[Frontiers in Bioscience 15, 1-11, January 1, 2010]

Alpha-hemoglobin stabilizing protein: molecular function and clinical correlation

Chairat Turbpaiboon, Prapon Wilairat

Department of Biochemistry, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand

TABLE OF CONTENTS

1. ABSTRACT

The discovery of alpha-hemoglobin stabilizing protein (AHSP), a chaperone for free alpha-hemoglobin (alpha-Hb), has provided a satisfactory solution to the perplexing problem of balanced globin levels for Hb production in erythroid cells in the face of a two-fold excess of alpha-globin to beta-globin gene dosage. Unmatched alpha-Hb is unstable and precipitates onto membranes, where the released heme exerts oxidative damages resulting in ineffective erythropoiesis and hemolytic anemia, the underlying causes of pathology in the hereditary anemia of beta-thalassemia. The interaction of alpha-Hb with AHSP involves surfaces normally employed in binding to beta-Hb. However, a conformational change to the AHSP-bound alpha-Hb results in an oxidized heme, but in a pocket that is now less exposed to the outside environment, thereby protecting against both peroxide-induced heme loss and iron-induced redox reaction. Studies in both mice and humans indicate that reduction in AHSP can result in hematological pathology. Conversely, alpha-Hb variants that are compromised in their ability to bind with AHSP produce beta-thalassemia-like symptoms. Disease conditions like some forms of thalassemia that are directly associated with AHSP structural and/or functional defects can now be included within the category of chaperonopathies.

2. INTRODUCTION

Adult hemoglobin (Hb A) is a heterotetramer composed of two different pairs of alpha- and beta-globin chains. However, the gene encoding alpha-globin, located in the short arm of chromosome 16 is duplicated (four copies in a diploid cell), whereas that for beta-globin exists as a single allele (two copies in diploid state) situated in the short arm of chromosome 11. An early explanation for the discrepancy between equality in amounts of the two types of globin chains and difference in gene dosage was that the rate of translation of alpha-globin mRNA is regulated in the red blood cell (rabbit reticulocyte) to be half of that of beta-globin mRNA (1). The study could be faulted as it was conducted in cell-free lysate under non physiological conditions. More recent studies employing quantitative polymerase chain reaction to measure the relative amounts of globin mRNA species in human reticulocytes indicate amounts more reflective of their gene copy numbers, namely, two-fold increase of alpha- over beta-globin mRNA (2, 3).

An imbalance of globin chains is the hallmark of thalassemia, a hereditary anemia caused by mutations in the human globin gene complex resulting in quantitative defect of globin chain synthesis (4). In alpha-thalassemia, deletion of three out of the four copies of the duplicated

alpha-globin genes results in the production of excess unmatched beta-globin chains that form relatively less stable homotetrameric beta-hemoglobin, Hb H. However, in beta-thalassemia (caused by a variety of point mutations in beta-globin gene) excess unmatched alpha-globin chains are unable to form stable homotetramers and they bind to surface of the red cell cytoplasmic and plasma membranes, where heme released from denatured alpha-hemoglobin (alpha-Hb) generates oxidative damage, resulting in apoptosis of bone marrow erythroid precursor cells (causing ineffective erythropoiesis) and reduced lifespan of circulating thalassemic erythrocytes (hemolytic anemia) (4).

According to the above scenario, normal red blood cells should manifest a beta-thalassemia phenotype. How do such cells protect themselves against the cytotoxic effects of unmatched alpha-Hb chains? The unexpected answer came from the recent identification of a chaperone for alpha-Hb, known as alpha-Hb stabilizing protein (AHSP), which has the ability to bind with monomeric alpha-Hb until it can be transferred to a beta-Hb subunit to form a heterodimer, which then binds with another heterodimer to form stable tetrameric Hb (See Figure 1 for a schematic diagram of AHSP function under normal (Wild Type) condition).

AHSP was first identified in 2002 by Weiss and colleagues (5) using subtractive hybridization to search for genes whose expression is induced by GATA-1, an essential erythroid transcription factor. AHSP had been designated previously as erythroid differentiation-related factor (EDRF), an erythroid-specific molecular marker for detecting transmissible spongiform encephalopathies (TSE) (6). Interaction between AHSP and prion protein (PrP) remains unclear although AHSP can be assumed to affect PrP conformation that underlies the molecular pathogenesis of TSE.

3. BIOPHYSICAL AND FUNCTIONAL STUDIES

3.1. AHSP function

AHSP forms a stable complex with free alpha-Hb but not with beta-Hb or Hb A (5). Nevertheless, this complex then can be disrupted by free beta-Hb to form a heterodimer releasing free AHSP, indicating the function of AHSP as a molecular chaperone for the unstable free alpha-Hb, consistent with evidence showing that AHSP can prevent both oxidant-induced alpha-Hb precipitation and production of radical oxygen species by free alpha-Hb (5, 7). In an *Escherichia coli* heterologous expression system, recombinant alpha-Hb is synthesized as a soluble protein only when recombinant AHSP also is co-expressed (8). *In vivo* evidence from reticulocytes has demonstrated the existence of alpha-Hb/AHSP complex in support of the function of AHSP as a stabilizer of free alpha-Hb pool in the cytosol (9). Thus, the amount of intra-erythrocytic AHSP is sufficient to cope with the unmatched alpha-Hb in clinically-silent beta-thalassemic heterozygotes but inadequate in symptomatic homozygotes. In addition to its stabilizing role of free alpha-Hb, AHSP also assists in the proper folding of newly-synthesized alpha-globin chains in a manner typical of the classical molecular chaperones. In addition to these, there has been no documented evidence describing either any other molecules being chaperoned by AHSP or examples of inducible expression of AHSP gene.

In vitro properties of AHSP suggest it may have an important role in protecting cells from cytotoxic free alpha-Hb *in situ*. Although loss of AHSP has no effect on growth and development in a mouse model, certain erythroid defects in AHSP-null mice were observed that lend support to the notion that AHSP is required for normal erythropoiesis (5, 7). Absence of AHSP causes hemolytic anemia in the mice, implied from presence of reticulocytosis, with inclusion body-containing erythrocytes. These red cells have thalassemia-like morphology (hypochromia, micro- and anisocytosis), reduced lifespan and are more susceptible to oxidative stress, as observed from the presence of precipitated Hb A on red cell membrane. In addition, there is ineffective erythropoiesis as evidenced by a significant increase in apoptotic erythroid precursors. In order to compensate for the increased hemolytic state, erythroid precursors are significantly produced in hematopoietic tissue, which causes splenic enlargement. Heterozygous AHSP-null mutant mice show only subtle changes, suggesting that AHSP haplo-insufficiency may not exert a significant effect on red blood cells such that clinical pathology can be observed. More recently, an increase in apoptosis in the AHSP-knockdown cells in human erythroid cell culture system has been reported (10). As loss of AHSP shares similar consequences with beta-thalassemia in increasing the amounts of free alpha-Hb, co-existence of both conditions should produce a more severe hematological pathology than one condition alone (see Figure 1 for the schematic diagrams of Hb synthesis under different beta-globin and AHSP status).

On the other hand, if absence of AHSP produces a beta-thalassemia-like condition, then co-existence of both alpha-thalassemia and null AHSP conditions should ameliorate the clinical severity of each separate pathology as is in the case of co-inheritance of alpha- and beta-thalassemia, owing to a more balanced ratio of globin chains (11). However, contrary to expectation, the presence of both AHSP null genotype and heterozygous alpha-thalassemia resulted in a more severe pathology than with either mutation alone (9). In the case of a loss of AHSP alone, both alpha- and beta-globin chains are found on the red cell membrane even though there is a balance in alpha- to beta-globin ratio, suggesting that AHSP indirectly affects the fate of beta-Hb as well. When this condition is compounded with an imbalance of globin chains stemming from alpha-thalassemia, pathology of the AHSP-deficient cell then is worsened by the increase in amounts of free beta-Hb due to reduced availability of AHSP-stabilized alpha-Hb (see Figure 1 for a schematic diagram of Hb synthesis under different alpha-globin and AHSP status). Thus, defective AHSP expression can mimic both alpha- and beta-thalassemia pathophysiology, depending on which type of free unmatched globin chains predominates in the hematopoietic cell.

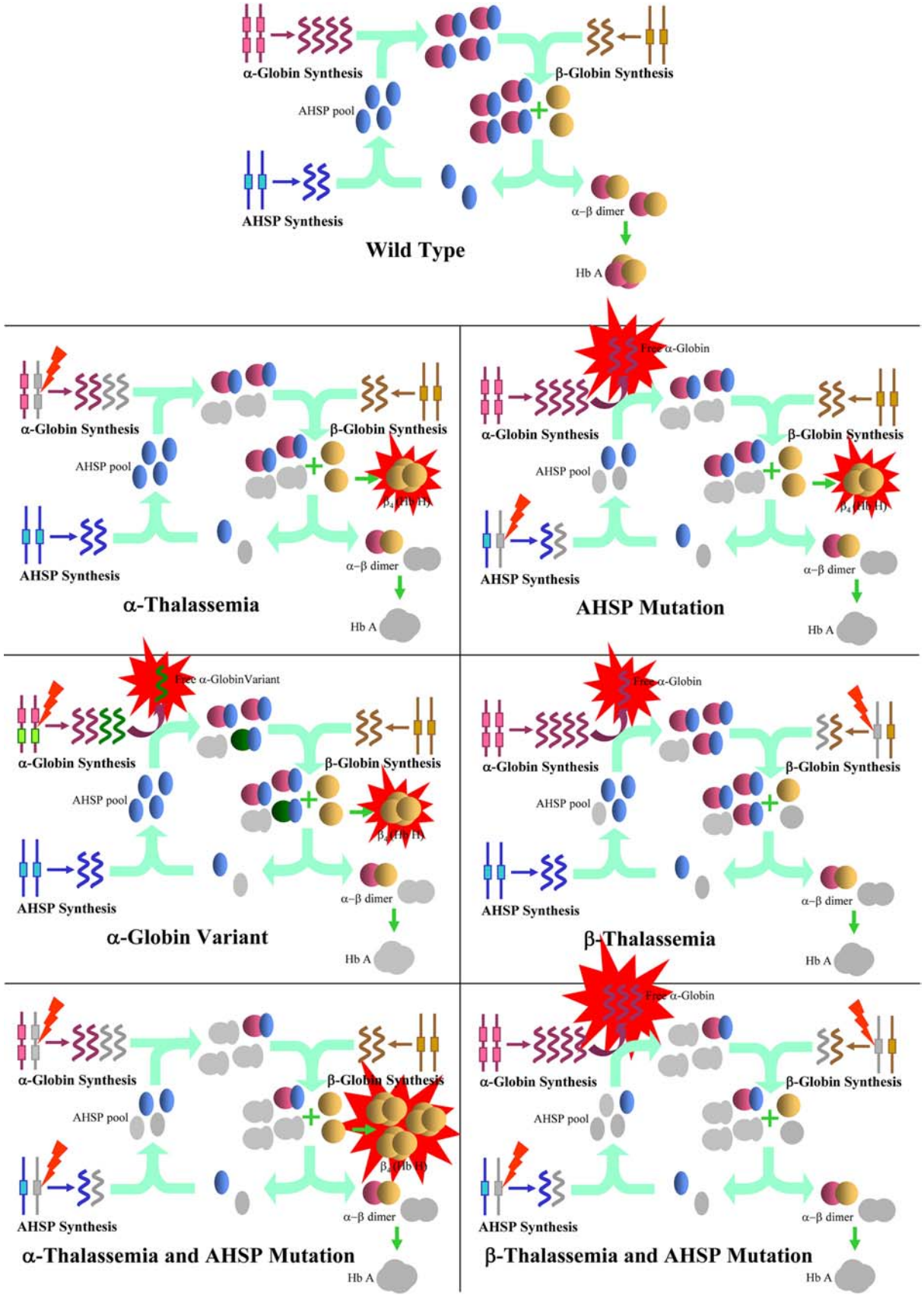
α-Globin Synthesis
β-Globin Synthesis
AHSP pool
AHSP Synthesis
α–β dimer
Hb A
Wild Type
Free α-Globin
α-Thalassemia
AHSP Mutation
Free α-GlobinVariant
α-Globin Variant
β-Thalassemia
α-Thalassemia and AHSP Mutation
β-Thalassemia and AHSP Mutation

Figure 1. Schematic diagram of hemoglobin synthesis under different AHSP, alpha-globin and β-globin status. A cascade of interactions among the three proteins (AHSP, alpha-globin and beta-globin) is shown, with gene, polypeptide chain and globular protein represented as box, curved string and sphere/oval, respectively. The grey models are used to imply their disappearance compared with the wild type condition. In the case of alpha-thalassemia, decreased production of alpha-globin chains produces reduced amounts of AHSP-alpha-Hb complex, resulting in reduction in alpha-beta-dimer and thus Hb A with the remaining unmatched beta-Hb tetramerizing into Hb H. In a similar manner to alpha-thalassemia, decreased amount of AHSP (due to AHSP mutation, which would be a case of genetic chaperonopathy (28)) reduces production of Hb A, but apart from the unmatched beta-Hb, excess alpha-globin not being stabilized by AHSP also exists, as found in beta-thalassemia. However, a combination of both alpha-thalassemia and AHSP mutation generates a more severe consequence owing to the extremely diminished availability of AHSP-alpha-Hb complex. Impaired interaction with AHSP of alpha-globin variant, giving rise to the presence of free alpha-globin variant chains, mimics alpha-thalassemia phenomenon of defective tetrameric Hb production, probably due to inadequate amounts of wild type AHSP-alpha-Hb complex, and also impaired dimerization of alpha-globin variant and beta-globin. Although beta-thalassemia does not directly affect the interaction between AHSP and alpha-globin, the depleted production of beta-Hb for heterodimerization with alpha-Hb reduces the pool of free AHSP available for further interaction with newly synthesized alpha-globin chains, increasing the pool of free alpha globin. In addition, if the pool of wild type AHSP is abnormally repleted due to presence of abnormal AHSP-alpha-Hb complex, then the beta-thalassemia condition can be worsened.

It is believed that the high prevalence of thalassemic genes in the human population is due to their selective advantage in heterozygous individuals in the face of malaria infection (12). It will be of interest to investigate whether AHSP-null genotypes have also been selected in regions of malaria transmission (past and present).

3.2. Molecular structure and interaction

AHSP consists of 102 amino acids (including the first methionine) with molecular mass of 11.84 kDa. Monomeric AHSP contains about 70 % alpha-helices (13) (Figure 2). The formation with alpha-Hb is an exothermic reaction with 1:1 stoichiometry and an affinity constant (K_A) of 10^7 M^{-1}. The heme group and ATP are not required for this binding process, indicating a possible role of AHSP in stabilizing alpha-globin polypeptide during state of heme shortage, e.g. iron deficiency anemia. X-ray crystallography and NMR spectroscopy show that the interacting complex comprises three and six helices of AHSP and alpha-Hb respectively (14, 15) (Figure 3). The three AHSP alpha-helices fold in an antiparallel fashion with the negatively-charged alpha-Hb binding domain, starting from C-terminus of the first helix to N-terminal of the second helix, interacting with helices G and H of alpha-Hb at the positively-charged intradimeric surface (alpha1-beta1 interface) (Figure 4). However, the main interacting interfaces of AHSP-alpha-Hb complex are contributed by hydrophobic interactions of about 30 amino acids with three specific hydrogen bonds (that are weaker than that in alpha1-beta1 interaction), thereby masking the hydrophobic surface of alpha-Hb and preventing free alpha-Hb from either precipitating in the cytosol or binding to cell membrane before being transferred to beta-Hb when available.

Amino acids of both proteins in the interacting interface are highly conserved among mammalian orthologs, indicating the crucial function of AHSP on stabilizing alpha-Hb. The alpha-Hb binding domain on AHSP structurally corresponds to the same domain found on beta-Hb molecule, explaining why they share the same binding interface on alpha-Hb chain. However, the conformation of alpha-Hb in the AHSP complex is much different from that in Hb A, especially the rearrangement of helix F, the heme- interacting helix of alpha-Hb in Hb A. In solution, AHSP has two isomeric forms (*cis/trans*) and proline at the 30^{th} residue, located in the intervening loop region between the first two helices, is thought to play a role in isomerization. Evidence~~s~~ from NMR spectroscopy suggest that the *trans* conformation should be involved in the interaction with alpha-Hb (15). Oxygen affinity of alpha-Hb in AHSP-alpha-Hb complex is 3-fold lower than free alpha-Hb, indicating an intermediate state between R (oxy) and T (deoxy) allosteric forms of Hb A (16) (see below).

3.3. Mechanism of alpha-Hb stabilization

The heme pocket is located inside the AHSP-alpha-Hb complex on the opposite side to the AHSP-binding region, implying no direct interference with any reaction involving iron and heme molecule (14) (Figure 3). Surprisingly, this heme moiety in the complex is coordinated at the 5^{th} co-ordinate position of the iron atom by a distal histidine residue (His-58 in helix E) instead of the proximal residue (His-87 in helix F) as is found in Hb A (see Figure 5 for a structural model of heme pocket rearrangement). Other heme-coordinating amino acids are significantly rearranged, but most of the heme-stabilizing residues are still preserved. Based on this structure, the oxy-reduced (ferrous) form of heme in this complex is now fully exposed to the cytoplasmic environment and can no longer be kept in the hydrophobic cavity as in Hb A, facilitating heme oxidation ($Fe^{2+} \rightarrow Fe^{3+}$ and deoxygenation) to occur in an auto-oxidation manner at physiologic conditions (17). To fulfill its function of preventing a redox reaction caused by heme Fe^{3+}, binding with AHSP should facilitate a conformational alteration of alpha-Hb to convert this prone-to-oxidize form of iron (high-spin state of Fe^{3+}) into a non-reactive form (low-spin state of Fe^{3+}). Evidence from Raman spectroscopy has confirmed this hypothesis and the proximal histidine would be the best possible choice for being liganded at the 6^{th} coordinate position of the heme iron atom, thereby forming an inert bis-histidyl ferric heme complex (14). X-ray crystallography has subsequently confirmed this configuration (18) (Figure 5). This conformational change to the AHSP-bound oxidized alpha-Hb results in a heme

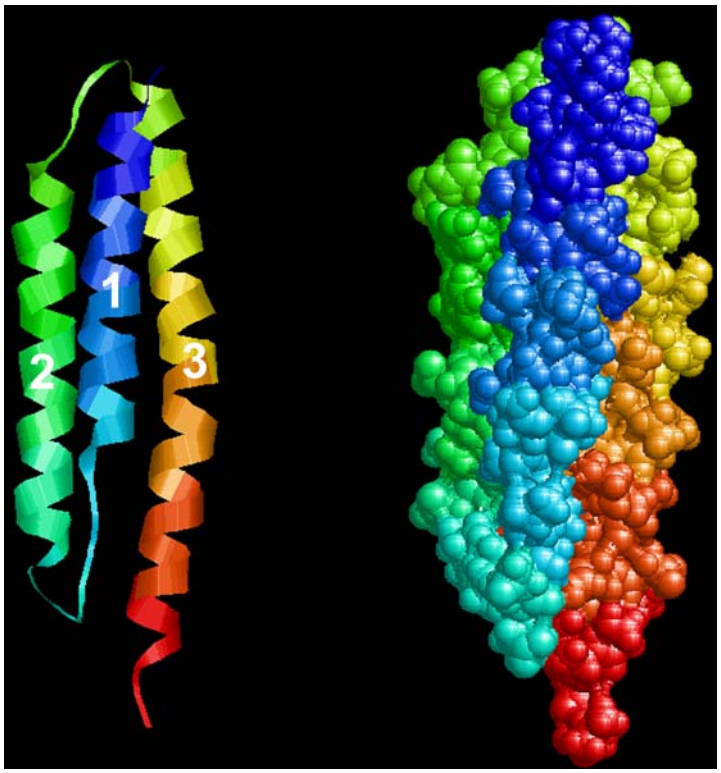

Figure 2. Structural model of AHSP molecule. AHSP molecule (solution structure of the *trans* form of AHSP; PDB ID: 1W0A) is demonstrated in both ribbon and molecular-surface models using RasMol structure viewer. The three helices were numbered in the N- to C-terminus fashion.

pocket that is now less exposed to the outside environment, giving rise to a decrease in both peroxide-induced heme loss and also iron-induced redox reaction. AHSP binding also may allow the heme pocket to become more flexible, subsequently affecting the whole structure and preventing aggregation (and precipitation) (19). Hence, binding of AHSP to alpha-Hb (i) stabilizes both ferrous and ferric forms of alpha-Hb before transfer to beta-Hb or from being reduced by cellular MetHb reductase (in case of Fe^{3+}), and (ii) protects cell from oxidative damage caused by free alpha-Hb by keeping the oxidized heme in its inactive state.

4. AHSP GENE AND ITS EXPRESSION

The structures of AHSP gene among mammals are highly conserved without any specific motif (5). In humans, the 922-bp AHSP gene, located at 16p11.2, comprises of 3 exons with one transcription initiation site (Reference Sequence Number: NC_000016). The translation initiation site is located in the 2nd exon starting at the 5th nucleotide and the termination codon is found in the 3rd exon (Figure 6). AHSP expression is specific for hematopoietic tissues starting at the embryonic stage (5). Expression of AHSP progressively increases at a nearly constant ratio to that of alpha-globin during erythroid development reaching a peak level at the polychromatic and orthochromatic stages (20). There are at least 10^7 AHSP molecules per late erythroid precursor (proerythroblast) (5).

The consensus TATA and CCAAT boxes are absent in the promoter region of AHSP gene (21). Instead, several potential binding sites for GATA-1 are located within the AHSP gene, including the 5' upstream sequence and intron 1, with some of them conserved among mouse and humans. The binding site for Oct-1, a universal factor required in embryogenesis and erythropoiesis, is situated in an AT-rich segment of intron 1 (Figure 6). Both GATA-1 and Oct-1 are necessary for AHSP promoter function. In erythroid precursor cells at the embryonic stage, AHSP expression is also dependent on EKLF, a global zinc-finger transcription factor recognizing CACC box of several gene promoters, as found in other housekeeping erythroid protein genes (22). Two CACC boxes are conserved among mammalian AHSP gene upstream sequences and are located between the first two GATA-1 binding sites (23) (Figure 6). Nevertheless, which CACC box is specific for EKLF binding is still under debate although *in vivo* binding of EKLF in this region and AHSP promoter activation by EKLF could be demonstrated (23, 24). Based on the proximity of GATA-1 and EKLF binding sites on the AHSP promoter and previous data suggesting an interaction between these two transcription factors, it may well be possible that both co-activate AHSP expression either synergistically or in a manner of an activation cascade.

Recently AHSP has been discovered as belonging to the family of iron-regulated genes (25). A stem-loop

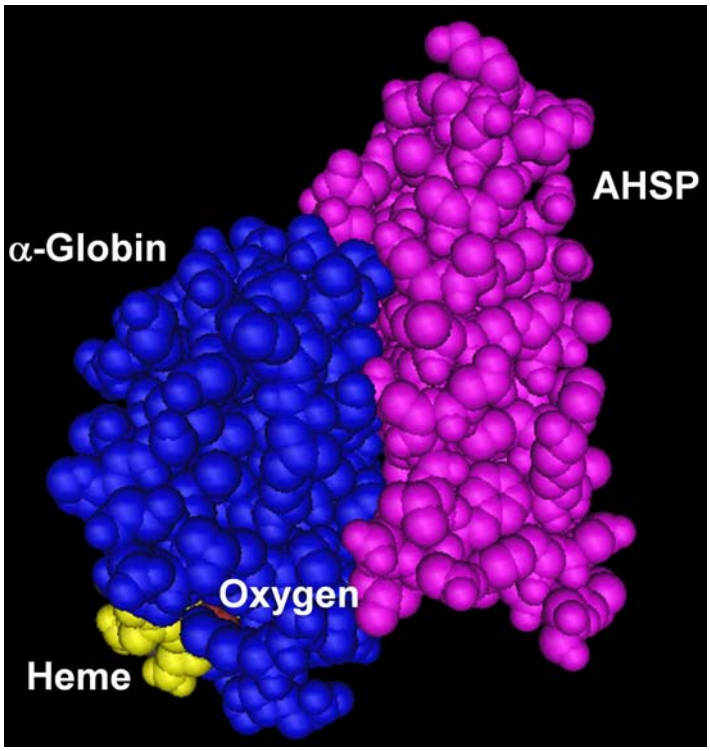

Figure 3. Structural model of AHSP-alpha-Hb complex. AHSP-alpha-Hb complex (crystal structure of AHSP bound to Fe(II) alpha-Hb; MMDB ID: 31668) is displayed in the space-filling model using Cn3D structure viewer. Note that both heme (yellow) and its bound oxygen (red) molecule are found on the opposite side to the AHSP-interacting surface of alpha-Hb molecule.

iron responsive element (IRE), restricted to only among primates, was identified in the 3'UTR of AHSP transcript and its *in vivo* interaction with cellular iron regulatory protein (IRP) demonstrated. In the presence of iron, IRP is released from IRE, thereby destabilizing AHSP transcript. This interesting finding supports the role of AHSP in preventing excess alpha-globin from precipitation during the pause of Hb synthesis *in situ*ation of iron depletion. Nevertheless, in an iron-replete situation, there must be sufficient amounts of AHSP to stabilize alpha-Hb.

5. CLINICAL CORRELATION

As described above, absence of AHSP can have a modifying effect on cellular pathology caused by imbalance of both alpha- and beta-globin chains (as demonstrated in a mouse model). It may be expected that in humans AHSP should be regarded as one of the important clinical modifiers in patients with hemoglobinopathies caused by either quantitative (thalassemia) or qualitative (Hb variant) defect of the globin chains.

5.1. AHSP-alpha-Hb interaction: quantitative aspect

Based on hematological data, thalassemic subjects with the same genetic defects in their globin gene manifest a wide variation in their clinical symptoms, ranging from mild anemia requiring no treatment to that requiring regular blood transfusions (26). It is possible that variations in AHSP expression may in part explain this phenomenon, especially in beta-thalassemia disease caused by presence of excess alpha-Hb. On the other hand, no significant association between AHSP gene variations and disease severity was found after sequencing the coding regions extending up to about 0.5 kb of upstream sequence, conducting variant haplotyping among 120 patients having clinical variability in beta-thalassemia/Hb E disease and excluding other known genetic modifiers such as alpha-thalassemia and high Hb F expression (27). Thus, variations in clinical severity in thalassemic individuals with the same defects in their globin genes could be examples of chaperonopathies due to chaperone-gene (AHSP) dysregulation (28, 29). Likewise, chaperonopathies associated with chaperone-gene polymorphisms have been described (28-30), a situation that might also occur with AHSP as discussed below.

AHSP gene polymorphism was studied in a thalassemic population (compound heterozygote of beta-thalassemia and triplicated alpha-globin gene) whose red cell pathology was caused by increased alpha- to beta-globin ratio (31). Six loci of polymorphisms composed of five SNPs (one in the upstream sequence, two in intron 1 and two in exon 3) and one poly-T repeat in the upstream sequence with significant population frequency were found giving rise to several haplotypes, of which four variants (two in the upstream sequence, one in intron 1 and a synonymous SNP in exon 3), are in a strong linkage disequilibrium (LD) state (the underlined variants in Figure 6). Interestingly, statistically significant association

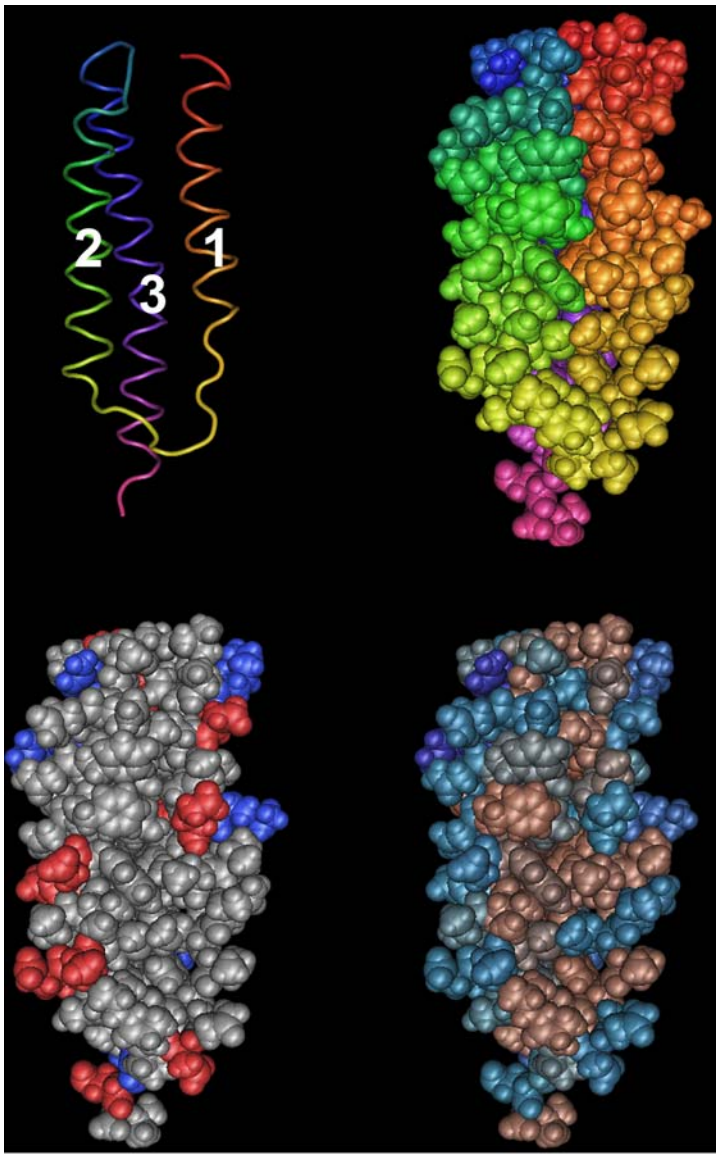

Figure 4. Alpha-Hb-interacting interface of AHSP. AHSP molecule viewed by Cn3D structure viewer (solution structure of the *trans* form of AHSP; MMDB ID: 28454) is turned to show the alpha-Hb-interacting interface (from C-terminus of the first helix to N-terminus of the second helix) in front (string model on the upper left and space-fill model on the upper right). Charge properties on this surface are drawn in blue, red and grey for positively-charged, negatively-charged and neutral residues on the lower left model, respectively. Hydrophobicity is also represented in colors from red (most) to blue (least) on the lower right model.

between these LD loci with ASHP (and also with AHSP/alpha-globin ratio) transcription levels was demonstrated both in haplotypic manner and separately. Two variants, one SNP and the homopolymer of T repeats located in the upstream regulatory region, out of these four influence gene expression both *in vitro* and *in vivo*. The longer allele (T18) of T repeats is associated with higher expression of AHSP and thereby leading to less clinical severity than the shorter allele (T15). In addition to these upstream variants, a SNP located in intron 1 (A allele) was also shown to have an effect on promoter activity *in vitro* by reducing the binding of Oct-1 although there has been no *in vivo* evidence so far for such binding (21, 32). More studies in beta-thalassemia populations in other ethnic groups and investigations of other upstream cis-regulatory regions of the AHSP gene and trans-regulating factors are still required. Besides beta-thalassemia, alpha-thalassemia in which AHSP was previously shown to act as a phenotype modifier in mice (9) should also be a focus of attention (see below).

5.2. AHSP-alpha-Hb interaction: qualitative aspect

Apart from beta-thalassemia in which the amount of unmatched free alpha-Hb is quantitatively affected by

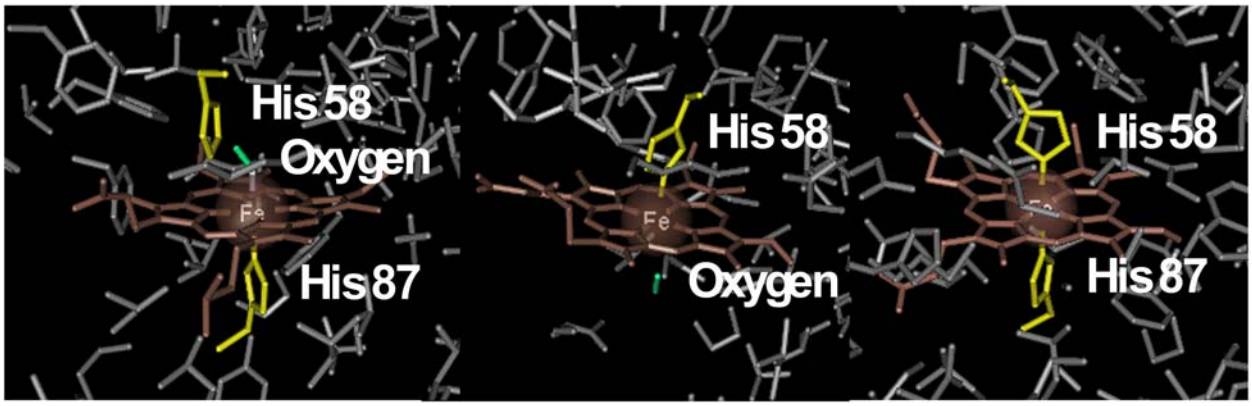

Figure 5. Structural model of heme pocket rearrangement. Heme pocket structures in different condition are illustrated using Cn3D structure viewer. Heme, histidine residues and oxygen are in brown, yellow and green colors, respectively. The left structure represents the heme pocket arrangement in oxygenated (Fe(II)) alpha-Hb inside Hb A (MMDB ID: 39205) while the middle and the right structures are found in oxygenated (Fe(II)) alpha-Hb of the AHSP-alpha-Hb complex (MMDB ID: 31668) and in deoxygenated (Fe(III)) alpha-Hb of the AHSP- alpha-Hb complex (MMDB ID: 33819), respectively.

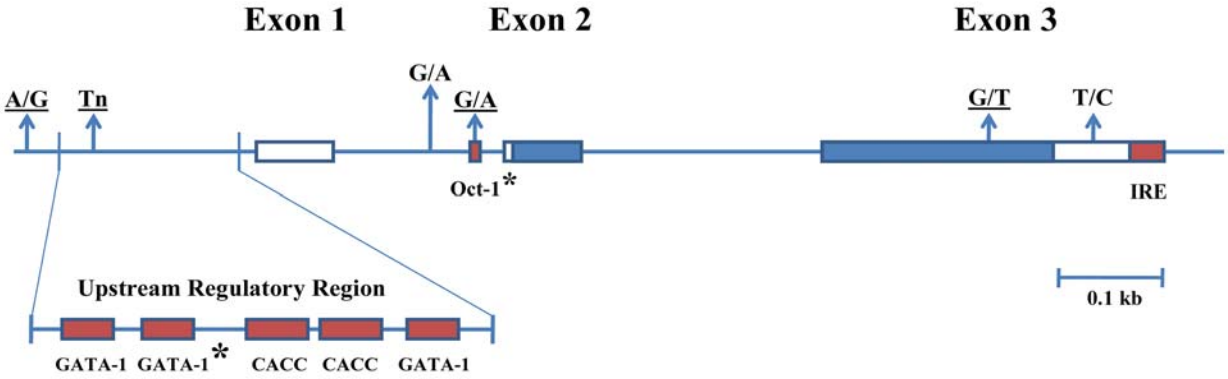

Figure 6. Schematic diagram of AHSP gene and its variants. AHSP gene composed of 3 exons is demonstrated as boxes with coding regions labeled in blue and UTRs (untranslated regions) in white. Consensus transcription factor binding sites are shown in red: GATA-1 for GATA-1 binding site, CACC for EKLF binding region and Oct-1 for Oct-1 binding sequence. Asterisks mark the sites of *in vitro* proven binding interaction. IRE represents iron responsive element. Six polymorphisms (5 SNPs and one poly-T (Tn) repeats) are denoted by the arrows and the SNP alleles are also indicated. Four variants found in the linkage disequilibrium state are underlined.

variations in AHSP expression, clinical status of patients with alpha-globin variants, either with or without alpha-thalassemia phenotype, can also be modified by AHSP. The first evidence supporting this postulation was demonstrated in two common alpha2-globin-gene termination codon mutations (alpha^T-globin genes), Hb Constant Spring (Hb CS; *U*AA → *C*AA (Gln)) and Hb Pakse (Hb PS; UA*A* → UA*U* (Tyr)) (33). These mutations lead to the production of alpha-globin chains elongated by 31 amino acids before the next in-frame termination codon. However, the presence of these alpha^T-globin variants (even in reduced amounts) results in alpha-thalassemia phenotype. Pathogenesis of this non-deletional alpha-thalassemia has been variously explained by the inherent instability of mRNA (34) and of the elongated globin chains (35-37). The instability of these alpha^T-globins was attributed to impaired interaction with AHSP, possibly due to interference by the elongated C-terminus as the C-terminal helices of wild-type alpha-Hb are required for AHSP interaction (33). The failure in being stabilized and transferred to beta-Hb by AHSP may explain the presence of membrane-bound alpha^T-globin chains, which generate oxidative damage to these variant red cells, thereby producing the observed pathology (see Figure 1 for schematic diagrams of Hb synthesis under alpha-globin variant status). This explanation may be applied to other elongated alpha-globin variants with unexpected severe clinical phenotypes, viz. Hb Pak Num Po (T insertion after the 131st codon) (38). Another example of this defect in alpha-Hb/AHSP interaction is the case of Hb Groene Hart (119; *C*CT (Pro) → *T*CT (Ser)), whose mutation is situated in the alpha-Hb-AHSP contact surface (39). Thus it would be expected that Hb variants with mutations located in the interaction region of alpha-Hb-AHSP complex and expressing alpha-thalassemia phenotype may be the consequence of impaired stabilization by its AHSP chaperone (see (40) for a recent comprehensive review of unstable and thalassemic alpha-chain hemoglobin variants).

Very recent studies of 6 known human alpha-globin variants involving mutations in the AHSP-interacting surface showed that only mutations of K99 affected binding exclusively to AHSP, whereas the other mutations (at positions 103, 117 and 119) inhibited binding to both AHSP and beta-Hb (41).

6. CONCLUSIONS

The discovery in erythroid cells of AHSP, a molecular chaperone whose function is to stabilize alpha-Hb for subsequent transfer to beta-Hb to form heterotetrameric Hb, provides a satisfactory explanation for the presence of equal amounts of alpha- and beta-globin chains in the face of a 2:1 ratio of their respective gene copies. Variations in ASHP expression levels can have a significant effect on the clinical outcome of hematological disorders in which free alpha-Hb is a causative factor. Conversely, impaired interaction of alpha-globin chain variants with AHSP can account for the instability and thalassemic phenotypes of these unstable variant Hbs. In view of recent findings that demonstrate the role of malfunctional chaperones in pathogenesis causing diseases named chaperonopathies, some forms of thalassemia in which the chaperone AHSP is altered structurally or quantitatively due to gene changes or dysregulation ought to be included within the chaperonopathies.

7. REFERENCES

1. Lodish, H. F.: Alpha and beta globin messenger ribonucleic acid. Different amounts and rates of initiation of translation. *J Biol Chem*, 246, 7131-8 (1971)

2. Chaisue, C., S. Kitcharoen, P. Wilairat, A. Jetsrisuparb, G. Fucharoen & S. Fucharoen: alpha/beta-Globin mRNA ratio determination by multiplex quantitative real-time reverse transcription-polymerase chain reaction as an indicator of globin gene function. *Clin Biochem*, 40, 1373-7 (2007)

3. Irenge, L. M., A. Robert & J. L. Gala: Quantitative assessment of human beta-globin gene expression *in vitro* by TaqMan real-time reverse transcription-PCR: comparison with competitive reverse transcription-PCR and application to mutations or deletions in noncoding regions. *Clin Chem*, 51, 2395-6 (2005)

4. Weatherall, D. J. & J. B. Clegg: The thalassaemia syndromes. Blackwell Science, Oxford (2001)

5. Kihm, A. J., Y. Kong, W. Hong, J. E. Russell, S. Rouda, K. Adachi, M. C. Simon, G. A. Blobel & M. J. Weiss: An abundant erythroid protein that stabilizes free alpha-haemoglobin. *Nature*, 417, 758-63 (2002)

6. Miele, G., J. Manson & M. Clinton: A novel erythroid-specific marker of transmissible spongiform encephalopathies. *Nat Med*, 7, 361-4 (2001)

7. Kong, Y., S. Zhou, A. J. Kihm, A. M. Katein, X. Yu, D. A. Gell, J. P. Mackay, K. Adachi, L. Foster-Brown, C. S. Louden, A. J. Gow & M. J. Weiss: Loss of alpha-hemoglobin-stabilizing protein impairs erythropoiesis and exacerbates beta-thalassemia. *J Clin Invest*, 114, 1457-66 (2004)

8. Vasseur-Godbillon, C., D. Hamdane, M. C. Marden & V. Baudin-Creuza: High-yield expression in Escherichia coli of soluble human alpha-hemoglobin complexed with its molecular chaperone. *Protein Eng Des Sel*, 19, 91-7 (2006)

9. Yu, X., Y. Kong, L. C. Dore, O. Abdulmalik, A. M. Katein, S. Zhou, J. K. Choi, D. Gell, J. P. Mackay, A. J. Gow & M. J. Weiss: An erythroid chaperone that facilitates folding of alpha-globin subunits for hemoglobin synthesis. *J Clin Invest*, 117, 1856-65 (2007)

10. Pinho, F. O., D. M. de Albuquerque, S. T. Olalla Saad & F. F. Costa: Reduction of AHSP synthesis in hemin-induced K562 cells and EPO-induced CD34(+) cells leads to alpha-globin precipitation, impairment of normal hemoglobin production, and increased cell death. *Exp Hematol*, 36, 265-72 (2008)

11. Sripichai, O., T. Munkongdee, C. Kumkhaek, S. Svasti, P. Winichagoon & S. Fucharoen: Coinheritance of the different copy numbers of alpha-globin gene modifies severity of beta-thalassemia/Hb E disease. *Ann Hematol*, 87, 375-9 (2008)

12. Williams, T. N.: Human red blood cell polymorphisms and malaria. *Curr Opin Microbiol*, 9, 388-94 (2006)

13. Gell, D., Y. Kong, S. A. Eaton, M. J. Weiss & J. P. Mackay: Biophysical characterization of the alpha-globin binding protein alpha-hemoglobin stabilizing protein. *J Biol Chem*, 277, 40602-9 (2002)

14. Feng, L., D. A. Gell, S. Zhou, L. Gu, Y. Kong, J. Li, M. Hu, N. Yan, C. Lee, A. M. Rich, R. S. Armstrong, P. A. Lay, A. J. Gow, M. J. Weiss, J. P. Mackay & Y. Shi: Molecular mechanism of AHSP-mediated stabilization of alpha-hemoglobin. *Cell*, 119, 629-40 (2004)

15. Santiveri, C. M., J. M. Perez-Canadillas, M. K. Vadivelu, M. D. Allen, T. J. Rutherford, N. A. Watkins & M. Bycroft: NMR structure of the alpha-hemoglobin stabilizing protein: insights into conformational heterogeneity and binding. *J Biol Chem*, 279, 34963-70 (2004)

16. Baudin-Creuza, V., C. Vasseur-Godbillon, C. Pato, C. Prehu, H. Wajcman & M. C. Marden: Transfer of human alpha- to beta-hemoglobin via its chaperone protein: evidence for a new state. *J Biol Chem*, 279, 36530-3 (2004)

17. Zhou, S., J. S. Olson, M. Fabian, M. J. Weiss & A. J. Gow: Biochemical fates of alpha hemoglobin bound to alpha hemoglobin-stabilizing protein AHSP. *J Biol Chem*, 281, 32611-8 (2006)

18. Feng, L., S. Zhou, L. Gu, D. A. Gell, J. P. Mackay, M. J. Weiss, A. J. Gow & Y. Shi: Structure of oxidized alpha-

haemoglobin bound to AHSP reveals a protective mechanism for haem. *Nature*, 435, 697-701 (2005)

19. Hamdane, D., C. Vasseur-Godbillon, V. Baudin-Creuza, G. H. Hoa & M. C. Marden: Reversible hexacoordination of alpha-hemoglobin-stabilizing protein (AHSP)/alpha-hemoglobin Versus pressure. Evidence for protection of the alpha-chains by their chaperone. *J Biol Chem*, 282, 6398-404 (2007)

20. dos Santos, C. O., A. S. Duarte, S. T. Saad & F. F. Costa: Expression of alpha-hemoglobin stabilizing protein gene during human erythropoiesis. *Exp Hematol*, 32, 157-62 (2004)

21. Gallagher, P. G., R. I. Liem, E. Wong, M. J. Weiss & D. M. Bodine: GATA-1 and Oct-1 are required for expression of the human alpha-hemoglobin-stabilizing protein gene. *J Biol Chem*, 280, 39016-23 (2005)

22. Drissen, R., M. von Lindern, A. Kolbus, S. Driegen, P. Steinlein, H. Beug, F. Grosveld & S. Philipsen: The erythroid phenotype of EKLF-null mice: defects in hemoglobin metabolism and membrane stability. *Mol Cell Biol*, 25, 5205-14 (2005)

23. Keys, J. R., M. R. Tallack, D. J. Hodge, S. O. Cridland, R. David & A. C. Perkins: Genomic organisation and regulation of murine alpha haemoglobin stabilising protein by erythroid Kruppel-like factor. *Br J Haematol*, 136, 150-7 (2007)

24. Pilon, A. M., D. G. Nilson, D. Zhou, J. Sangerman, T. M. Townes, D. M. Bodine & P. G. Gallagher: Alterations in expression and chromatin configuration of the alpha hemoglobin-stabilizing protein gene in erythroid Kruppel-like factor-deficient mice. *Mol Cell Biol*, 26, 4368-77 (2006)

25. Dos Santos, C. O., L. C. Dore, E. Valentine, S. G. Shelat, R. C. Hardison, M. Ghosh, W. Wang, R. S. Eisenstein, F. F. Costa & M. J. Weiss: An iron responsive element-like stem-loop regulates alpha hemoglobin stabilizing protein mRNA. *J Biol Chem*, 283, 26956-64 (2008)

26. Winichagoon, P., S. Fucharoen, P. Chen & P. Wasi: Genetic factors affecting clinical severity in beta-thalassemia syndromes. *J Pediatr Hematol Oncol*, 22, 573-80 (2000)

27. Viprakasit, V., V. S. Tanphaichitr, W. Chinchang, P. Sangkla, M. J. Weiss & D. R. Higgs: Evaluation of alpha hemoglobin stabilizing protein (AHSP) as a genetic modifier in patients with beta thalassemia. *Blood*, 103, 3296-9 (2004)

28. Macario, A. J. L. & E. Conway de Macario: Sick chaperones, cellular stress and disease. *New Eng J Med* 353, 1489-501 (2005)

29. Macario, A. J. L. & E. Conway de Macario: Chaperonopathies by defect, excess, or mistake. *Ann New York Acad Sci*, 1113, 178-91 (2007)

30. Singh, R., S. Kolvraa & S. I. S. Rattan: Genetics of human longevity with emphasis on the relevance of *HSP70* as candidate genes. *Front Biosci* 12, 4504-13 (2007). http://www.bioscience.org/2007/v12/af/2405/fulltext.htm

31. Lai, M. I., J. Jiang, N. Silver, S. Best, S. Menzel, A. Mijovic, S. Colella, J. Ragoussis, C. Garner, M. J. Weiss & S. L. Thein: Alpha-haemoglobin stabilising protein is a quantitative trait gene that modifies the phenotype of beta-thalassaemia. *Br J Haematol*, 133, 675-82 (2006)

32. dos Santos, C. O., S. Zhou, R. Secolin, X. Wang, A. F. Cunha, D. R. Higgs, J. L. Kwiatkowski, S. L. Thein, P. G. Gallagher, F. F. Costa & M. J. Weiss: Population analysis of the alpha hemoglobin stabilizing protein (AHSP) gene identifies sequence variants that alter expression and function. *Am J Hematol*, 83, 103-8 (2008)

33. Turbpaiboon, C., T. Limjindaporn, W. Wongwiwat, U. P. Y, N. Siritanaratkul, P. T. Yenchitsomanus, S. Jitrapakdee & P. Wilairat: Impaired interaction of alpha-haemoglobin-stabilising protein with alpha-globin termination mutant in a yeast two-hybrid system. *Br J Haematol*, 132, 370-3 (2006)

34. Weiss, I. M. & S. A. Liebhaber: Erythroid cell-specific determinants of alpha-globin mRNA stability. *Mol Cell Biol*, 14, 8123-32 (1994)

35. Peerapittayamongkol, C., L. F. Bernini & P. Wilairat: Presence of alpha^{CS}-globin on membrane of red cell containing hemoglobin Constant Spring (CS). *J Sci Soc Thailand* 22, 117-20 (1996)

36. Schrier, S. L., A. Bunyaratvej, A. Khuhapinant, S. Fucharoen, M. Aljurf, L. M. Snyder, C. R. Keifer, L. Ma & N. Mohandas: The unusual pathobiology of hemoglobin constant spring red blood cells. *Blood*, 89, 1762-9 (1997)

37. Turbpaiboon, C., A. Siritantikorn, W. Thongnoppakhun, D. Bunditworapoom, C. Limwongse, P. T. Yenchitsomanus, N. Siritanaratkul & P. Wilairat: Hemoglobin Pakse: presence on red blood cell membrane and detection by polymerase chain reaction-single-strand conformational polymorphism. *Int J Hematol*, 80, 136-9 (2004)

38. Viprakasit, V., V. S. Tanphaichitr, G. Veerakul, W. Chinchang, S. Petrarat, P. Pung-Amritt & D. R. Higgs: Co-inheritance of Hb Pak Num Po, a novel alpha1 gene mutation, and alpha^{0} thalassemia associated with transfusion-dependent Hb H disease. *Am J Hematol*, 75, 157-63 (2004)

39. Vasseur-Godbillon, C., M. C. Marden, P. Giordano, H. Wajcman & V. Baudin-Creuza: Impaired binding of AHSP to alpha chain variants: Hb Groene Hart illustrates a mechanism leading to unstable hemoglobins with alpha

thalassemic like syndrome. *Blood Cells Mol Dis*, 37, 173-9 (2006)

40. Wajcman, H., J. Traeger-Synodinos, I. Papassotiriou, P. C. Giordano, C. L. Harteveld, V. Baudin-Creuza & J. Old: Unstable and thalassemic alpha chain hemoglobin variants: a cause of Hb H disease and thalassemia intermedia. *Hemoglobin*, 32, 327-49 (2008)

41. Xiang Yu, X., T. L. Mollan, A. Butler, A. J. Gow, J. S. Olson & M. J. Weiss: Analysis of human alpha globin gene mutations that impair binding to the alpha hemoglobin stabilizing protein. *Blood*, 113, 5961-69 (2009)

Key Words: Hemoglobin, Hb, Alpha-Hemoglobin, alpha-Hb, Hemoglobin Variant, Thalassemia, Molecular Chaperone, Alpha-Hemoglobin Stabilizing Protein, AHSP, Review

Send correspondence to: Prapon Wilairat, Department of Biochemistry, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand, Tel: 662-3547140, Fax: 662-3547174, E-mail: scpwl@mahidol.ac.th

http://www.bioscience.org/current/vol15.htm