# Regulating Fetal Hemoglobin and Erythropoiesis

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# Hemoglobinopathies

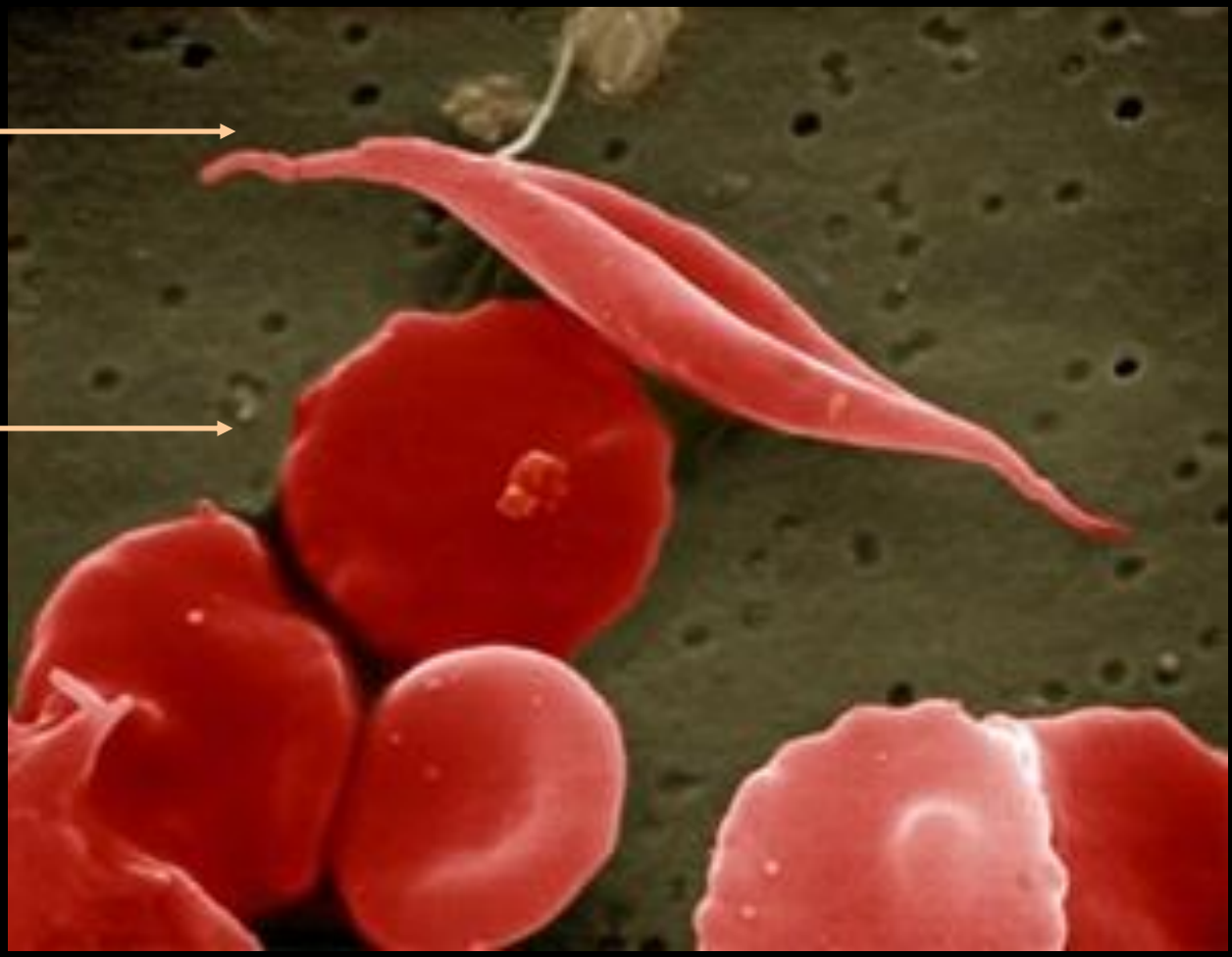
- Most common monogenic diseases
  - Sickle cell disease (SCD), Cooley's anemia, thalassemias
- Recessive disorders
- Symptoms range from mild anemia to death
- Currently treatments:
  - regular blood transfusion
  - hydroxyurea administration
- Gene therapy target – β-thalassemia

# Sickle Cell Disease

- Caused by a point mutation in the adult β-globin gene
  - Glu6 → Val
- Symptoms:

- low red blood cell counts (anemia)
- pain episodes/ strokes
- increased infections
- bone damage
- yellow eyes or jaundice
- early gallstones
- lung blockage
- kidney damage and loss of body water in urine
- painful erections in men (priapism)
- eye damage
- delayed growth

# Sickled Red Blood Cells



# Sickle Cell Disease Variants

- **HbSS or Sickle Cell Anemia (SCA/ SCD):** People who have this form of SCD inherit two sickle cell genes (“S”), one from each parent. This is usually the most severe form of the disease.

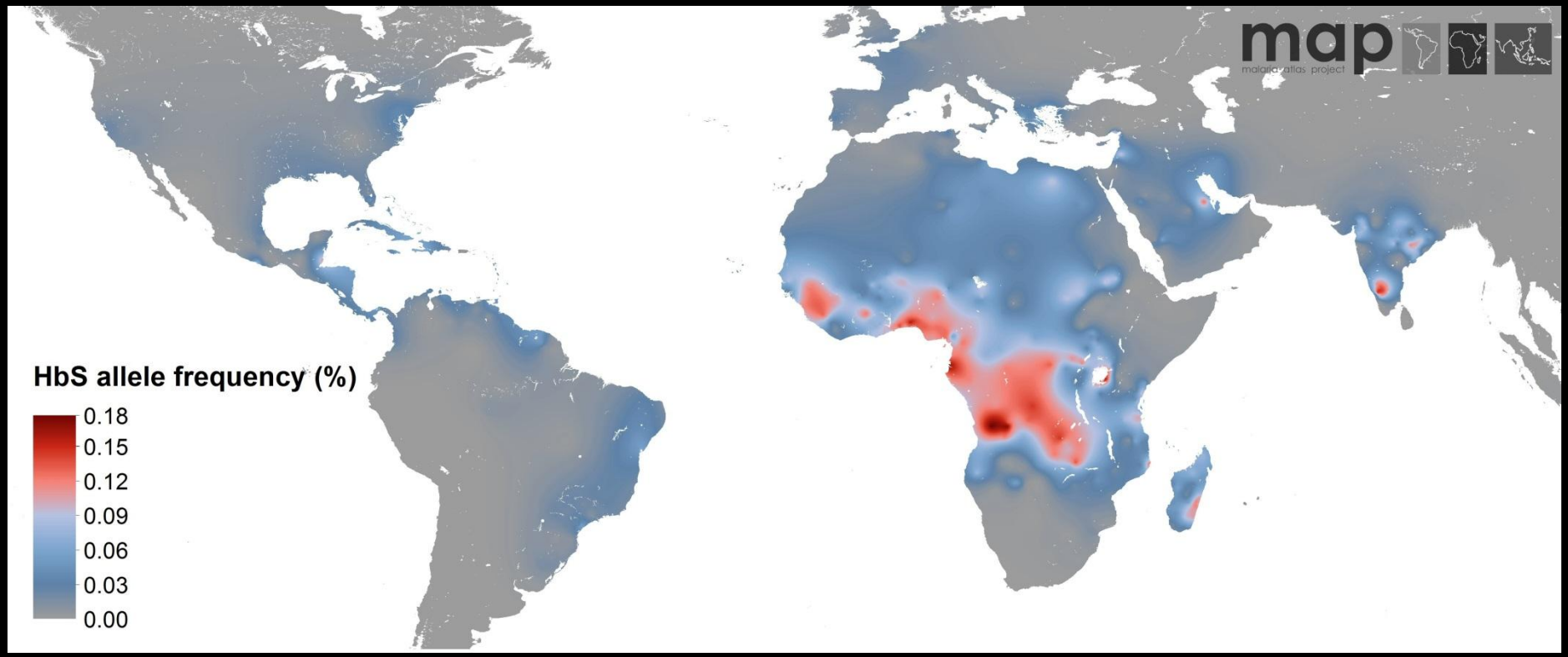
- **HbAS or Sickle Cell Trait (SCT):** People who have SCT inherit one sickle cell gene (“S”) from one parent and one normal gene (“A”) from the other parent. People with SCT usually do not have any of the signs of the disease and live a normal life, but they can pass the trait on to their children. Additionally, there are a few, uncommon health problems that may potentially be related to sickle cell trait.

# Sickle Cell Trait

Most people with SCT do not have any symptoms of SCD, although, people with SCT might experience complications of SCD, such as *pain crises*. The following conditions could be harmful for people with SCT:

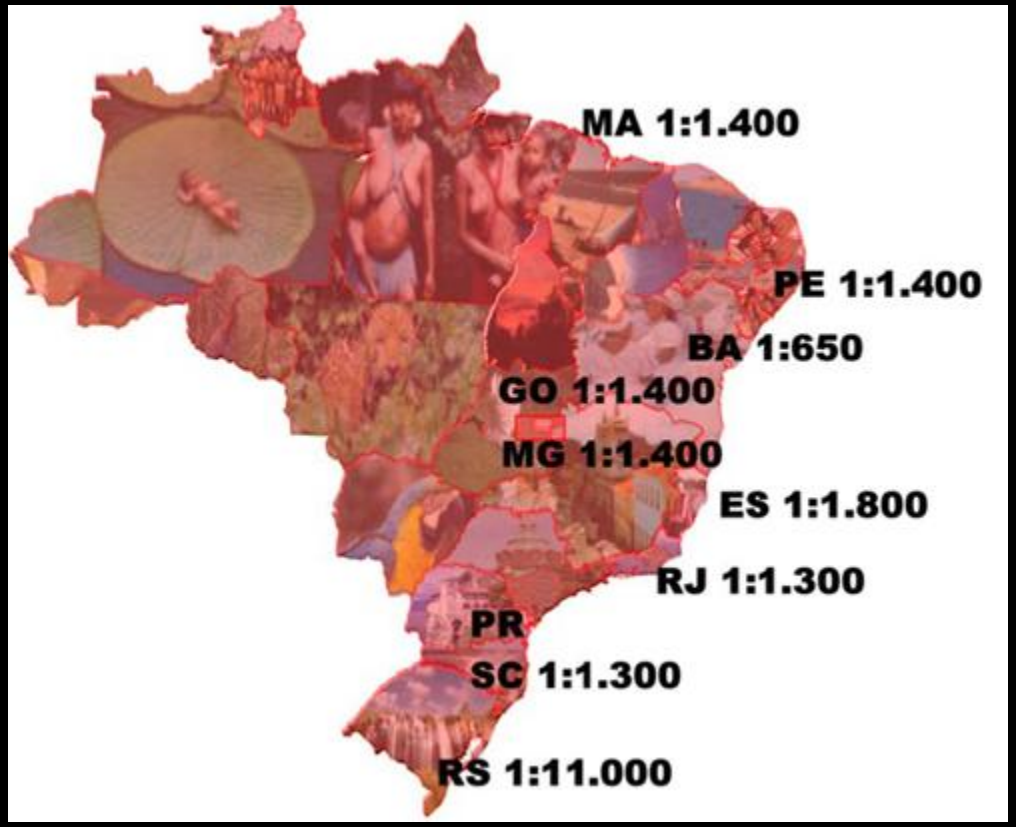
- Increased pressure in the atmosphere (scuba diving for example);
- Low oxygen levels in the air (surgery, mountain climbing, exercising extremely hard in military boot camp, or training for an athletic competition);
- Dehydration (for example, when one has too little water in the body);
- High altitudes (flying, mountain climbing, etc).

# Sickle Cell Disease in the World




http://www.ox.ac.uk/images/hi_res/15799_Sickle_cell_anaemia_MAP.jpg

# Sickle Cell Disease in Brazil



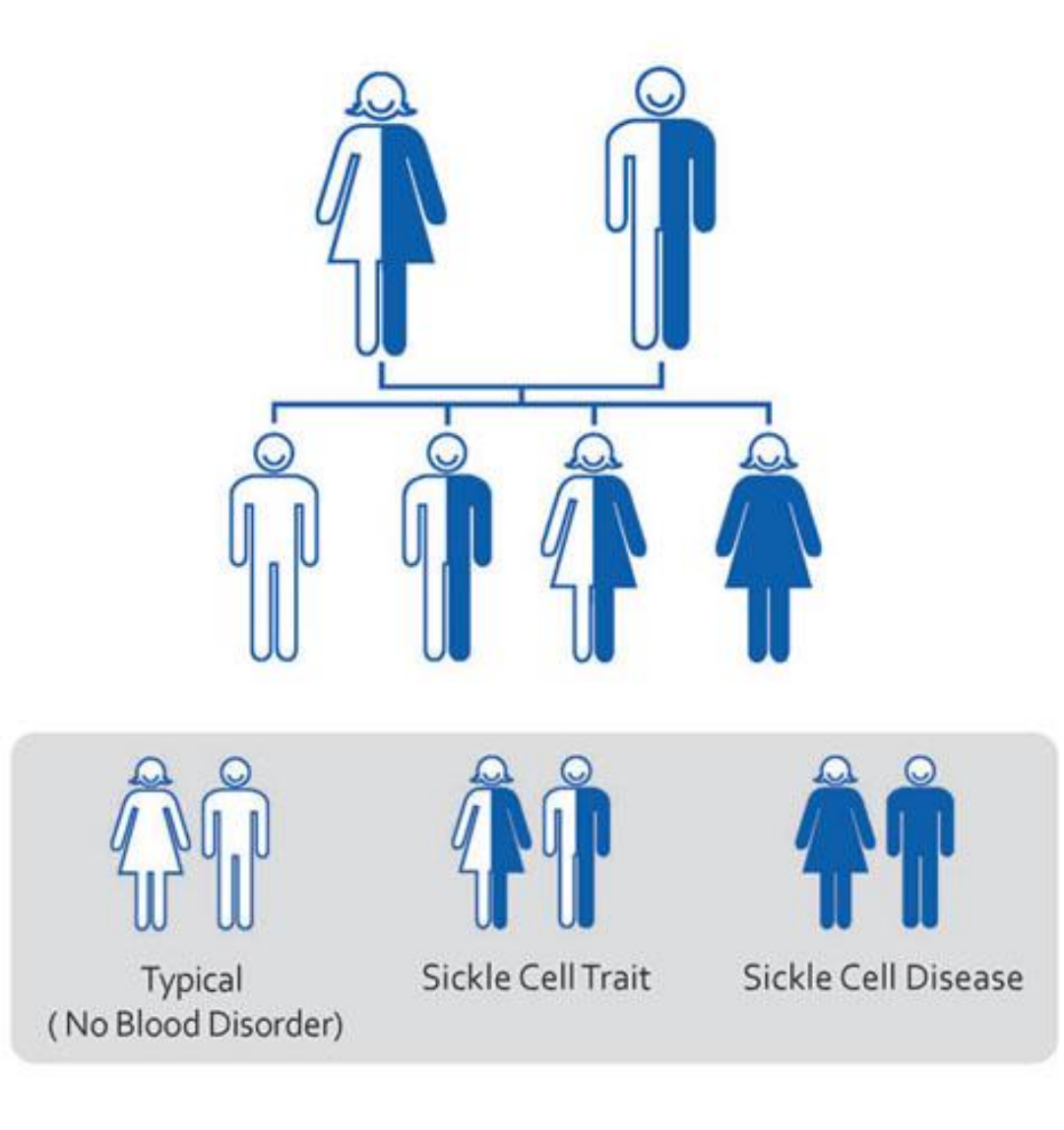

| State | Sickle Cell Trait |
|---|---|
| BA | 1:17 |
| RJ | 1:20 |
| PE and MA | 1:23 |
| GO | 1:25 |
| ES | 1:28 |
| MG | 1:30 |
| SP | 1:40 |
| PR, SC, RS | 1:65 |

# Sickle Cell in USA

- SCD affects 90,000 to 100,000 Americans.
- SCD occurs among about 1 out of every 500 African-American births.
- SCD occurs among about 1 out of every 36,000 Hispanic-American births.
- SCT occurs among about 1 in 12 African Americans.
- From 1989 through 1993, an average of 75,000 hospitalizations due to SCD occurred in the United States, costing approximately $475 million.
- During 2005, medical expenditures for children with SCD averaged $11,702 for children with Medicaid coverage and $14,772 for children with employer-sponsored insurance

http://www.cdc.gov/ncbddd/sicklecell/data.html

# Sickle Cell Trait

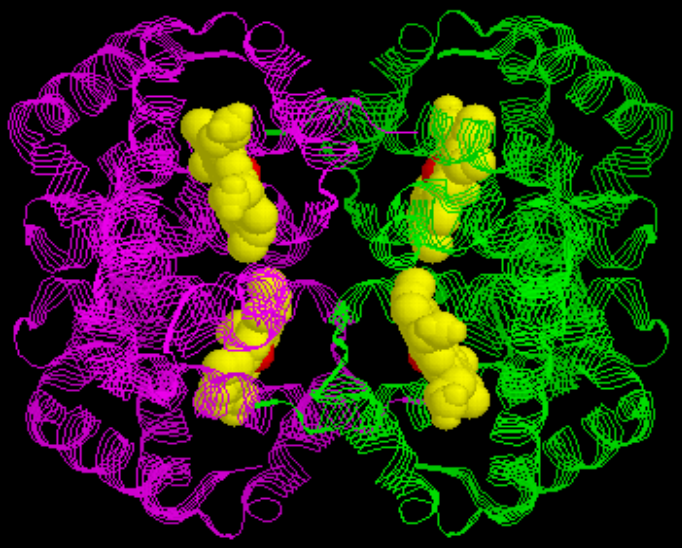



**Children and Sickle Cell Disease: Average Annual Medical Expenses by Insurance Type**

| | Medicaid | Private Insurance |
|---|---|---|
| Children with SCD* | $11,075 | $14,722 |
| Children without SCD | $1,706 | $1,293 |
| Expenses due to SCD | $9,369 | $13,469 |

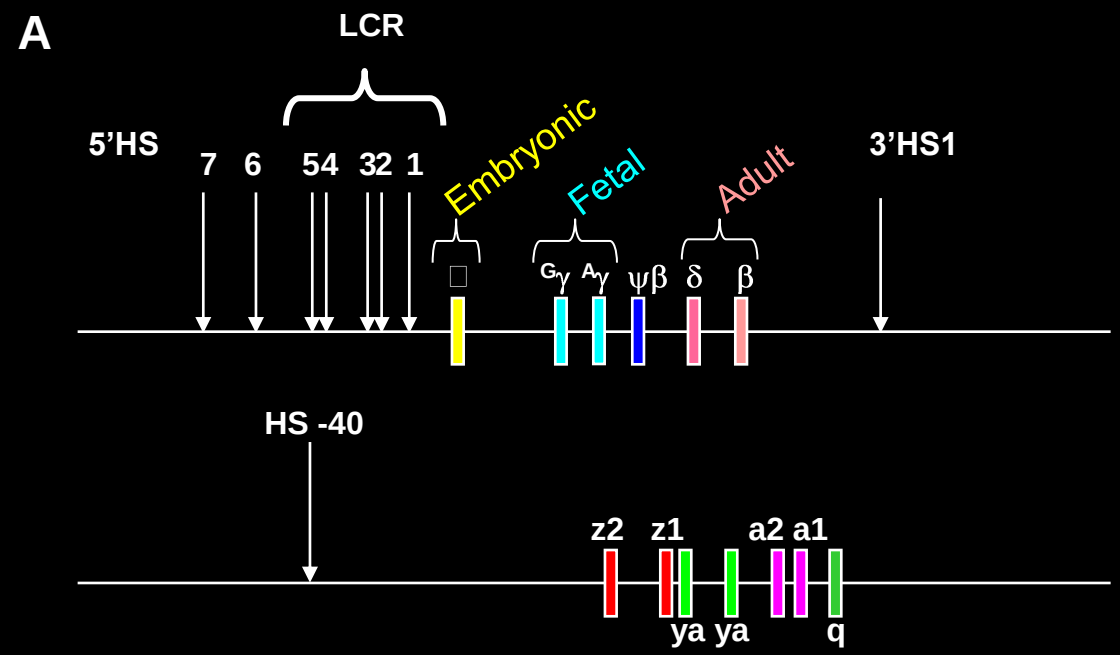
*All expenses are average per child

http://www.cdc.gov/ncbddd/sicklecell/data.html



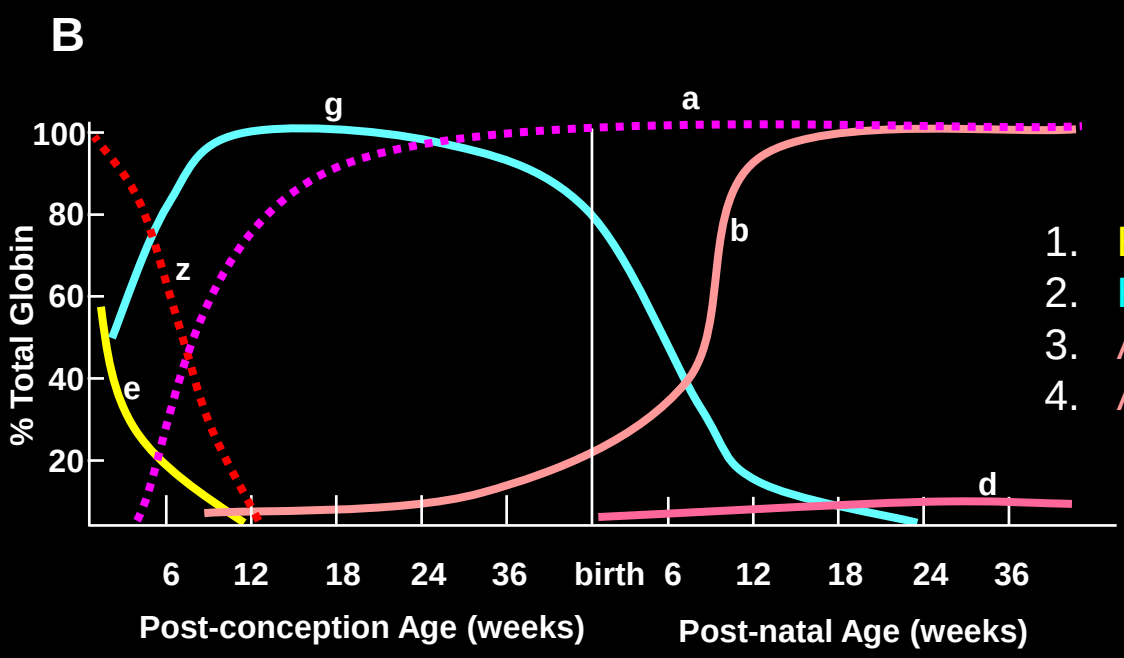
α-globin β-globin

- Composition of the hemoglobin tetramer varies during development. Different α- and β-like globin chains are incorporated into the molecule as ontogeny proceeds. This process is called globin gene switching.




## Sites of Hemoglobin Production

- Embryonic yolk sac
- Fetal liver
- Adult bone marrow



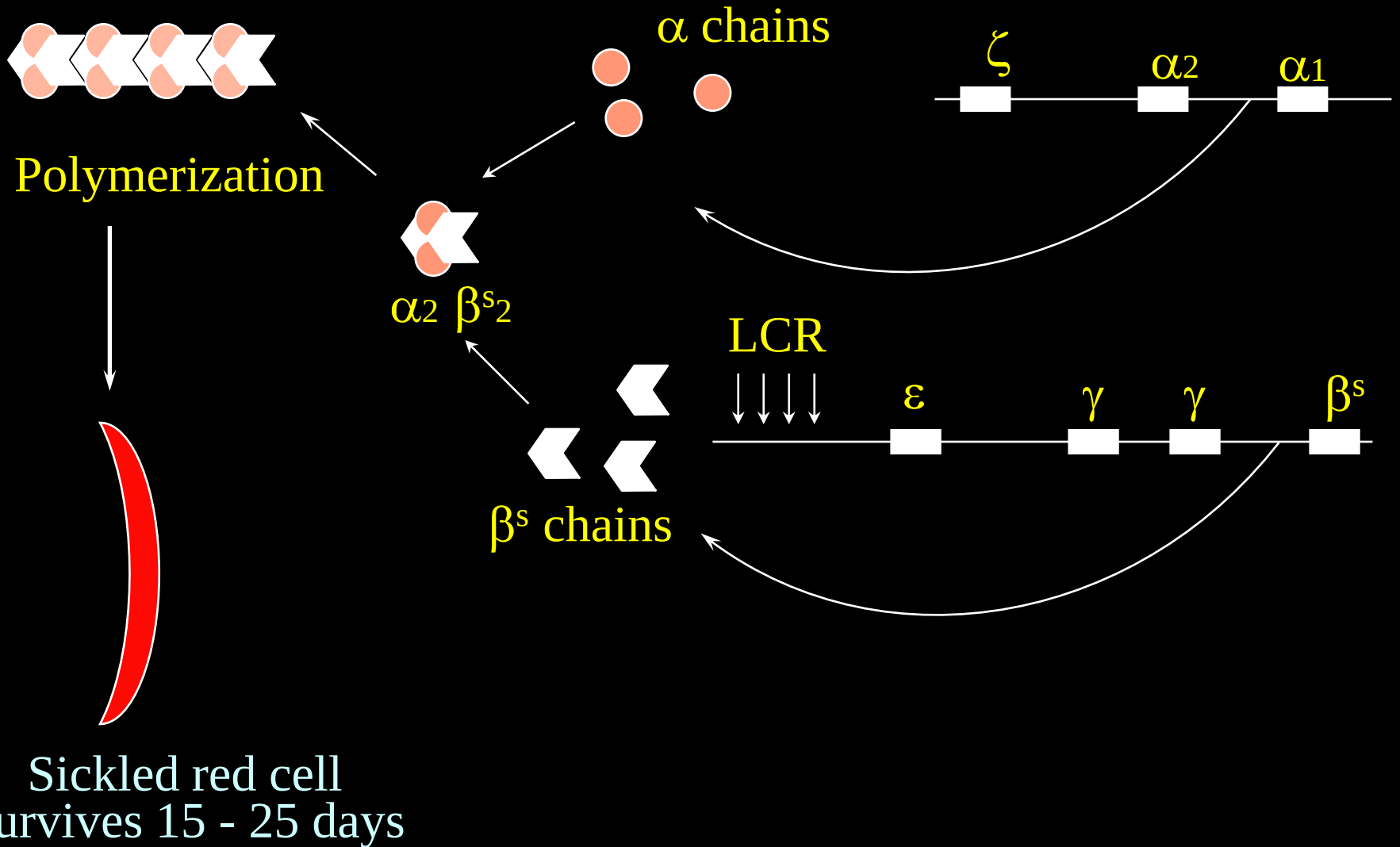

1. Embryonic Hemoglobin = HbE = $\zeta_2\varepsilon_2$
2. Fetal Hemoglobin = HbF = $\alpha_2\gamma_2$
3. Adult Hemoglobin = HbA = $\alpha_2\beta_2$
4. Adult Hemoglobin 2 = $HbA_2$= $\alpha_2\delta_2$

# Importance of Understanding Regulation of the β-globin Locus

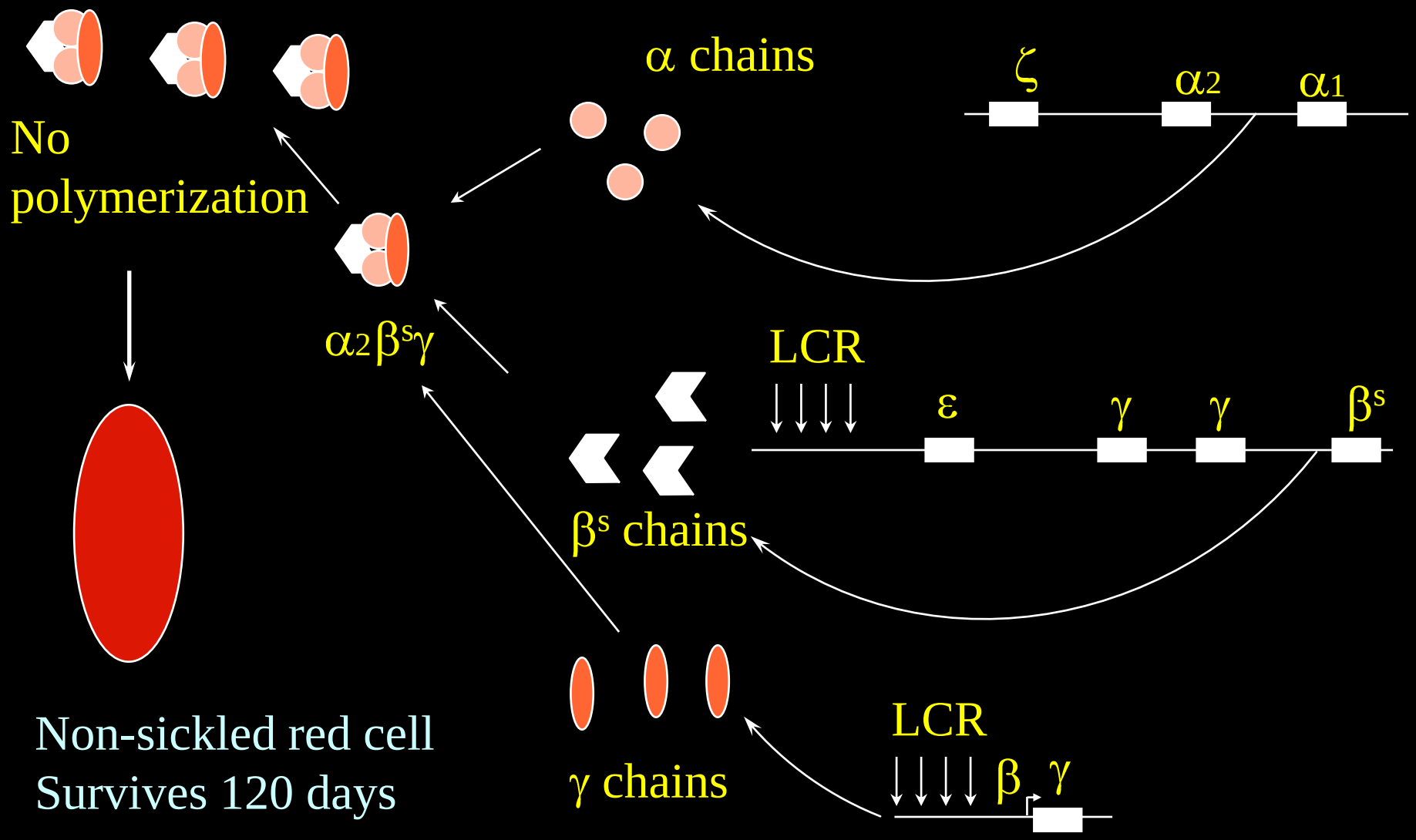
- Natural mutations exist that sustain fetal γ-globin expression during adulthood.
  - Hereditary persistence of fetal hemoglobin (HPFH)
  - Ameliorate SCD and thalassemia phenotypes

- Will lead to design of better drug and gene therapies.
  - Reactivation of γ-globin gene expression by drugs or gene therapy will cure SCD
  - Important to understand regulation of γ-globin to identify therapeutic targets.

# The Molecular Basis of Sickle Cell Anemia



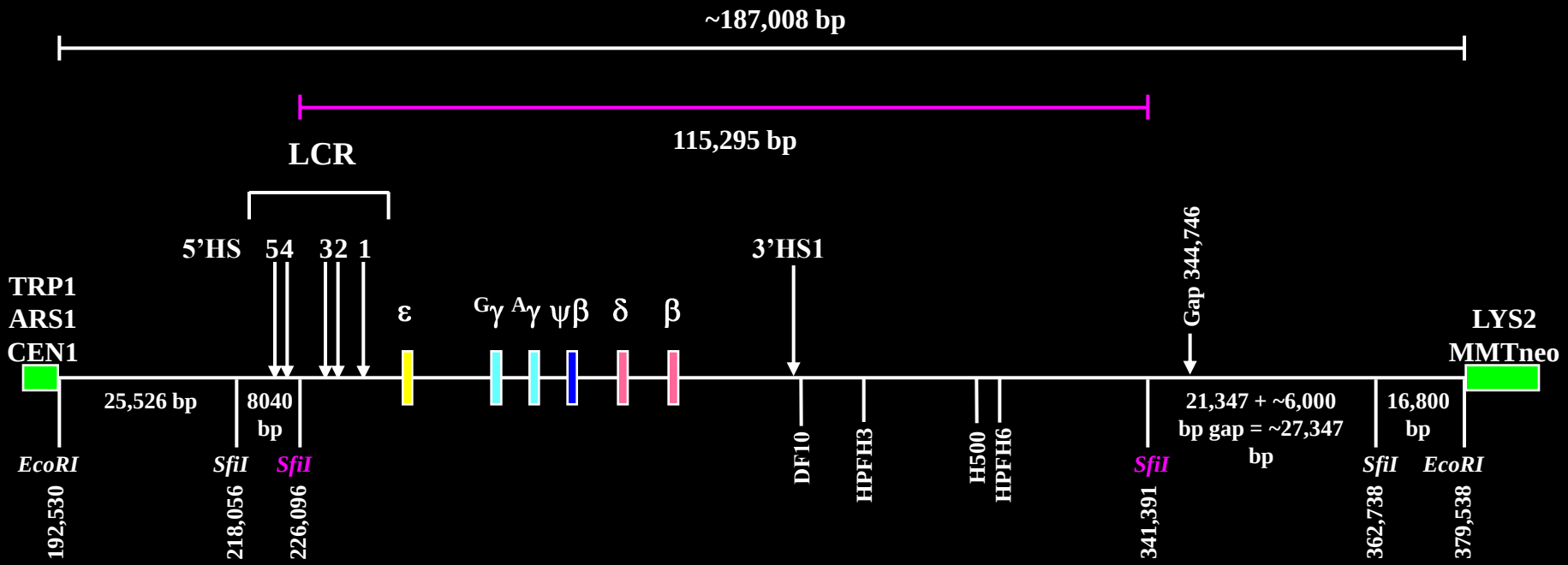
# Sickle Cell Anemia Therapy



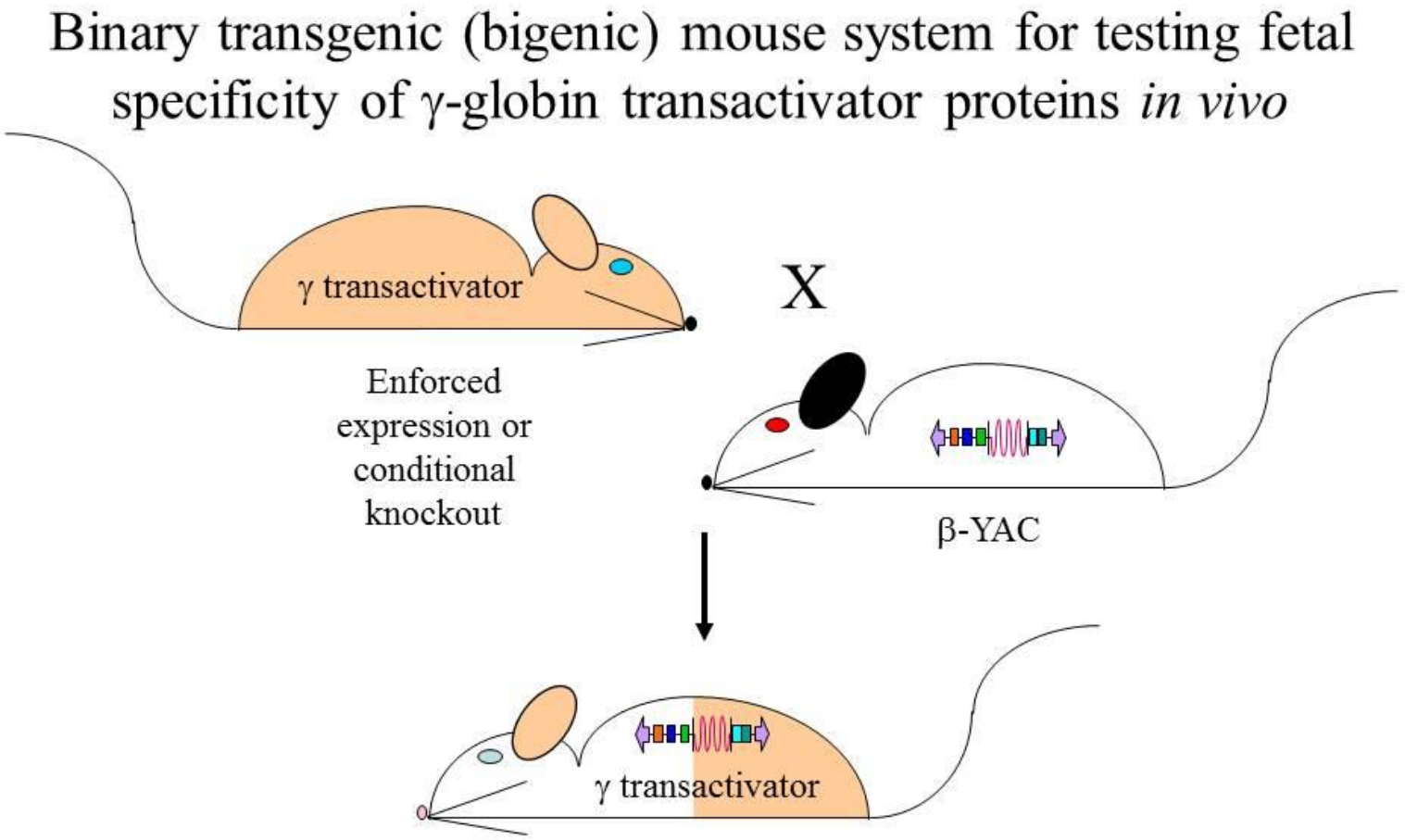
# Transactivate or Alleviate Repression of γ-globin (HbF)

- Transactivation: Mechanisms of fetal globin gene activation.
  - MTF-1 transcription factor
  - High-Throughput Screening of new drugs

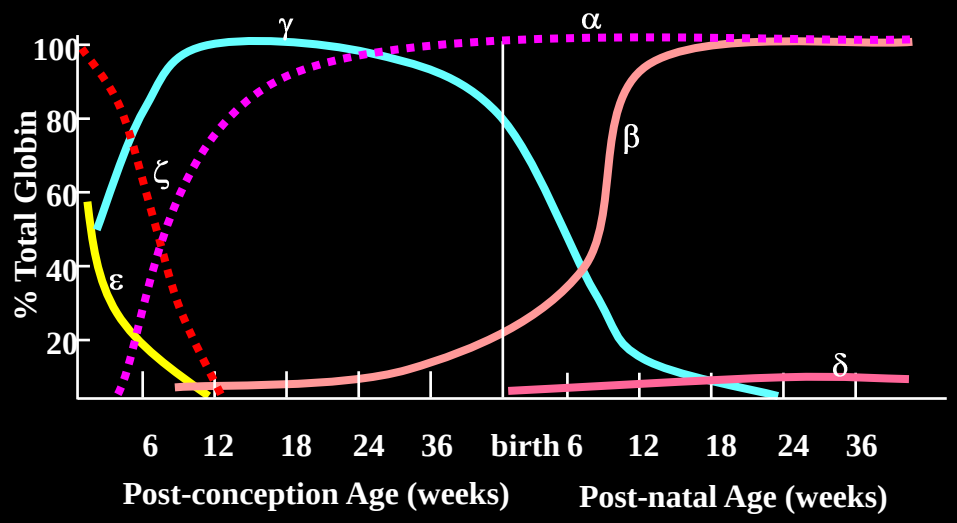
# Human β-globin Locus in a Yeast Artificial Chromosome (β-YAC)



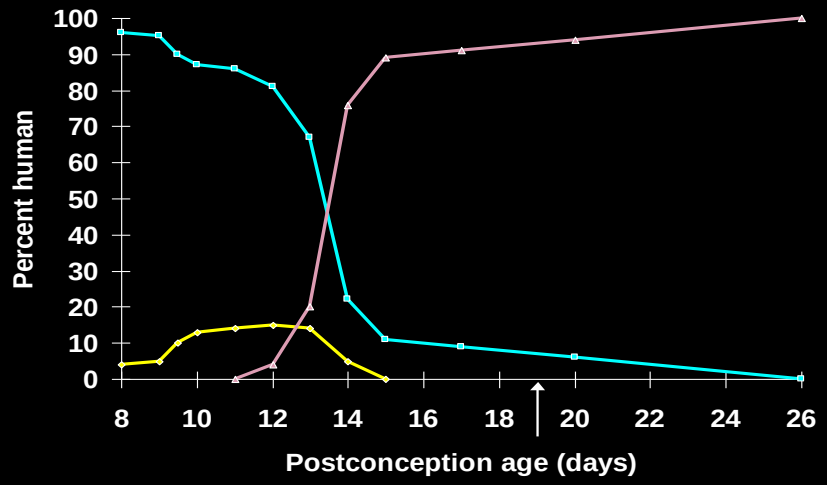
# TSPYL1 β-YAC Transgenic mice



# Hemoglobin Switching

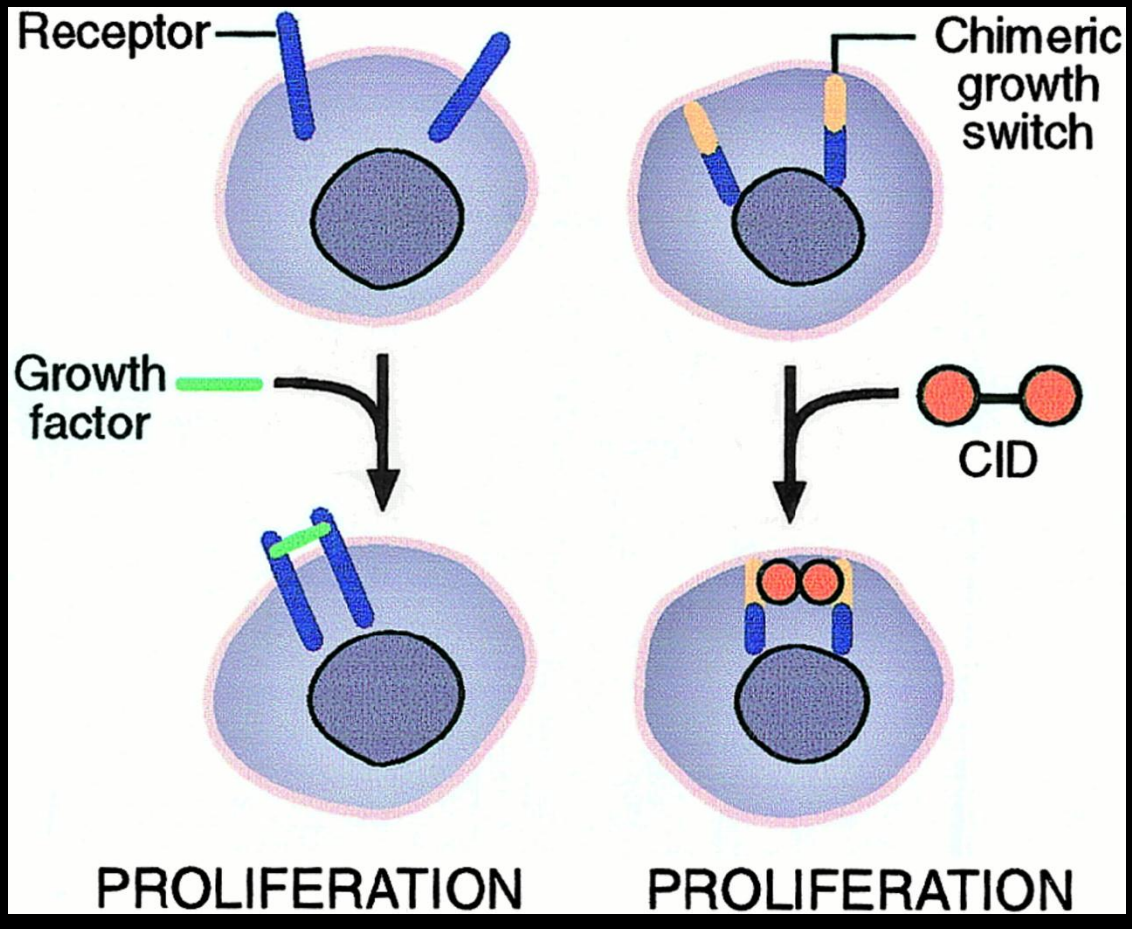



*Normal Human*




*Wild type β-YAC Mouse*

# CID Dependent Bone Marrow Cells

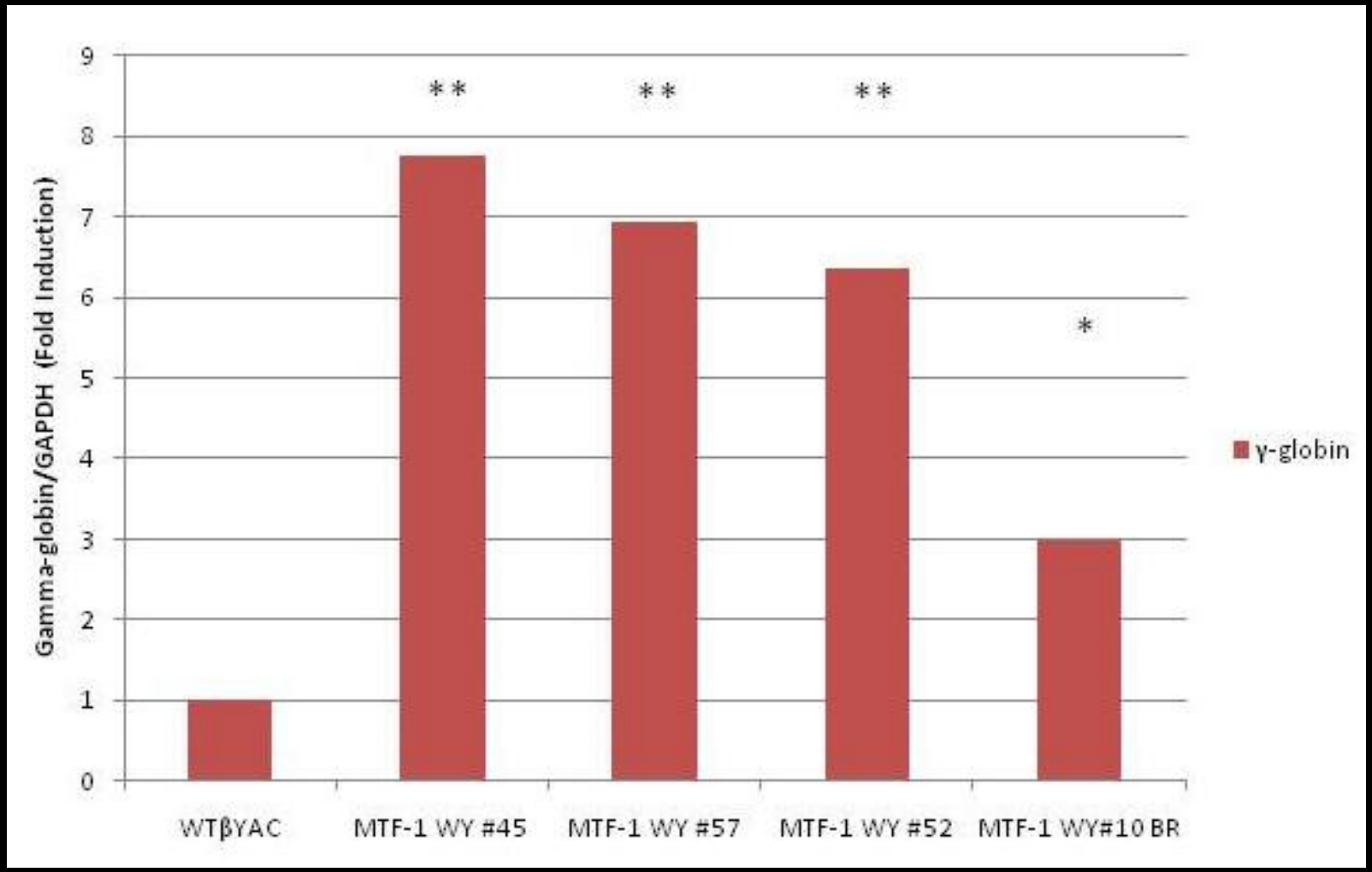



**Induction of dimerization by a CID**. A chimeric growth switch consisting of receptor sequences and a dimerization domain is activated on addition of a CID. The CID enforces dimerization by binding 2 dimerization domains on 2 neighboring molecules with a 1:2 stoichiometry. Dimerization causes signaling from the receptor sequences. This principle applies to the FK1012-FKBP system. The molecule shown also carries a myristylation domain derived from c-src for targeting to the inner cell membrane.

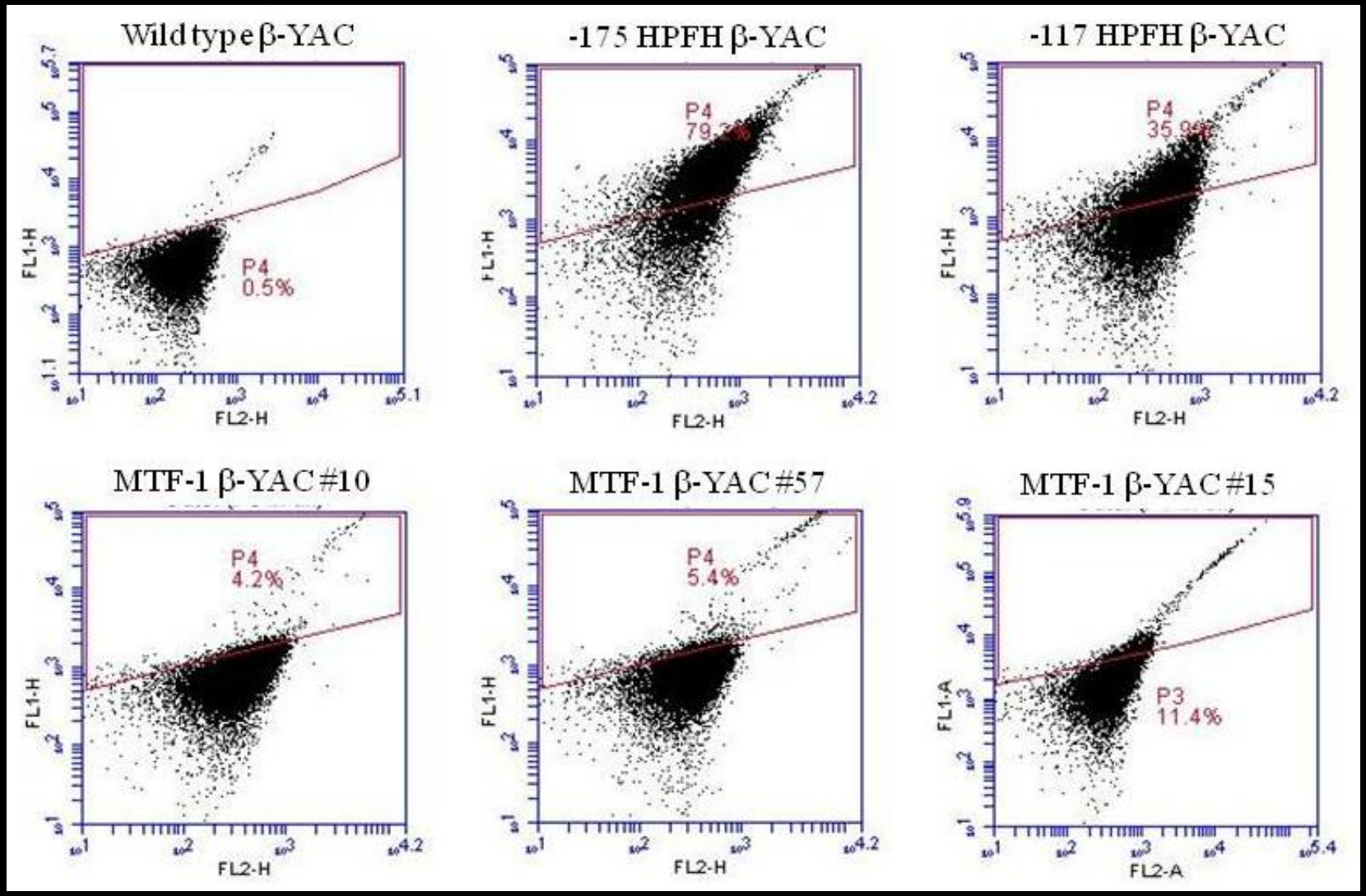
# MTF-1 and Sickle Cell Disease

- MTF-1 is a transcription factor and is expressed in fetal liver but not in the adult blood.
- Activates genes such as metallothioneins in response to zinc uptake.
- Sickle cell disease patients have low zinc levels. Zinc treatment reduces vaso-occlusion, hospitalization and transfusion requirements.

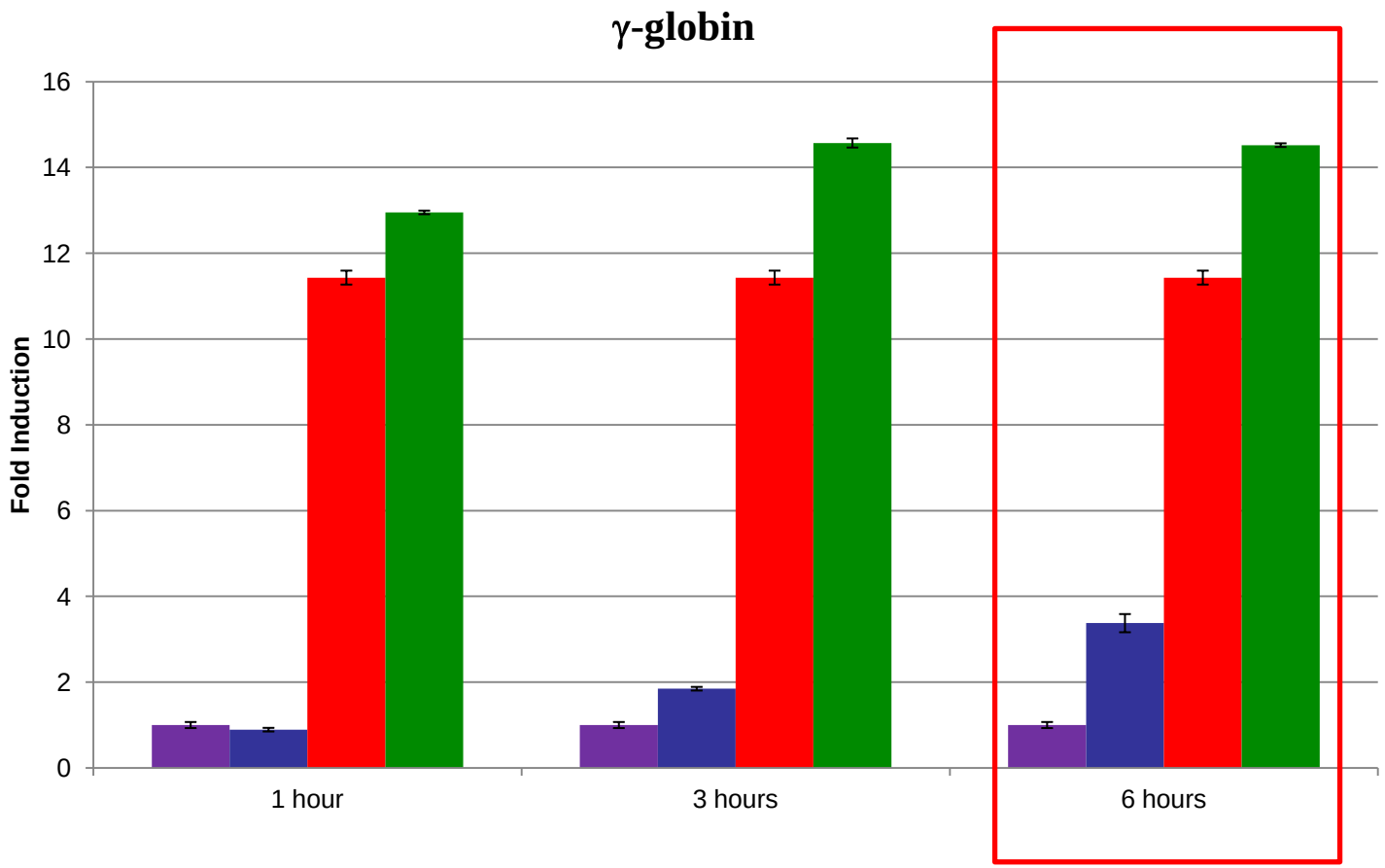
# γ-globin gene expression is increased in MTF-1 β-YAC bigenic lines



# Increased HbF in adult blood of MTF-1 β-YAC Bigenic Mice



# Zinc increases γ-globin in bone marrow cells of transgenic mice




β-YAC only: no Zn, purple; + Zn, blue
MTF-1 β-YAC: no Zn, red; + Zn, green

# New compounds to treat sickle cell disease

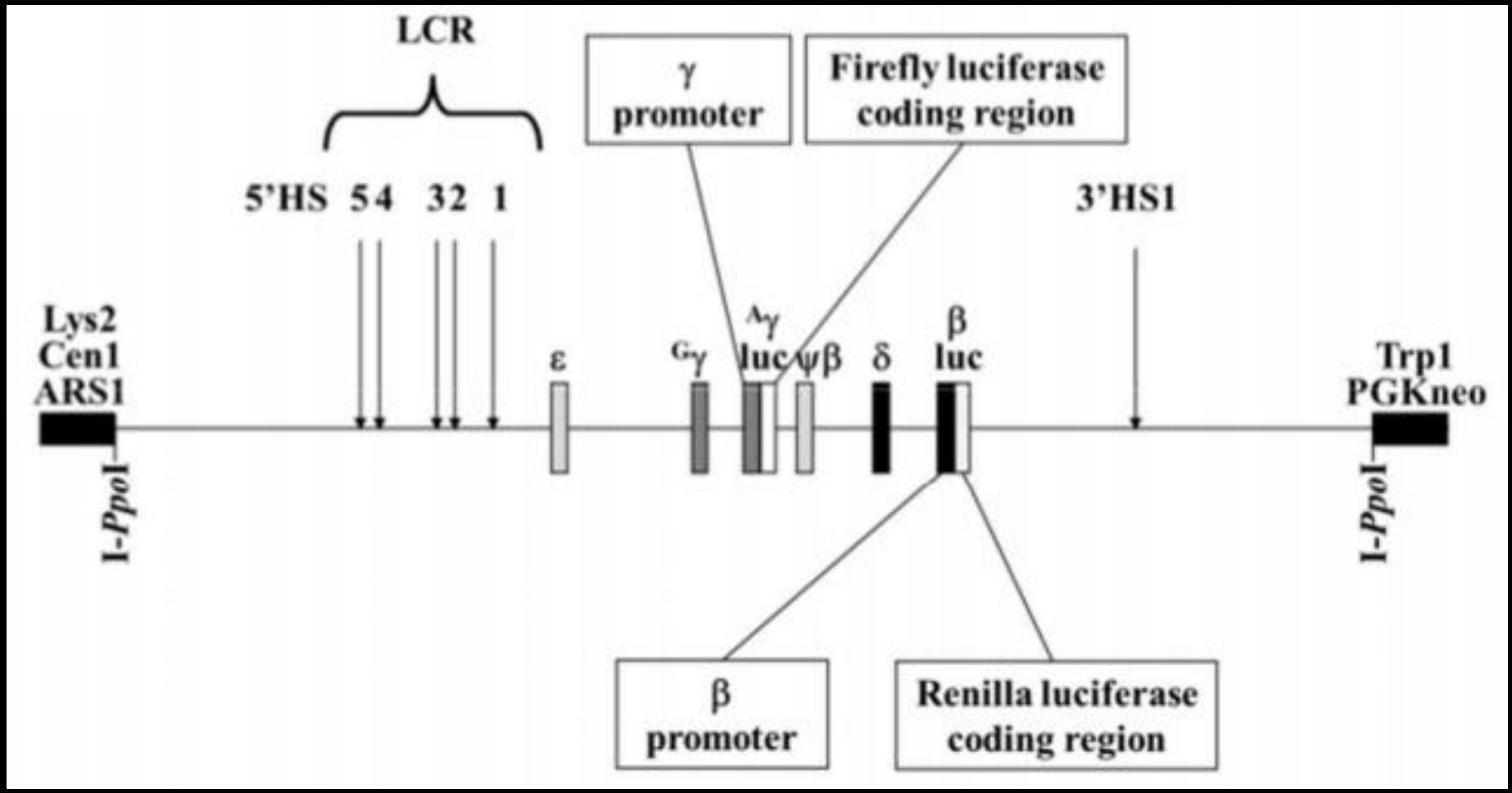
# Screening of New HbF Inducers

- Regular blood transfusion;

- Hydroxyurea:

  - Not all the patients respond to this treatment;
  - Side effect with long term use;
  - Need for new drugs.

# Screening of New HbF Inducers



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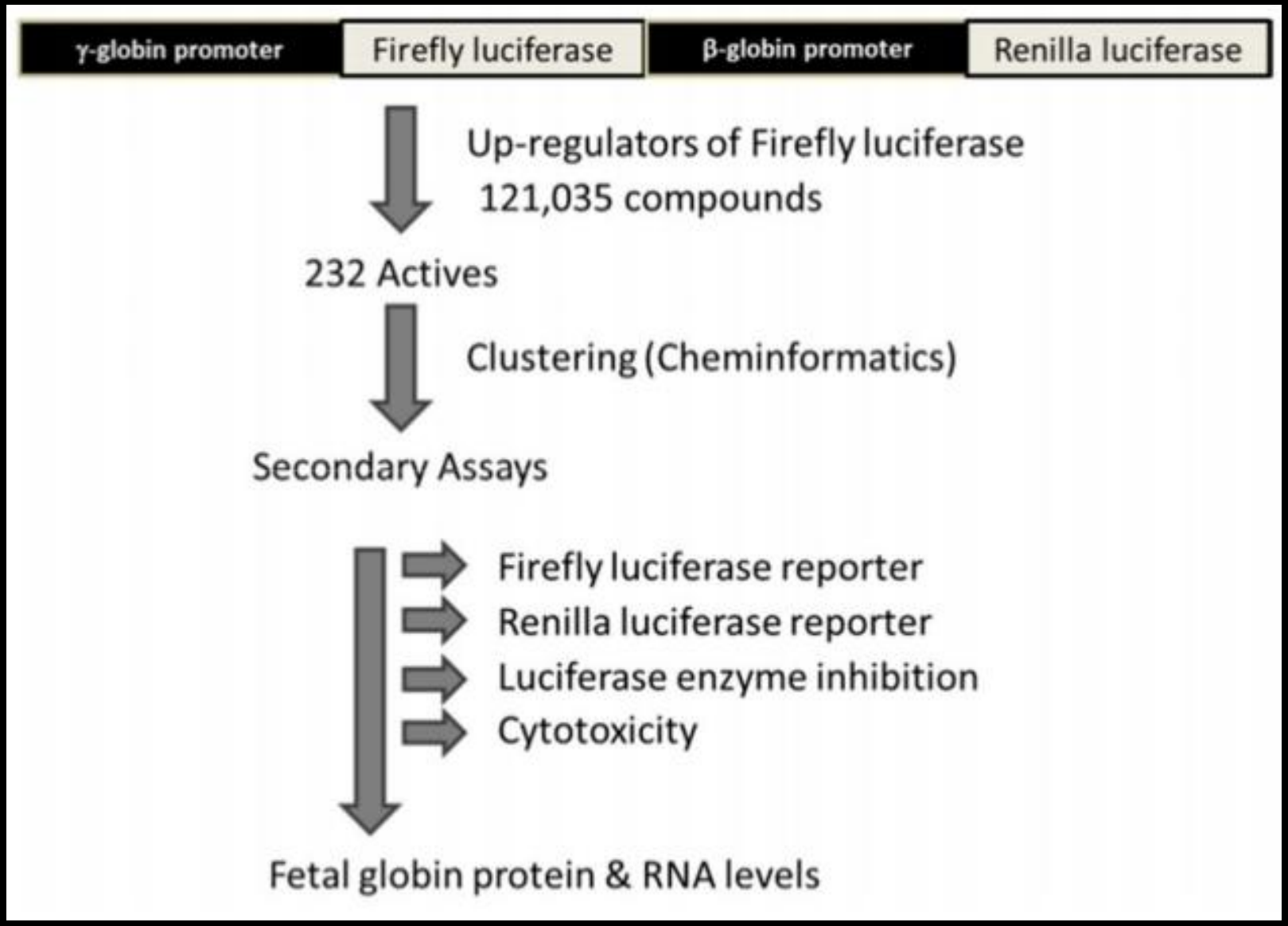

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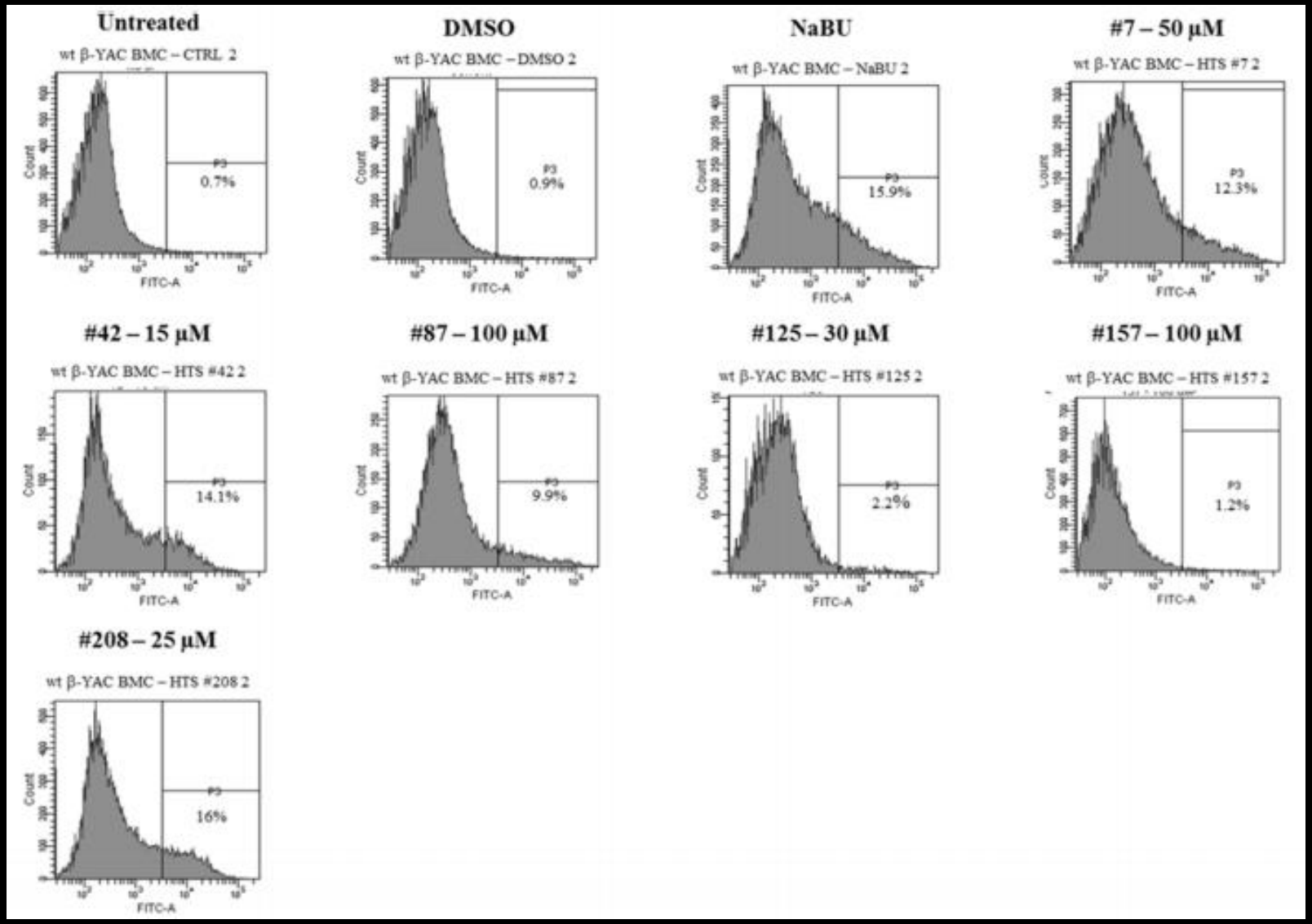

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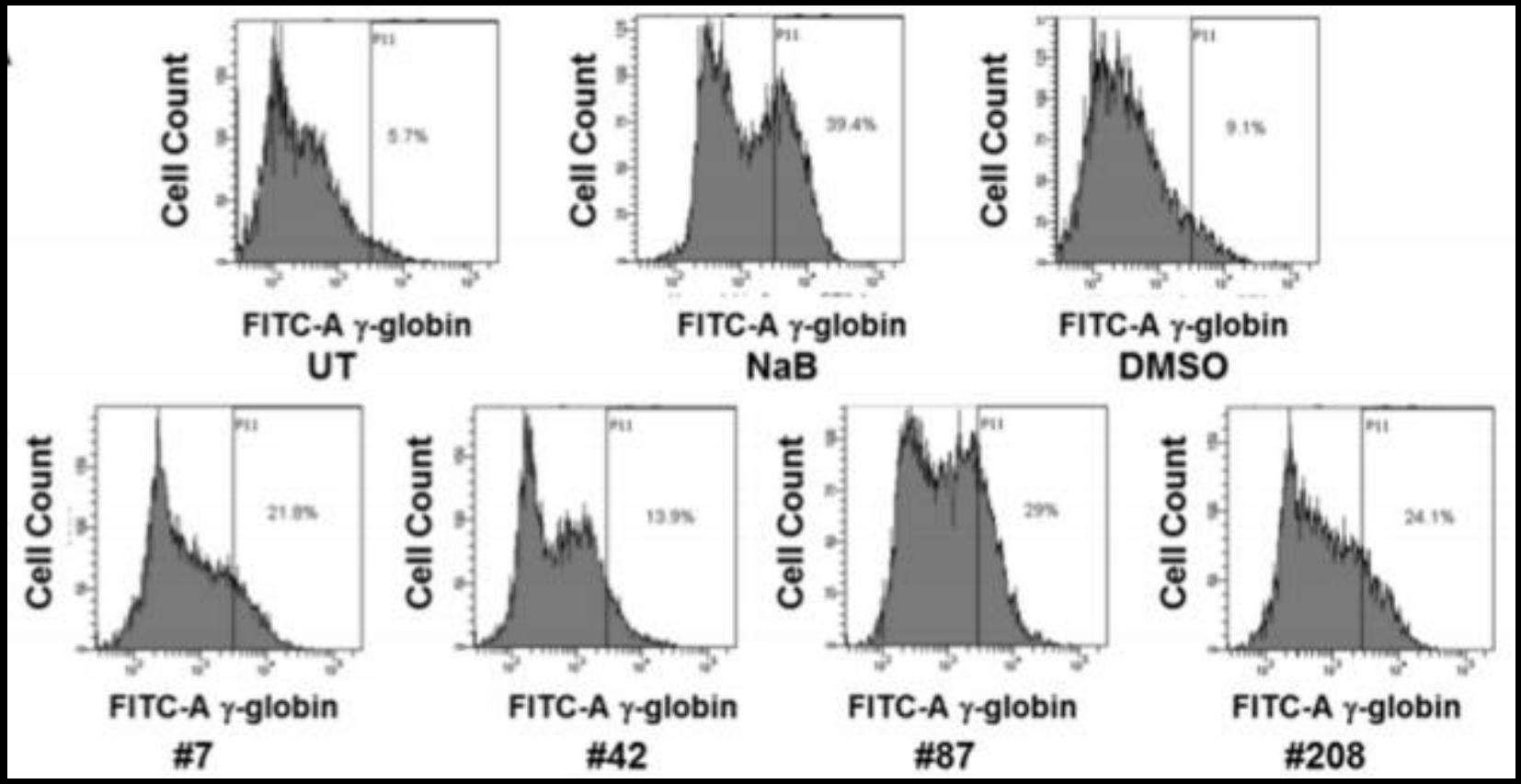

# Sickle Cell and Transfusion

# Sickle Cell and Transfusion

- Stroke - Chronic transfusions are used to prevent further strokes and brain damage, and they are usually given for many years.

- Acute chest syndrome (pneumonia) - Chronic transfusions may be used to prevent further episodes of acute chest syndrome, and they are usually given for one (1) or 2 years.

- Abnormal transcranial Doppler ultrasound (TCD) - TCD is a sound wave test that measures the blood fl ow in blood vessels of the brain. Very fast blood fl ow indicates that a child is at high risk for having a stroke. Chronic red blood cell transfusion has been proven to greatly decrease the risk for strokes in these patients.

# Sickle Cell and Transfusions

- The good
  - Increase O2 carrying capacity & organ perfusion
  - Decrease percentage of HbS
  - Prevent organ damage

- The bad
  - Infection
  - Volume overload
  - Transfusion reactions
  - Alloimmunization
  - Iron overload
  - Hyperviscosity

# Induction of HbF in adulthood will cure sickle cell disease!

# Acknowledgments


- ✓ Dr. Ken Peterson
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