The Structure and Evolution of the Spider Monkey Delta-Globin Gene[1]

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We have isolated the δ-globin gene of the New-World spider monkey, *Ateles geoffroyi,* and compared its nucleotide sequence with those of other primate δ- and β-globin genes. Among primate δ-globin genes, the rate of nonsynonymous substitutions is much less than the rate of synonymous substitutions. This suggests that primate δ-globin genes may remain under evolutionary conservation, perhaps because hemoglobin A_2 has an as yet unknown physiological importance.

Introduction

The δ-globin gene encodes the β-like globin chain of the minor adult hemoglobin A_2 (Hb A_2; $\alpha_2\delta_2$). Hb A_2 is found in man, the great apes, and New-World monkeys (NWM) but not in Old-World monkeys (OWM; Kunkel et al. 1957) and Prosimii. In man, Hb A_2 composes only ~2.5% of total normal adult hemoglobin. Among higher primates, the highest known level of Hb A_2, ~6% of total hemoglobin, occurs in the New-World spider monkey, *Ateles geoffroyi* (Boyer et al. 1971). However, Hb A_2 has no known physiological importance. The proto-δ- and proto-β-globin genes apparently arose by means of duplication of a single ancestral gene ~100 Myr before the present (Mybp), prior to the mammalian radiation (Hardison 1984). In lemurs (Prosimii), recombination between the η- and δ-globin genes created a hybrid ηδ-globin pseudogene (Jeffreys et al. 1982). Approximately 40 Mybp (Efstratiadis et al. 1980), prior to the divergence of the catarrhine and platyrrhine evolutionary lineages (Giebel et al. 1985), a portion of the δ-globin gene 5′ to the second intervening sequence (IVS2) was apparently converted by the β-globin gene (Jeffreys et al. 1982; Martin et al. 1983; Hardies et al. 1984; Hardison and Margot 1984). In the lineage leading to the OWM, the δ-globin gene was silenced.

To extend the analysis of δ-globin gene evolution in primates, we cloned the δ-globin gene of *A. geoffroyi* and determined its nucleotide sequence. This permitted identification of structural features of the *Ateles* δ-globin gene that might account for its somewhat enhanced expression. Furthermore, the pattern of nucleotide substitutions in the *Ateles* and human δ-globin genes is typical of genes under evolutionary conservation, suggesting that Hb A_2 may have an as yet unknown physiologic importance.

1. Key words: globin genes, hemoglobin A_2, gene evolution, substitution rates, primate genes, New-World monkeys. Abbreviations: R = purine; Y = pyrimidine; K = guanine or thymine; M = adenine or cytosine; S = guanine or cytosine; and V = adenine, cytosine, or guanine.

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Material and Methods

Molecular Cloning

Ateles geoffroyi DNA, prepared from cultured skin fibroblasts of a healthy 5-year-old male spider monkey, was partially digested with *Sau*3AI, and 20-kb fragments were inserted into the *Bam*HI cloning site of bacteriophage vector λ Charon 35 (Loenen and Blattner 1983). Two such recombinant phage libraries were prepared and propagated in *Escherichia coli* K-12 strain ED8767 and screened (Benton and Davis 1977), with a 2.25-kb *Pst*I DNA fragment containing the human δ-globin gene (Spritz et al. 1980) being used as probe. Two recombinant phage, 3-3 and 6-7, were isolated. Because these phage recombinants grew several 100-fold more slowly than typical recombinant phage, selected fragments of phage 3-3 were subcloned in pBR322 or pBR328 and propagated in *E. coli* HB101.

Southern Blot Analyses

Genomic *A. geoffroyi* DNA was analyzed by means of the method of Southern (1975), with either the 2.25-kb δ-globin *Pst*I fragment described above or a 380-bp *Nco*I-*Eco*RI fragment of human β-globin cDNA being used as probes. DNAs from recombinant phage 3-3 and recombinant phage 6-7 were analyzed by using as probes either the same 2.25-kb *Pst*I fragment or a 0.95-kb *Bam*HI-*Eco*RI fragment containing IVS2 of the *Ateles* δ-globin gene.

Nucleotide Sequence Analysis

Nucleotide sequences of subcloned phage 3-3 DNA fragments were determined according to the method of Maxam and Gilbert (1980).

Results and Discussion

Genomic Analyses

Southern blot analysis of *Ateles* genomic DNA, with human δ- and β-globin used as probes, demonstrated that the 5′ portions of the *Ateles* δ- and β-globin genes reside on *Eco*RI fragments of 2.4 and 2.8 kb, respectively (fig. 1). A weakly hybridizing 4.1-kb band probably represents the 5′ portion of the *Ateles* γ-globin gene (Giebel et al. 1985). A second weakly hybridizing band is observed at 3.5 kb. The *Ateles* η-globin gene resides on an *Eco*RI fragment ~10 kb in size (R. A. Spritz and L. B. Giebel, unpublished data); therefore, the 3.5-kb fragment may represent the 5′ portion of the ε-globin gene.

Detailed mapping of genomic *Ateles* and phage 3-3 and 6-7 DNAs showed that the linked *Ateles* δ- and β-globin genes are separated by ~6.4 kb (fig. 2) and have structures typical of β-like globin genes. However, the *Ateles* β-globin gene apparently lacks the *Bam*HI site that is within the second exon and occurs in all other expressed primate globin genes. The *Bam*HI recognition site (GGATCC) spans codons 98–100; however, *Ateles* δ- and β-globin chains are identical at these positions (Val-Asp-Pro). Therefore, the β-globin gene in the *Ateles* lineage must have undergone a silent substitution at Val 98 (GTG → GTN), Asp 99 (GAT → GAC), or both; all changes in the first two positions of codon 100 (Pro CCT) would produce an amino acid substitution. In addition, we observed an apparently polymorphic *Pst*I site ~850 bp 5′ of the δ-globin gene. This site was present in phage 6-7 but absent in phage 3-3, indicating that phage 3-3 and phage 6-7 derive from different alleles.

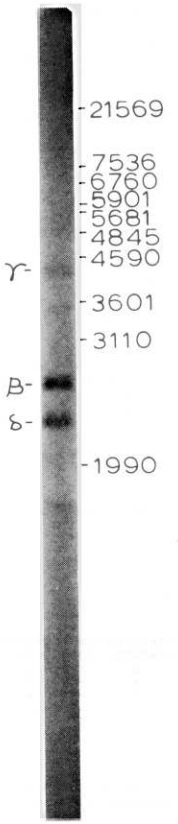

FIG. 1.—Autoradiograph from Southern hybridization of *Eco*RI-digested *Ateles geoffroyi* DNA hybridized to a 380-bp *Nco*I-*Eco*RI fragment of human β-globin cDNA. *Ateles* γ-, β-, and δ-globin DNA fragments and molecular-size standards are indicated.

Nucleotide Sequence Analysis

We determined the nucleotide sequence of 1,959 bases extending from 184 bases 5′ of the translational initiation codon to 218 bases 3′ of the translational termination codon (fig. 3). To establish the evolutionary relationships between the human, OWM, and NWM lineages, we aligned the δ-globin gene sequences of *Ateles* (Age δ), human (Hsp δ; Spritz et al. 1980; Poncz et al. 1983), baboon (Pan δ; Kimura and Takagi 1983), *Colobus* (Cpo δ; Martin et al. 1983), and *Rhesus* (Rhe δ; Martin et al. 1983)

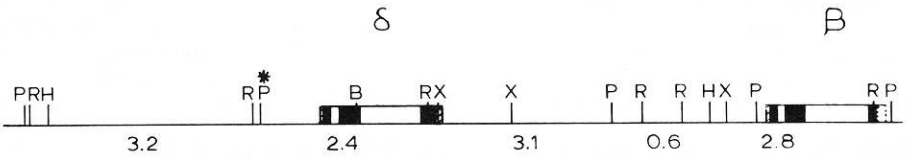

FIG. 2.—Partial restriction-endonuclease cleavage map of the *Ateles* δ- and β-globin gene region. Exons are represented by solid bars, intervening sequences by open bars, and untranslated regions by hatched bars. B = *Bam*HI; R = *Eco*RI; H = *Hin*dIII; P = *Pst*I; and X = *Xba*I. Sizes of the *Eco*RI fragments are indicated. *Xba*I cleavage patterns in the 3.2- and 0.6-kb *Eco*RI fragments have not been determined.

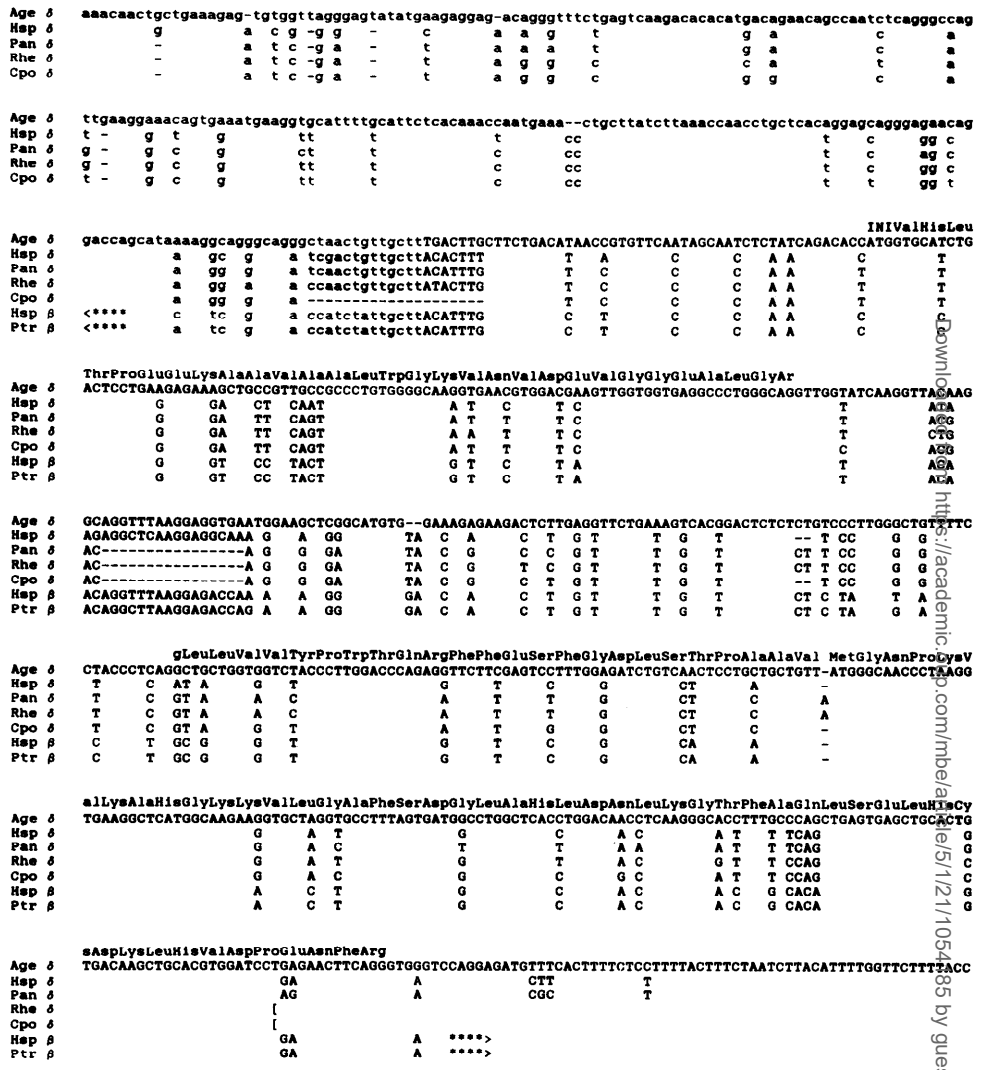

FIG. 3.—Nucleotide sequence comparison of the spider monkey (*Ateles geoffroyi;* Age δ), human (*Homo sapiens;* Hsp δ), baboon (*Papio anubis;* Pan δ), *Rhesus* monkey (Rhe δ), and *Colobus* monkey (*Colobus polykomos;* Cpo δ) δ-globin and the human (Hsp β) and chimpanzee (*Pan troglodytes;* Ptr β) β-globin genes. The complete DNA and amino acid sequences of the *Ateles* gene are illustrated. Divergent nucleotides of the human (Spritz et al. 1980; Poncz et al. 1983), baboon (Kimura and Takagi 1983; K. A. Vincent, personal communication), *Rhesus,* and *Colobus* (Martin et al. 1983) δ-globin and of human (Lawn et al. 1980; Spritz et al. 1981; Poncz et al. 1983) and chimpanzee (Savatier et al. 1985) β-globin genes are presented below the *Ateles* δ-globin gene sequence. Unpublished corrections of errors in the published human (Spritz et al. 1980) and baboon (Kimura and Takagi 1983) δ-globin gene sequences are incorporated here and in the Genbank listing. Gaps introduced to improve alignment are denoted by dashes. The translational initiation (INI) and termination (TER) codons are indicated. Lowercase letters denote presumed 5′- and 3′-flanking sequences. Brackets denote limits of published sequences. Asterisks denote endpoints of segments with minimal similarity.

and also the β-globin gene sequences of human (Hsp β; Lawn et al. 1980, Spritz et al. 1981; Poncz et al. 1983) and chimpanzee (Ptr β; Savatier et al. 1985).

The deduced amino acid sequence of *A. geoffroyi* δ-globin chains agrees with the previously determined sequence (Boyer et al. 1969*b,* 1971) at all but one residue. Codon 5, previously reported to encode glycine, encodes proline in this gene. This

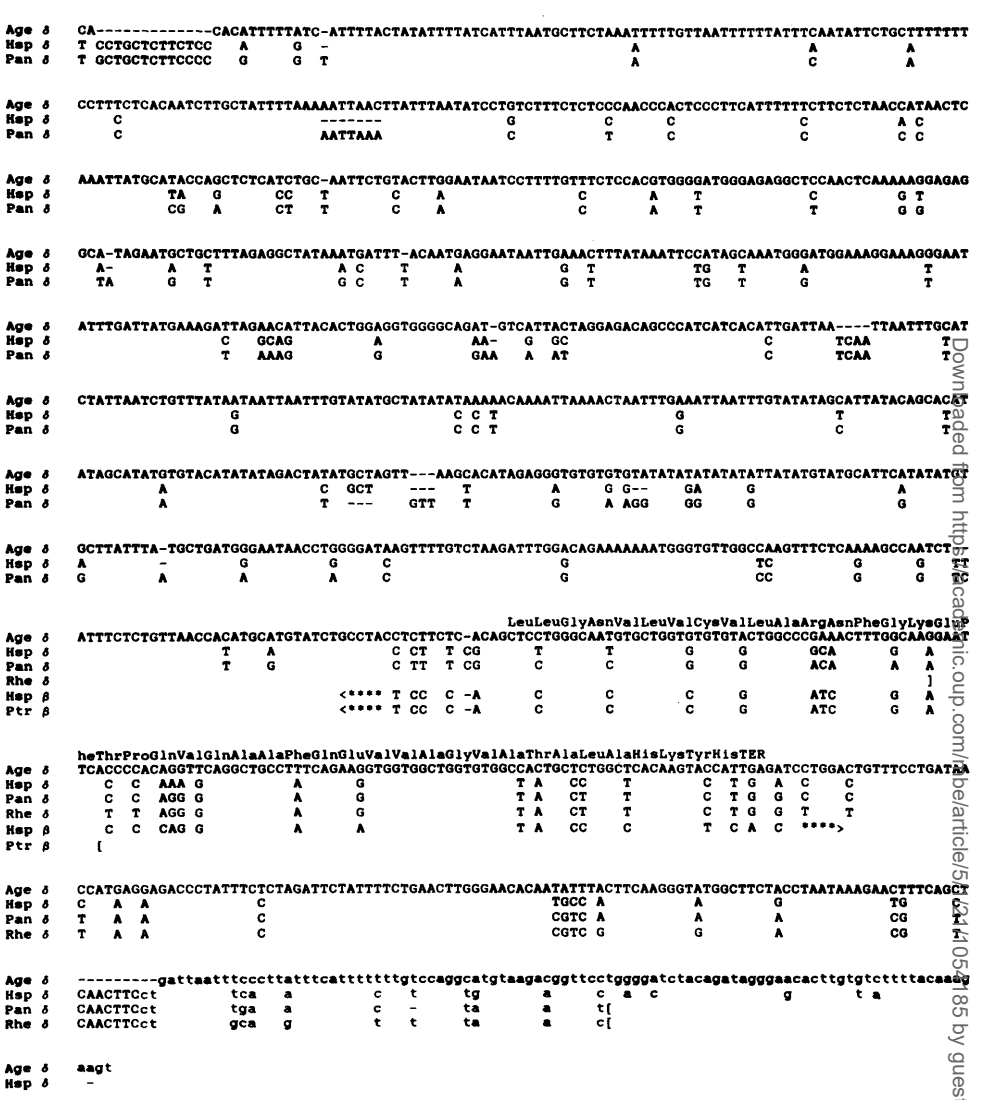

FIG. 3 (*Continued*)

residue is proline in human, ape, and OWM δ- and β-globin chains (table 1) but is glycine in δ- and β-globin chains of most NWM genera, including *Ateles* (Boyer et al. 1969*a*, 1969*b*, 1971, 1972). Residue δ 5 was originally determined to be glycine in multiple individuals of three different *Ateles* species (*geoffroyi, fusiceps,* and *paniscus;* Boyer et al. 1969*a*, 1971). Therefore, proline at residue δ 5 apparently represents an infrequent polymorphism in *Ateles.* This protein polymorphism cannot result from a single nucleotide substitution, since at least two base changes are necessary to convert the proline codon at position 5 (CCT) to a glycine codon (GGN). However, this might be accomplished in a single event by a 2-bp inversion of the sequence CC from codon 5, although such small inversions have not previously been identified as a source of sequence polymorphisms.

Two structural features of the *Ateles* δ-globin gene are evident that might account for the somewhat higher level of Hb A_2 in *Ateles* than in most other primates (Boyer

Table 1
Nucleotide Sequence Divergence of Primate δ-Globin Genes

	SUBSTITUTIONS/100 SITES (no. of sites)			
SITES	Age δ/Hsp δ	Age δ/Pan δ	Hsp δ/Pan δ	Age γ/Hsp γ[a]
Nonsynonymous	3.2 (331.0)	5.1 (331.3)	3.4 (333.3)	5.3 (336.3)
Synonymous	23.2 (107.0)	28.6 (106.7)	10.5 (104.7)	13.6 (101.7)
IVS1	13.3 (128.0)	11.6 (121.0)	7.5 (120.0)	9.6 (122.5)
IVS2	8.2 (892.5)	9.6 (903.5)	5.4 (911.0)	14.4 (887.5)
5′ Untranslated	15.1 (53.0)	15.1 (53.0)	7.6 (53.0)	9.2 (57.5)
3′ Untranslated	7.5 (133.0)	8.3 (133.0)	5.3 (133.0)	14.7 (90.0)
5′ Nontranscribed	9.4 (234.5)	11.1 (234.0)	5.5 (234.5)	7.5 (139.5)
3′ Nontranscribed	12.6 (95.5)	15.8 (50.5)	7.8 (51.5)	23.9 (68.5)

NOTE.—Sequences were compared according to the method of Li et al. (1985).
[a] Source: Giebel et al. (1985).

et al. 1971). First, the CCAAT box in the promoter region of the human δ-globin gene is atypical, having the sequence CCAAC (Efstratiadis et al. 1980). Mutations at the last base of the CCAAT box strongly depress transcription in vivo (Myers et al. 1985), probably accounting for low transcriptional activity of the human δ-globin gene (Proudfoot et al. 1980; Martin et al. 1983). In the *Ateles,* baboon, *Rhesus,* and *Colobus* genes, there is a second CCAAT sequence 23 or 25 bp upstream, possibly facilitating transcription of these δ-globin genes. The baboon and *Rhesus* genes contain a frameshift, and the *Colobus* gene contains a deletion that includes the mRNA 5′ terminus; therefore, these species cannot express δ-globin chains. However, *Ateles* δ-globin mRNA is apparently translatable; therefore, increased transcription could result in an increased level of δ-globin mRNA, with a consequent increase in δ-globin chain biosynthesis. Second, in the 3′-untranslated region, a 9-bp segment (CAACTTCCT) that includes the presumed polyadenylation site of (human) δ-globin mRNA has been deleted. This deletion creates a novel *Pvu*II cleavage site and has been confirmed by mapping with *Pvu*II. Three direct repeats of the sequence AACTTCC (or a closely related sequence) occur in this region, each repeat being separated by five dissimilar bases. The *Ateles* deletion includes the central copy of this repeat plus one flanking base on each side. Deletions of small tandem repeats probably result from strand slippage and mispairing between repeat units on the leading and trailing strands during DNA replication (Efstratiadis et al. 1980; Spritz 1981). This deletion, which alters the spatial relationship between conserved sequences upstream and downstream from the polyadenylation site (McLaughlan et al. 1985), obviously does not inhibit formation of functional δ-globin mRNA, since the level of Hb A_2 is greater in *Ateles* than in any other primate; on the contrary, this deletion might increase the efficiency of 3′-terminal processing or the stability of δ-globin mRNA in *Ateles.*

In IVS1, a 16-bp deletion (AGGYTYAAGGAGRYVA) apparently occurred in the catarrhine evolutionary line after divergence of the human and OWM lineages. A 2-bp difference (TA) within IVS1 resulted from either a deletion in the platyrrhine evolutionary line or from an insertion in the catarrhine lineage prior to divergence of humans from OWM. A 2-bp deletion (CT) within IVS1 has apparently occurred twice during primate evolution, probably because of its position within a tandem array of CT repeats (Spritz 1981).

In IVS2, two 1-bp (T, T) differences, one 2-bp (TY) difference, one 4-bp (TCAA) difference, and one 13-bp (SCTGCTCTTCYCC) difference resulted either from dele-

tions in the *Ateles* lineage after the platyrrhine/catarrhine divergence or from insertions in the catarrhine lineage after platyrrhine/catarrhine divergence but before divergence of the human and OWM lineages. Four 1-bp (T, A, A, A) differences and a 3-bp (GTT) difference resulted from insertions in the baboon lineage after divergence of the human and OWM lineages. A 3-bp difference (GCT) resulted from a deletion in the baboon lineage following human/OWM divergence. A 2-bp (KR) difference and a 7-bp (AATTAAM) difference resulted from deletions in the human lineage after human/OWM divergence. Apparently, no δ-globin IVS2 insertions occurred in the human lineage after its divergence from the OWM lineage. We also found no differences resulting either from deletions in the catarrhine lineage before human/OWM divergence or from insertions in the *Ateles* lineage after platyrrhine/catarrhine divergence. Other insertions and deletions have occurred in primate δ-globin-coding (Kimura and Takagi 1983; Martin et al. 1983), 5′-untranslated (Martin et al. 1983), and 5′-flanking regions.

Nucleotide Substitution Rates in Primate δ-Globin Genes

As shown in table 1, we performed pairwise comparisons of the nucleotide sequences of the *Ateles,* human, and baboon δ-globin genes by means of the method of Li et al. (1985). Because the *Rhesus* and *Colobus* sequences are incomplete, they were not included in these analyses. The overall rate of noncoding and synonymous substitutions in the *Ateles* and human δ-globin genes is $\sim 1.3 \times 10^{-9}$/site/year, similar to the rate of noncoding and synonymous substitutions in the *Ateles* and human γ-globin genes (1.7×10^{-9}/site/year; Giebel et al. 1985) and in the evolutionarily neutral primate η-globin genes (Chang and Slightom 1984; Koop et al. 1986). As shown in table 1, over virtually the entire δ-globin gene region there has been greater divergence between the spider monkey and baboon than between the spider monkey and humans, a finding generally consistent with a previously defined hierarchy of primate substitution rates (Koop et al. 1986).

For both the *Ateles*/human and *Ateles*/baboon comparisons, the apparent rate of nonsynonymous substitutions is considerably less than the apparent rate of synonymous substitutions, a characteristic of genes under evolutionary conservation. In fact, the number of nonsynonymous differences between the *Ateles* and human δ-globin genes is similar to the number of nonsynonymous differences between the *Ateles* and human γ-globin genes (Giebel et al. 1985), the principal β-like globin gene of the fetus. This suggests that the δ-globin gene may remain under selection in apes, NWM, and man. This is unexpected, because even though the oxygen-carrying properties of Hb A_2 are similar to those of Hb A (reviewed in Bunn and Forget 1986, pp. 62, 64, 345), Hb A_2 has no known physiologic importance. Humans with δ-globin-chain structural variants appear clinically and hematologically normal (reviewed in Vella 1977; Bunn and Forget 1986, pp. 62, 64, 345). Although many δ-chain structural variants apparently result from unidirectional conversions of the δ-globin gene by the β-globin gene (Petes 1982), making the variant δ-globin chains more β-like (and presumably not interfering with function), at least one known δ-chain variant produces a functionally abnormal Hb A_2 (Salkie et al. 1982). Furthermore, individuals with δ-thalassemia, i.e., partial (Pirastu et al. 1983) or complete (Ohta et al. 1971, 1980; Yasukawa et al. 1980) deficiency of δ-globin chains, also appear normal. It may be that δ-globin chains are not absolutely necessary for survival but play a marginally adaptive physiologic role. Only a small decrease in fitness of individuals heterozygous for δ-globin gene mutations might be sufficient to account for the observed conservation of this gene. However, the absence of δ-globin-chain biosynthesis cannot always be

disadvantageous, for silenced δ-globin genes were fixed in the prosimian and OWM lineages.

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