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Neutral Changes During Divergent Evolution of Hemoglobins

Thomas H. Jukes

Space Sciences Laboratory, University of California, Berkeley, CA 94720, USA

Summary. A comparison of the mRNAs for rabbit and human β-hemoglobins shows that synonymous changes in codons have accumulated three times as rapidly as nucleotide replacements that produced changes in amino acids. This agrees with predictions based on the so-called 'neutral theory'. In addition, seven codon changes that appear to be single-base changes (according to 'maximum parsimony') are actually two-base changes. This indicates that the construction of "primordial sequences" is of limited significance when based on inferences that assume minimum base changes for amino acid replacements.

Key words: Neutral changes - Hemoglobin evolution - Primordial sequences

Most changes in the third bases of codons do not change the corresponding amino acids and are hence 'synonymous.' It was stated by King and Jukes (1969) that 'If most DNA species divergence were due to adaptive evolution, then one should expect that the first two nucleotide positions of each codon would change more rapidly than the third position, since synonymous mutations are unlikely to be adaptive. But if DNA divergence in evolution includes the random fixation of neutral mutations, then the third-position nucleotides should change more rapidly, because synonymous mutations are more likely to be neutral.' Kimura (1968) estimated 1% amino acid substitution in 10^7 years for mammalian hemoglobins, corresponding to 0.4 nucleotide replacements per site per 10^9years, uncorrected for multiple hits and back mutations. Later, Kimura (1977) calculated more rapid rates of substitution in mRNA, and he noted that synonymous changes preponderated in histone IV mRNA sequences of two sea urchin species and in mRNA fragments of rabbit and human β hemoglobins.

Further opportunity to examine this point is now afforded by comparing the mRNAs of rabbit and human β hemoglobin chains, as published by Kafatos et al. (1977). The codon changes are summarized in Table 1. The number of amino acid replacements is 14. These are seen to have resulted from 18 nucleotide replacements that led to amino acid changes. The 18 nucleotide replacements have occurred in 283 sites that produce amino acid substitutions when changed. These 18 include four two-base changes in the first two positions of codons, showing that there have been two

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Table 1. Nucleotide Replacements in Divergence between Rabbit and Human Beta-Hemoglobin mRNAs, as Related to Amino-Acid Replacements and Silent Changes

Amino Acid Assignment			Third Base (Silent) Changes					
Codon		Changes	Codon			Codon		
4	(a)	UCC-ACU, Ser-Thr	2		CAU-CAC	86		GCU-GCC
5	(a)	AGU-CCU, Ser-Pro	4	(a)	UCC-ACU	87	(a)	AAG-ACA
21		GAA-GAU, Glu-Asp	10		GUC-GUU	90		GAA-GAG
50		UCU-ACU, Ser-Thr	19		AAU-AAC	111		GUU-GUC
51	(a)	GCA-CCU, Ala-Pro	33		GUU-GUG	115	(a)	UCU-GCC
52		AAU-GAU, Asn-Asp	36		CCA-CCU	117		CAU-CAC
56		AAC-GGC, Asn-Gly	42		UUC-UUU	123		ACU-ACC
69		GCU-GGU, Ala-Gly	47		GAC-GAU	124		CCU-CCA
73		GAG-GAU, Glu-Asp	51	(a)	GCA-CCU	125	(a)	CAG-CCA
76	(a)	AGU-GCU, Ser-Ala	53		GCU-GCA	132		AAG-AAA
87	(a)	AAG-ACA, Lys-Thr	57		AAU-AAC	138		GCC-GCU
112		AUU-UGU, Ile-Cys	66		AAG-AAA	142		GCU-GCC
115	(a)	UCU-GCC, Ser-Ala	68		CUG-CUC	144		AAA-AAG
125	(a)	CAG-CCA, Gln-Pro	71		UUC-UUU	145		UAC-UAU
			74		GGU-GGC	147	(b)	UGA-UAA
			82		AAA-AAG			

(a) Two-base changes that simulate one-base changes by "maximum parsimony"
(b) Silent change in chain-terminating codon

Data from Kafatos et al. (1977)

evolutionary amino acid changes at each site corresponding to these four codons. There are 31 synonymous or 'silent' nucleotide replacements in the remaining 158 nucleotide sites (411 minus 283) that can change without producing replacements of amino acids. These 31 replacements are neutral changes in the sense of protein evolution, although it may be speculated that they could produce changes in RNA secondary structure, or (in some cases) changes in usage of iso-acceptor tRNAs (earlier references and discussion are in Kafatos, et al., 1977). The rates of nucleotide replacement k_{nuc} (Kimura, 1977) are for replacements producing amino acid changes, 0.41×10^{-9} and, for synonymous replacements, 1.4×10^{-9}. Clearly, neutral changes predominate 3.4:1, in the divergence of these two homologous proteins.

Most of the synonymous changes have taken place in codons that are for the same amino acids at homologous sites in both hemoglobin chains. However, five of them occurred in codons 4, 51, 87, 115 and 125 that include two changes, one of which produced a change in the cognate amino acid. This enables a second point to be examined: 'maximum parsimony.' Many studies (e.g., Fitch and Farris, 1974) are based on postulations that amino acid replacements represent the minimum number of base replacements. The β-hemoglobin mRNA comparisons show 2 two-base changes, serine to proline, AGU to CCU at residue 5, and serine to alanine, AGU to GCU at residue 76, that would be represented as UCN to CCN and UCN to GCN in the 'minimum' computation. Thus, a total of seven replacements of two bases per codon has taken place in substitutions of amino acids requiring only a single-base change.

Construction of primordial sequences that depend on minimum descent phylogenies is therefore open to question.

For example, the ancestral codon for serine and proline, residue 5, would be assumed to be UCU or CCU, rather than ACU or CGU, either of which could change to AGU and CCU in the two lines of descent to residue 5.

The actual evolutionary rate of nucleotide change, 11%, in coding regions of mRNA for rabbit and human β-hemoglobins, is far higher than in former calculations based on amino acid replacements (e.g., Fitch and Langley, 1976). This higher rate results from the large number of previously unperceived 'silent' nucleotide replacements. It will necessitate a reappraisal of rates of evolutionary divergence at the DNA level as calculated from hybridization (see Wilson, et al., 1977, pp. 592-593 for discussion) or from amino acid data.

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