# CHAPTER 1

## INTRODUCTION

A hemoglobin molecule is consisted of heme and globin moieties. One globin protion consists of four polypeptide chains, each chain being attached to one heme. The amino acid sequences of each globin are specific i.e. 141 amino acid for the α globin chain and 146 amino acid for the β, δ and γ globin chains.

There are three normal adult hemoglobin: hemoglobin A (HbA), hemoglobin F (HbF) and hemoglobin $A_2$ ($HbA_2$). Hemoglobin A which has two α-chains and two β-chains($\alpha_2\ \beta_2$) is the major component and present at about 97% of the total adult hemoglobin. Hemoglobin F which has two α-chains and two γ-chains ($\alpha_2\ \gamma_2$) is the major hemoglobin in the fetal life. It is rapidly decreased in postneonatal period and present in less than 1% at one year of age. Hemoglobin $A_2$, is consisted of two α-chains and two δ-chains ($\alpha_2\ \delta_2$) is the minor hemoglobin in adult life.

Hemoglobinopathies can be classified into two groups. One consists of hemoglobin varients in which the amino acid sequences of each globin component are changed from the normal one. For example Hb Constant Spring, the elongated α globin chain, occurs from the mutation at the terminator codon of $\alpha_2$ globin gene. The other group is the thalassemia, which is characterized by a reduced rate of synthesis of one or more of the globin chains leading to the imbalanced globin chain production (Bunn et al, 1977).

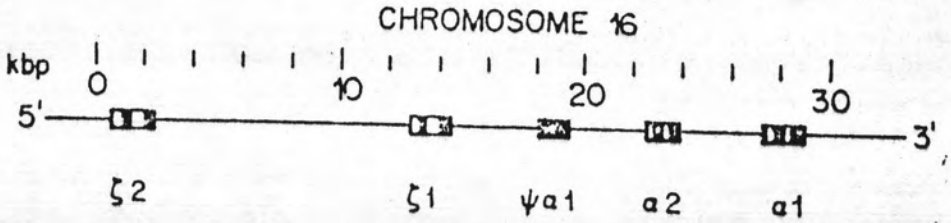


Figure 1. The α-globin gene complex. The arrangement of α-like globin loci on chromosome 16. The embryonic ζ-chain loci are 5' to the duplicated α-loci. An α-pseudogene lies between the $\alpha_2$ and $\zeta_1$ loci.

The common groups of thalassemias are (classified into 2 groups, namely) α and β thalassemia depending on whether α or β chain synthesis is defective. (Beale and Lehmann, 1965). The β-thalassemia is characterized by a reduced rate of β-chain production, results in excessive α-chains which are unstable and precipitate in the red cell precursors. The elevated $HbA_2$ and or increased HbF are also detected due to the compensation by δ- and γ-chain production (Kunkel et al, 1957). The α-thalassemia, the deficient α globin chain, causes the excess of γ and β-chains and these aggregate to produce the abnormal tetramers: Hbs Bart's ($\gamma_4$) and H ($\beta_4$) respectively. These abnormal tetramers do not precipitate in the bone marrow, but in red cells, leading to the production of the intracellular inclusion bodies (Wasi et al, 1974).

In Thailand, thalassemia and abnormal hemoglobins are highly prevalent. An incidence of α-thalassemias, based on cord blood studies, is about 20% of the population; 10% α-$thal_1$, and 10% α-$thal_2$ (Pootrakul et al, 1970). Hb Constant spring is found in more than 4% of the population. In addition the incidence of β-thalassemia and abnormal HbE is 5-9% and 13% respectively (Wasi et al, 1974).

Human α-like globin gene cluster

The α and α-like globin genes are arranged on the chromosome 16 in the order of their expression during development: 5' - $\zeta_2$ - $\psi\zeta$ - $\psi\alpha_1$ - $\alpha_2$ - $\alpha_1$ - 3' (Orkin et al, 1978; Lauer et al, 1980). Human α globin

chains are encoded by the two adjacent genes, $\alpha_1$ and $\alpha_2$. The $\zeta 2$ gene encoded for the embryonic $\zeta$ globin chain. The $\psi\alpha_1$ and $\psi\zeta$ are pseudogenes which do not encode any of the α-like globin polypeptides. (Figure 1).

Comparison of the $\alpha_1$ and $\alpha_2$ coding sequences has revealed no differences in the coding region. However, the 110 base pair 3'-non coding region contains 18 base differences and a single base insertion/deletion (Michelson et al, 1980). Relative levels of $\alpha_1$ and $\alpha_2$ globin mRNA have been determined. It is found that there is a 2 to 3 fold excess of $\alpha_2$ over $\alpha_1$ globin mRNA (Leibhaber and Kan 1982; Orkin and Goff 1981). However, the levels of their translated α globin protein products are equal. This is because the translational efficiency of $\alpha_1$ globin mRNA is higher than the $\alpha_2$ globin mRNA (Leibhaber and kan, 1982). The 3' nontranslated region of the α globin gene may play a significant role in translational control.

α-thalassemia

There are normally four α globin genes per diploid genome. Loss or dysfunction of one or more of these genes leads to deficient α-globin production and result in thalassemia. There are at least two different types of α-thalassemia genes designated as α-thalassemia 1 ($\alpha$-thal$_1$) and α-thalassemia 2 ($\alpha$-thal$_2$)

α-thalassemia 1 ($\alpha$-thal$_1$):

$\alpha$-thal$_1$ is caused by a deletion of both α-globin genes on one chromosome leading to complete suppression of α globin chain synthesis (Wasi et al, 1974).

α-thalassemia 2 ($\alpha$-thal$_2$) :

$\alpha$-thal$_2$ occurs from a deletion of one of the two α genes on one chromosome resulting in a decreased α globin chain production (Wasi et al, 1974 ; Dozy et al, 1979). Two different deletions that are responsible for the α-thalassemia 2 haplotypes have been characterized. In the so called left-ward deletion α-thalassemia 2 genotype, a 4.2 kb deletion involves the $\alpha_2$ globin gene (Embury et al, 1980, Higgs et al, 1980). In the right-ward deletion α-thalassemia 2 genotype, a 3.7 kb fragment involving the region bridging the $\alpha_2$ globin and $\alpha_1$ globin gene is deleted leading to the $\alpha_2$ $\alpha_1$ fusion gene (Orkin et al, 1979; Phillips et al, 1980). Both of these deletions are presumably the result of apparent nonhomologous crossover events, either inter or intra chromosomal in nature. However, the right-ward deletion type is more common than the other.

However, α-thalassemia is not solely a result of loss α globin genes. Nondeletion defects in which the α-globin gene complex appear to be intact is also described (Whitetaw et al, 1980; Orkin et al, 1979; Kan et al, 1977; Pressley et al, 1980). For example, Hb Constant Spring which is the abnormal α globin chain has the low level of $\alpha^{cs}$ synthesis resulting in α-thalassemia 2 effect. This $\alpha^{cs}$-variant occurs from a TAA ⟶ CAA mutation at the termination codon of the $\alpha_2$ globin gene leading to the elongated α globin chain (Bunn et al, 1977; Milner et al, 1971; Clegg et al, 1971; Wealtherall & Clegg, 1975; Proudfoot et al, 1977). The low level of $\alpha^{cs}$ synthesis is associated with instability of $\alpha^{cs}$ mRNA (Hunt et al, 1980).

Hemoglobin H disease.

Hemoglobin H disease is a mild to moderately severe α-thalassemia disease resulting from a defective α globin chain synthesis. The excessive β chains polymerize to from $\beta_4$ tetramers or Hb H which are unstable, precipitate and cause a shortened red cell survival (Weatherall et al, 1972). Hemoglobin H desease most frequently occurs from the interaction of $\alpha\text{-thal}_1$ and $\alpha\text{-thal}_2$ and therefore it is usually found in populations where both of these haplotypes are common. In Thailand where Hb CS is also common, Hb H disease occurs from the following genotypes:

a) $\alpha\text{-thal}_1/\alpha\text{-thal}_2$ (4.2 kb). Only the $\alpha_1$ globin gene is left functional on the homologous chromosome.

b) $\alpha\text{-thal}_1/\alpha\text{-thal}_2$ (3.7 kb). The functioned α globin gene is the hybrid gene ($\alpha_2\ \alpha_1$ gene)

c) $\alpha\text{-thal}_1$/Hb Constant Spring $\alpha^{cs}$ gene produced instability of α mRNA therefore the only functional globin gene is $\alpha_1$ gene.

About half of the Hb H patients have the genotype $\alpha\text{-thal}_1/\alpha\text{-thal}_2$ (Wasi et al, 1974) and most of them have the rightward deletion $\alpha\text{-thal}_2$ hyplotype (Winichagoon, 1981). Evidences from clinical and hematological studies showed that $\alpha\text{-thal}_1$/Hb CS is more severe than $\alpha\text{-thal}_1/\alpha\text{-thal}_2$ (Fucharoen et al, 1981).

Since the $\alpha_2$ globin gene synthesizes more α globin mRNA than the $\alpha_1$ globin gene, it is expected that $\alpha\text{-thal}_1$/Hb CS which only the

$\alpha_1$ gene is functional should have less α globin mRNA than the α-thal$_1$/α-thal$_2$ (3.7 kb). This should lead to the more imbalanced α/β globin synthesis ratio and consequently, severe clinical presentation in α-thal$_1$/Hb CS. However, the previous study showed no clear cut difference in the relative amounts of α and β mRNA between α-thal$_1$/Hb CS and α-thal$_1$/α-thal$_2$ (Hunt et al, 1980)

The purpose of this study is to determine the α globin mRNA produced from α-thal$_1$/Hb CS and α-thal$_1$/α-thal$_2$. The amount of the α globin mRNA in the two genotypes will also be compared.