- Only HBA can be glycosylated specialy HBA1.

ADULT HEMOGLOBIN GLYCOSYLATION

- HBA can be glycosylated with a hexose and it's desginated as HBAc.
- The major form HBA1c has glucose molecules attached to valine of beta chains.
- HBA1c is present at higher levels in patients with diabetes mellitus.

Two types of tests for diabetic patients

1. Blood fasting glucose level.

   Blood fasting test: the patient would fasts overnight and next day a blood test is made for total glucose level. it should be between 80 and 120.

   Advantage :
   Tells you the exact concentration of glucose at that exact time.

2. HBA1c glycosylation: provides long term trend, of how high your blood sugar has been over a period of time (2-3 months)

-It's like calculating the average of blood sugar level.

- Disadvantage: Doesn't capture short term variation like hyperglycemia and hypoglycemia
- Advantage :

- Used to check if diabetic patients are adherent to their medication or not
- Used to check how effective is the medication

Measurement of HBA1c:

HBA1C can be expressed as :

- **Percentage (DCCT UNIT)**
  the percentages are the ratio of glycosylated Hb to the total Hb molecules.
  Normal range: 5-6%
  Pre-diabetic : 6-7%
  Diabetic: >7%
- mmol/mol: value of mmol of a hemoglobin molecule per total mol of hemoglobin → mmol/mol (IFCC unit)→ IFCC is a newly recommened measurement.
  Advantage of IFCC :
  1- provides a larger range
  2- more accurate

GENETICS OF HEMOGLOBIN SYNTHESIS

Types of globin chains: (Alpha, Beta, zeta, epsilon, gamma)

- The genes exist in clusters on two different chromosomes (11,16)

We have two clusters:

1. Alpha gene cluster: contains two alpha genes (alpha 1, alpha2) and one zeta gene.

-Located on chromosome 16

2.Beta gene cluster: contains epsilon, two gamma, one delta, and one beta gene.

-Located on chromosome 11

- <u>The gene order parallels/reflects the order of expression .</u>

Side-note/ we have 2 alleles for each gene → (1 allele from mom, the other from dad)

Two alpha gene on a chromosome16 so -

Each person has 4alleles for alpha gene.

Notice: that the genes are located on two different chromosomes and you have genetic switching of:

zeta gene → alpha gene

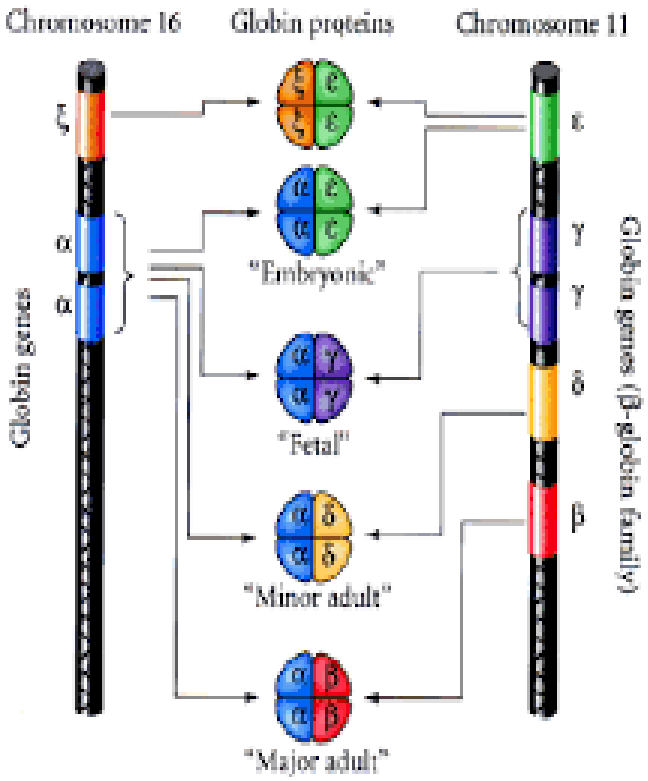
Epsilon gene→… →beta gene

These are all determined by:

1.Transcription factors

2.Genetic factors

3.Developmental clock (time controlled or age)



How does this globulin gene switching works?

The switching expressions of the genes from one to another is controlled by:

1. The gene's promoter.

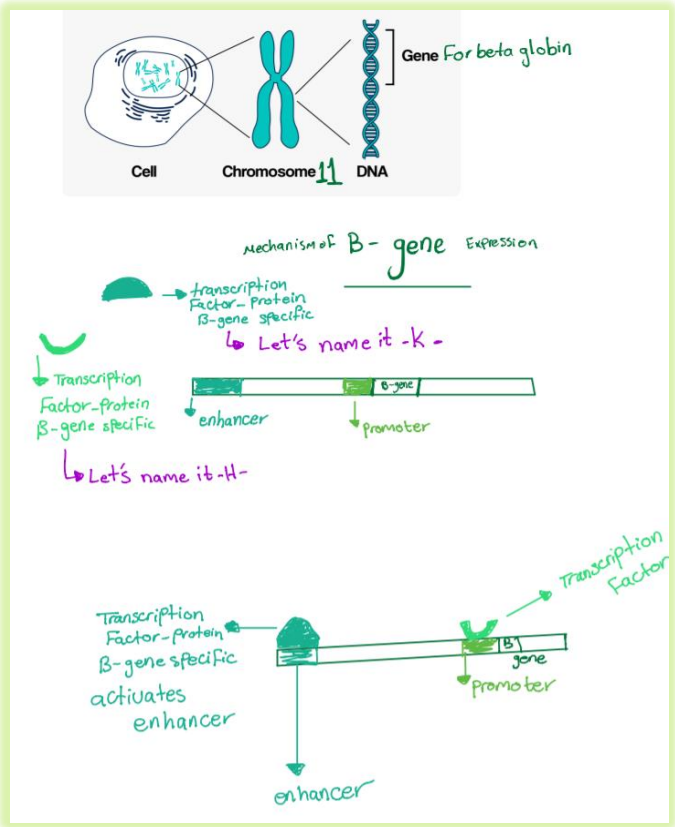
2. The gene's regulatory sequences (activators, silencers).

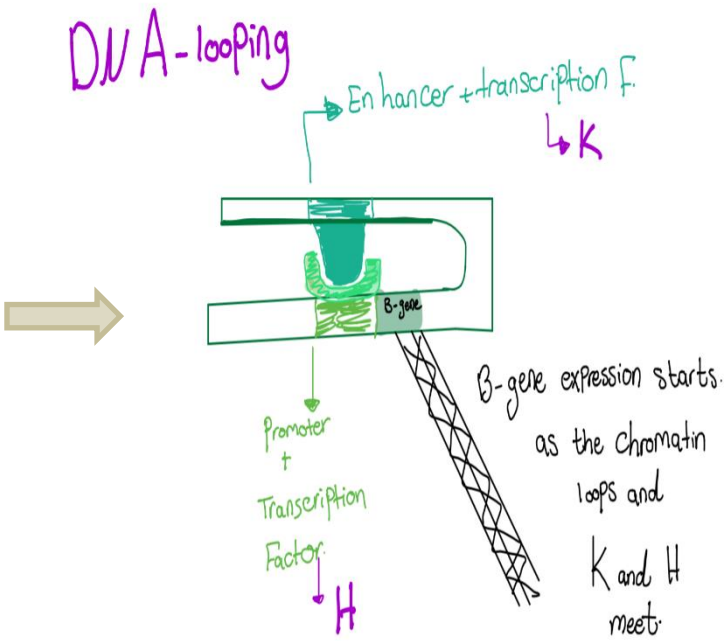
The alpha gene cluster is controlled by → HS40 region→ upstream to alpha gene cluster.

The beta-globin cluster is controlled by a master enhancer called→ locus control region (LCR) → upstream of B-cluster → controls the expression of chromosome 11 clusters.

THE MECHANISM OF REGULATION:

Each gene has its own promoter and transcription factor, and they are all controlled by their enhancer as we will see in the following illustration:





- This binding is all timed and programmed and starts with fertilization.
- This specific timing is achieved by Epigenetics. acetylation, methylation chromatin compactness and covering which lead to inactivation of the promoter region.

- we can USE this idea in treatment:

If someone can't synthesize beta globin, patients with beta thalassemia for example. We can treat them by inducing gamma gene's expression.

Good Luck!!