HLS

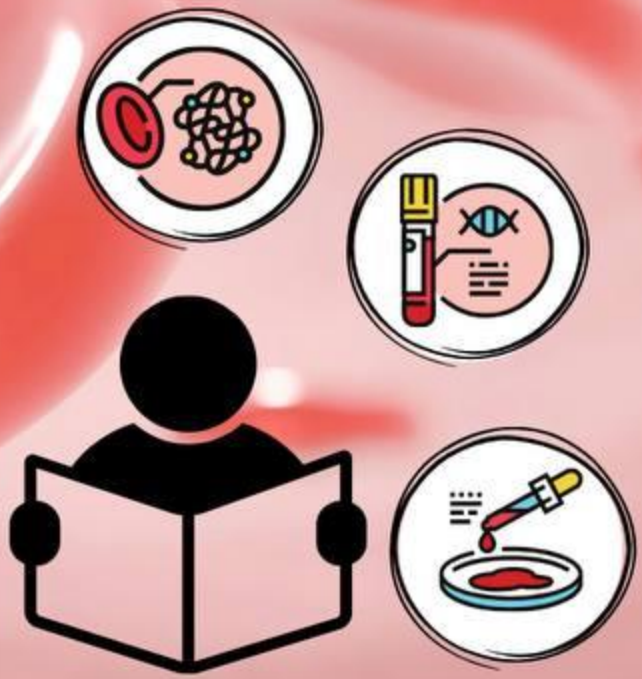
MODIFIED NO. 1 part 2

BIOCHEMISTRY

كتابة: سارة عمر

تدقيق: اسماعيل العارضة

الدكتور: مأمون أهرام



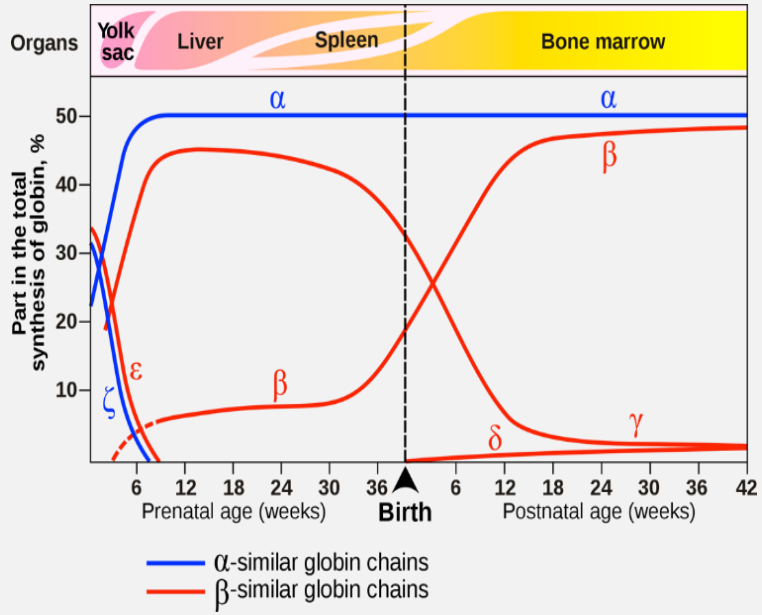
Hemoglobin
An overview and more

Prof. Mamoun Ahram
Hematopoietic-lymphatic system

Color code
Slides
Doctor
Additional info
Important

Genetics of globin synthesis

RECALL: Notice the order and the timing of hemoglobin synthesis , both are almost fixed



Alpha	Α α
Beta	Β β
Gamma	Γ γ
Delta	Δ δ
Epsilon	Ε ε
Zeta	Ζ ζ

The genes

The genes of globin proteins are found on 2 chromosomes (11,16) and they are presented as clusters of genes.

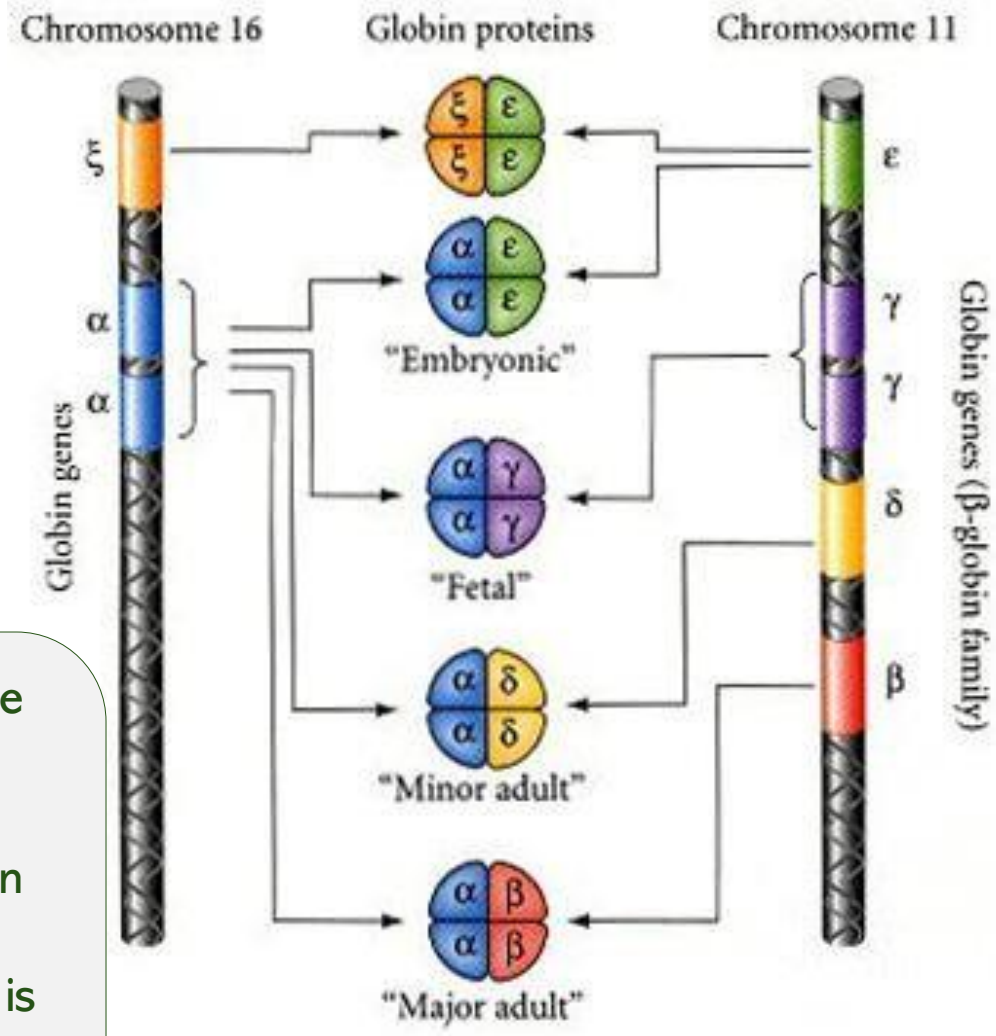
- **On chromosome 16**

  The α gene cluster contains three genes: Two identical α genes (α1 and α2), and ζ (zeta) gene.

- **On chromosome 11**

  The β gene cluster contains five genes:

  β gene,ε (epsilon) gene, two identical γ (gamma) genes, and δ (delta) gene.



Notice that the order of genes on each chromosome are the same as their order of synthesis (expression).

Chromosome 16 / **alpha cluster**: zeta then alpha.

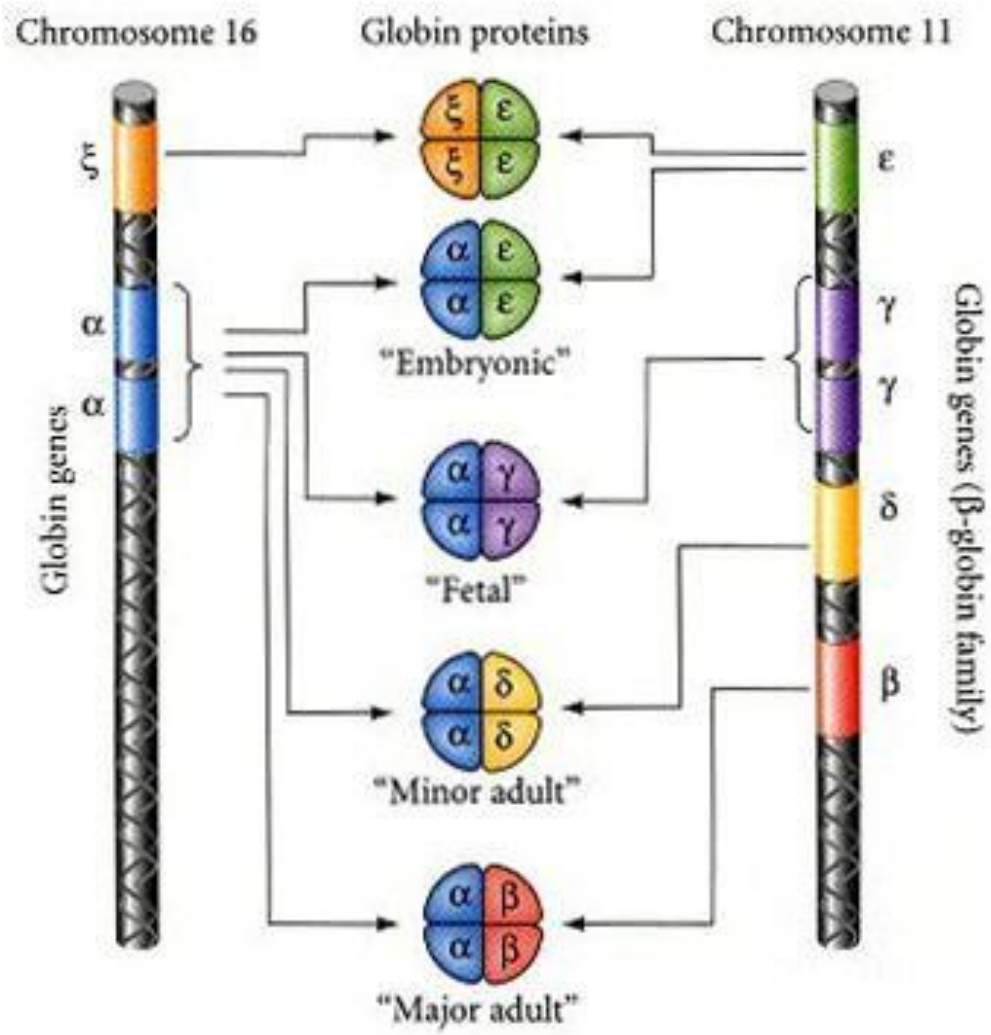
Chromosome 11/ **beta cluster**: epsilon then gamma then delta then beta.

Concerning the delta gene, as we know in previous slide that beta is expressed before delta, however here it is observed that it is expressed sooner than the beta gene; yet, it remains undetectable.

The genes

- Genetic switching is controlled by a transcription factor-dependent developmental clock, independent of the environment.

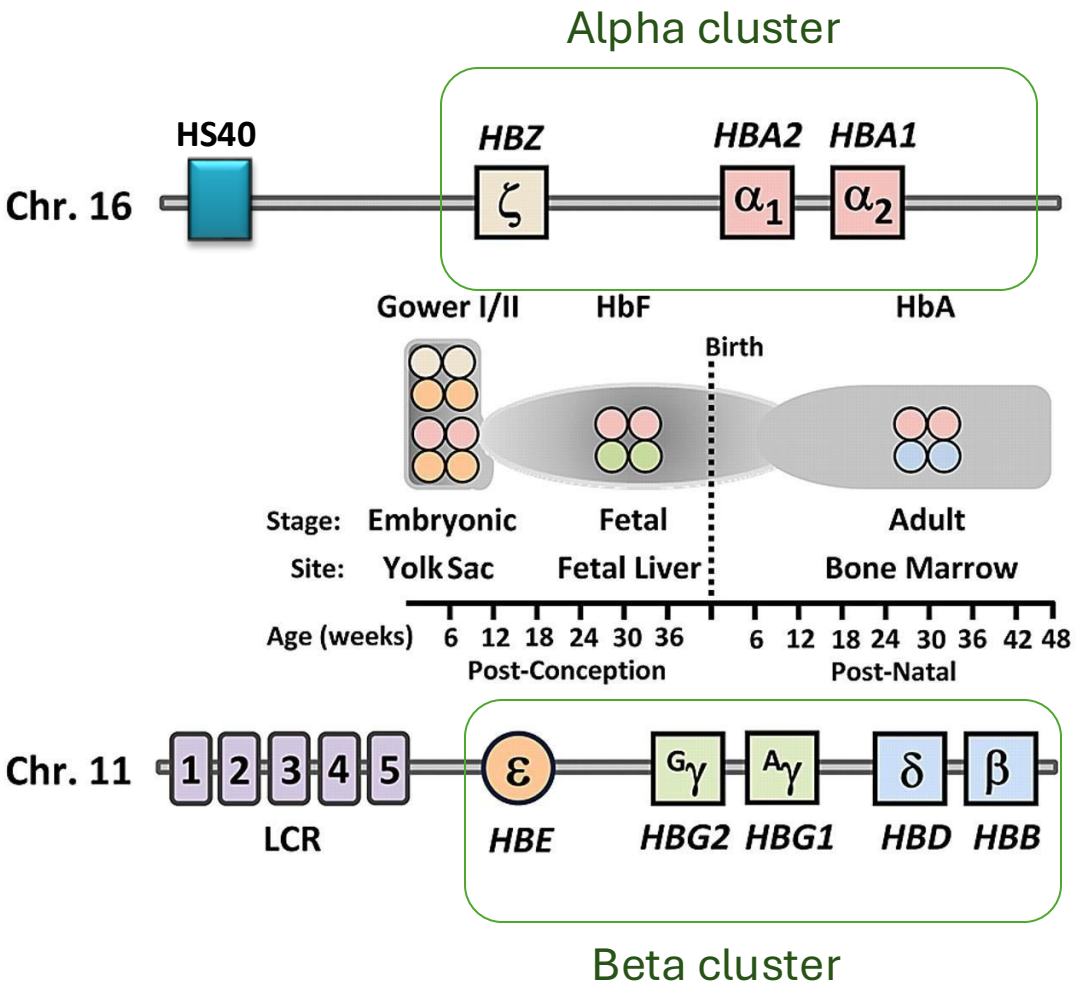
- *Premature newborns follow their gestational age.*



• Premature newborns follow their gestational age/stage, (which is 9 months after the time of fertilization). If a baby was born prematurely, the transition of gene expression from the fetal to the adult genes will wait until the 9-month mark hits (no matter when the birth did happen).
Meaning If a baby was born after 7 months of pregnancy, the transition of expression to the adult form will happen when he is 2 months old (he will have 60% HbF and 40% HbA1) which is what a mature born child would have before he was born.

Locus structure

- Each gene has its promoter and regulatory sequences (activators, silencers).
- The α gene cluster is controlled by the HS40 region (enhancer).
- The β-globin cluster is controlled by a master enhancer called locus control region (LCR).



A ***promoter*** is a region of DNA upstream of a gene where relevant proteins (such as RNA polymerase and transcription factors) bind to initiate transcription of that gene, each gene has its own promoter which means epsilone gene has its own promoter which is different from Gamma , and Beta also has its own promoter and so on.

Certain genes are incapable of being transcribed; in order for RNA polymerase to function, it requires an enhancer in addition to its own promoter.

Enhancer is a regulatory element (a part of the DNA sequence) activates transcription over long distances that can be located near the gene or far away from it, enhancers' positions can be changed according to the next activated gene's location because of the DNA looping.
On chromosome 11, epsilon ,gama ,delta and beta genes has it's own promotors.

So how that happens? Check the next slide
1.Transcription regulatory proteins **bind to enhancer** DNA sequence (HS40 or LCR)
2. **DNA looping** so these regulatory proteins with the enhancer can reach the wanted gene's promoter
3. These regulatory proteins **interacts** with the other proteins located on the promoter
4.**Activating** RNA polymerase
These regulatory proteins with the enhancer loop and bind with each different promoter independently.

The mechanism of regulation

It's timed; once the fertilization happens the clock starts,

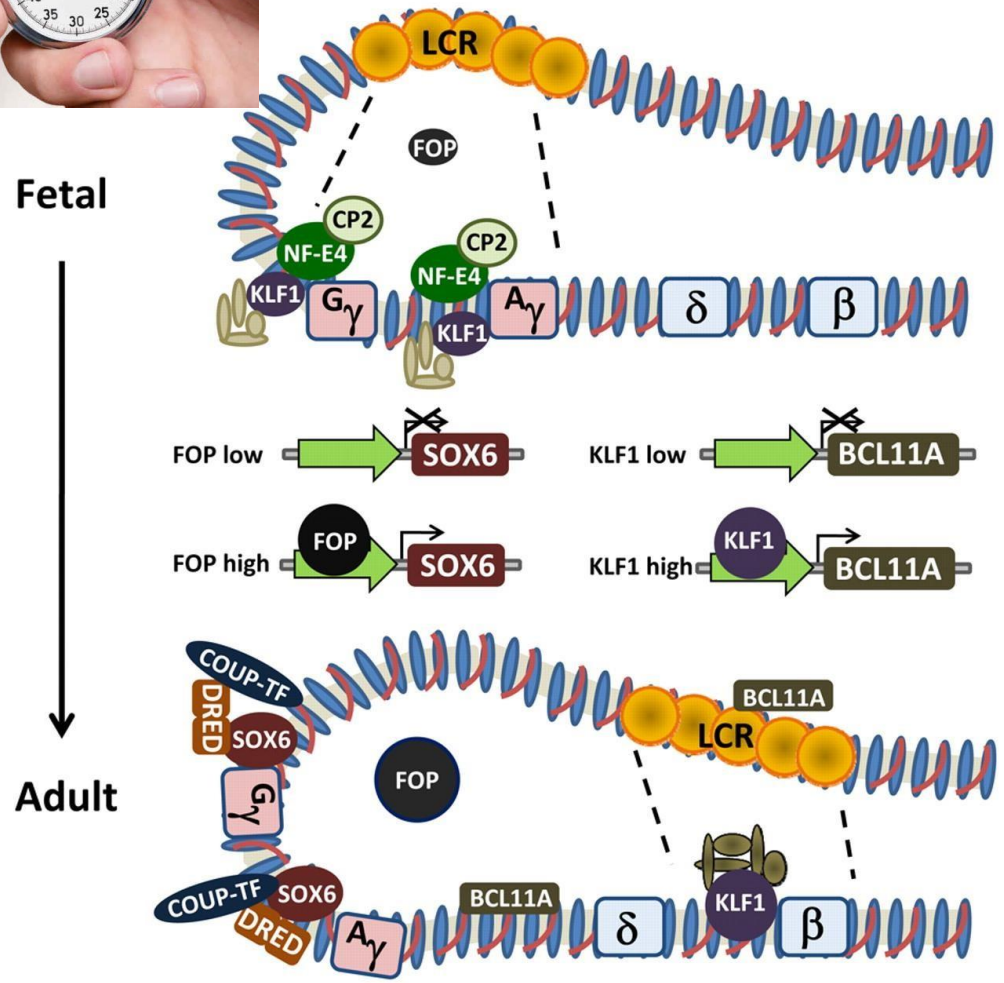
So as it is explained before, the proteins (in the yellow color) binds to the enhancer (LCR) then the DNA loops, so the proteins on the enhancer interacts with the other proteins on Gamma Gene's so The Gamma polypeptide is produced. Same thing happens to alpha gene.

By time, the looping will change due to the change of proteins. The Binding proteins on gamma gene will change and new proteins will bind to beta gene ,so the regulatory proteins on the enhancer now will bind to Beta gene regulatory proteins rather than gamma.

By time, gamma peptide production will decrease and Beta peptide will increase, so **the percentage of fetal hemoglobin (HbF) will decrease while Adult hemoglobin (HbA) increases with time.**

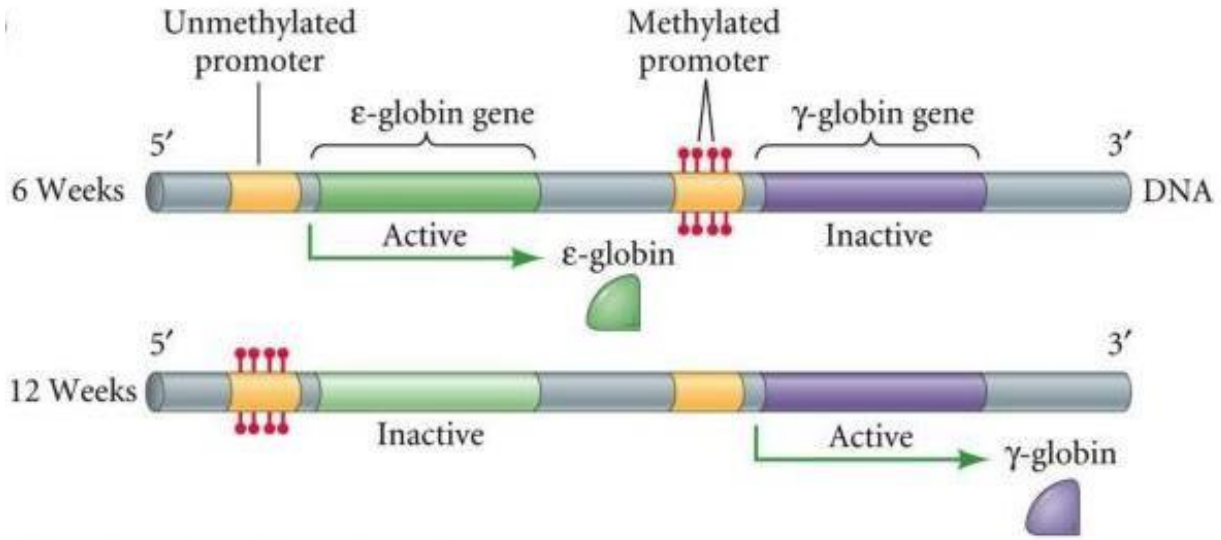
The mechanisms of regulation

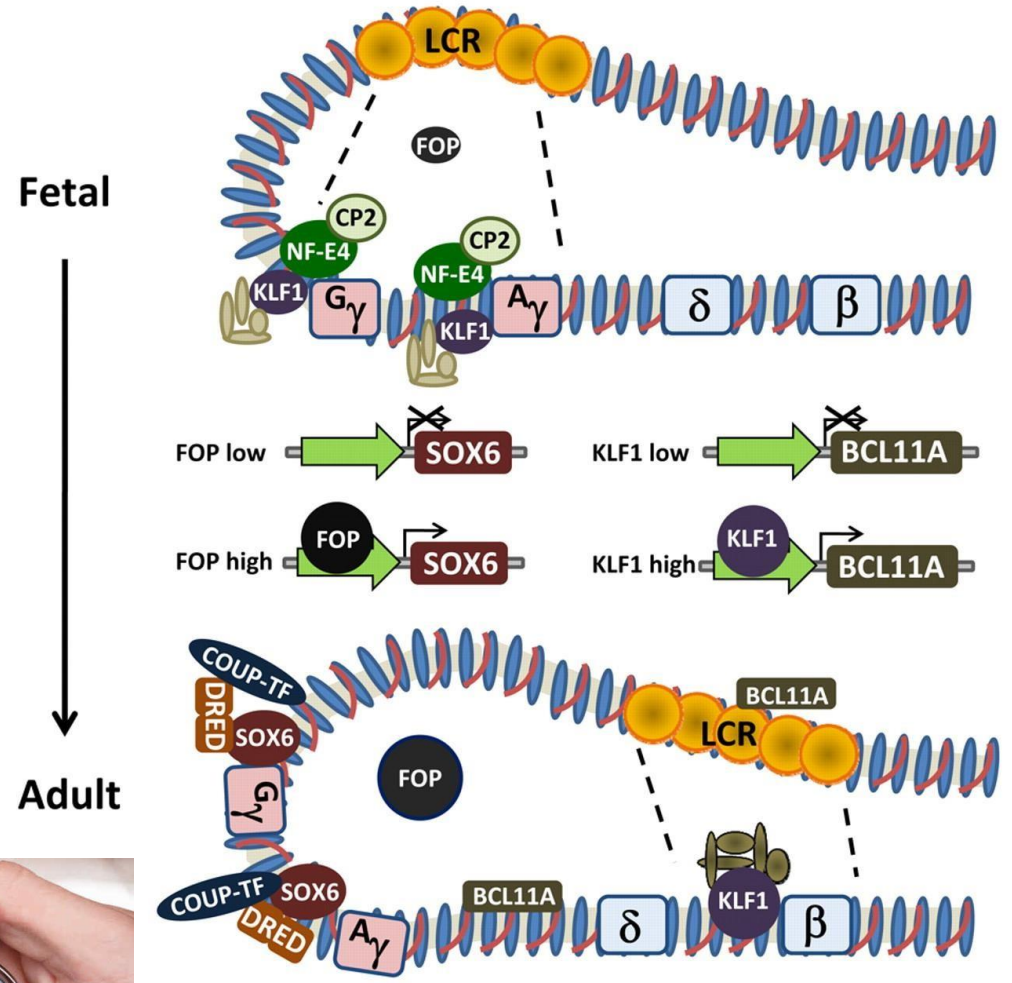
1. Changing in DNA loop due to change of proteins.
2. Epigenetic regulation



The mechanism of regulation

- The mechanism requires *timed* expression of regulatory transcription factors for each gene, epigenetic regulation (e.g., acetylation, methylation), chromatin looping, and non-coding RNA (e.g., long non-coding RNA, microRNA, etc.).
- Note: treatment!!





Epigenetic regulation: how cells control gene activity without changing the DNA sequence, modifications to DNA that regulate whether genes are turned on or off, eg changing chromatin shape to be compact / Condensed (heterochromatin) ,so regulatory proteins can not access to enhancer ,so DNA is inactive, or It could be (euchromatin) Relaxed ,so DNA sequence can be accessable for regulatory proteins.

Treatment !!

Researchers tried to **treat beta thalassemia**, which is highly prevalent in our region, by making some epigenetic modifications. This disease is related to abnormal beta globins, so they induced the expression of gamma globin instead of beta (which works similar to beta globins but has more affinity for O2) **using CRISPR-Cas9, long noncoding RNAs, and enhancers.**

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.

أريد أن أُذكِّرك بشيء .. أقدارنا مكتوبة قبل أن نرى نور الدنيا من الأساس، فأي قلق بخصوص أي شيء قادم ما هو إلا هَلكة للنفس والأعصاب، استثمر المتاح، واستغل كل الممكن، وسلِّم أمورك لله .🕊

Additional sources

1. **Promoter and enhancer**

https://youtu.be/aq8PAM5Sa0s?si=tSj9qLq5jAl3djAT

2. **Transcription and enhancers**

https://youtu.be/ZlTRplvasQs?si=cCAH_8jvFUVmeXLM

VERSIONS	SLIDE #	BEFORE CORRECTION	AFTER CORRECTION
V1→ V2			
V2→V3			

امسح الرمز و شاركنا بأفكارك لتحسين أدائنا !!