Evolutionary Rate of Immunoglobulin Alpha Noncoding Region Is Greater in Hominoids than in Old World Monkeys[1]

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Recent studies on the molecular evolution of primates show that the evolutionary rate among hominoids is considerably slower than that among nonhominoid primates. However, this observation at the nucleotide-sequence level is restricted to the β-globin family region. In this study, we sequenced orthologous immunoglobulin alpha (Cα) genes of chimpanzee, gorilla, orangutan, and crab-eating macaque (an Old World monkey) and compared them with that of the human by using noncoding regions for analysis. Since significant differences in rates among hominoids were not found by using the relative rate test, we evaluated the ratio (R) of the evolutionary distance between Old World monkey and human to the distance between orangutan and human. The R value (1.12) for the Cα gene was much smaller than the expected value (1.38–2.33), showing that the nucleotide substitution rate (= mutation rate per year under selective neutrality) of the Cα gene is greater in the human lineage than in the Old World monkey lineage. We also did a similar analysis for the γ1-, γ2-, ψη -, and δ-globin genes and found a considerable heterogeneity (1.12–2.37) among the R values, including that for the Cα gene. This indicates that the hominoid slowdown of the evolutionary rate is not a universal phenomenon in primate evolution.

Introduction

The debate concerning rate constancy of molecular evolution began immediately after the molecular clock was first proposed by Zuckerkandl and Pauling (1962), and it continues today. Wu and Li (1985), Britten (1986), Li and Tanimura (1987), and Li et al. (1987) argued that the rate of nucleotide substitution is significantly higher in rodents than in primates and artiodactyls. Easteal (1990), however, detected no systematic variation in evolutionary rates among those mammalian orders when he applied a relative-rate test and used marsupial orthologues for reference. Recently, Li et al. (1990) accepted Easteal's (1990) view that rodents diverged first, but they still argued that the evolutionary rate of rodents is at least 1.5 times higher than those in other major eutherian orders. As for the rate variation within primates, Koop et al. (1986, 1989*b*), Li and Tanimura (1987), Li et al. (1987), and Maeda et al. (1988) argued for a rate slowdown in hominoids, on the basis of comparison of noncoding regions or synonymous sites of DNA sequence data. However, a statistically significant slowdown of evolutionary rate in the hominoid lineage has been observed only in the

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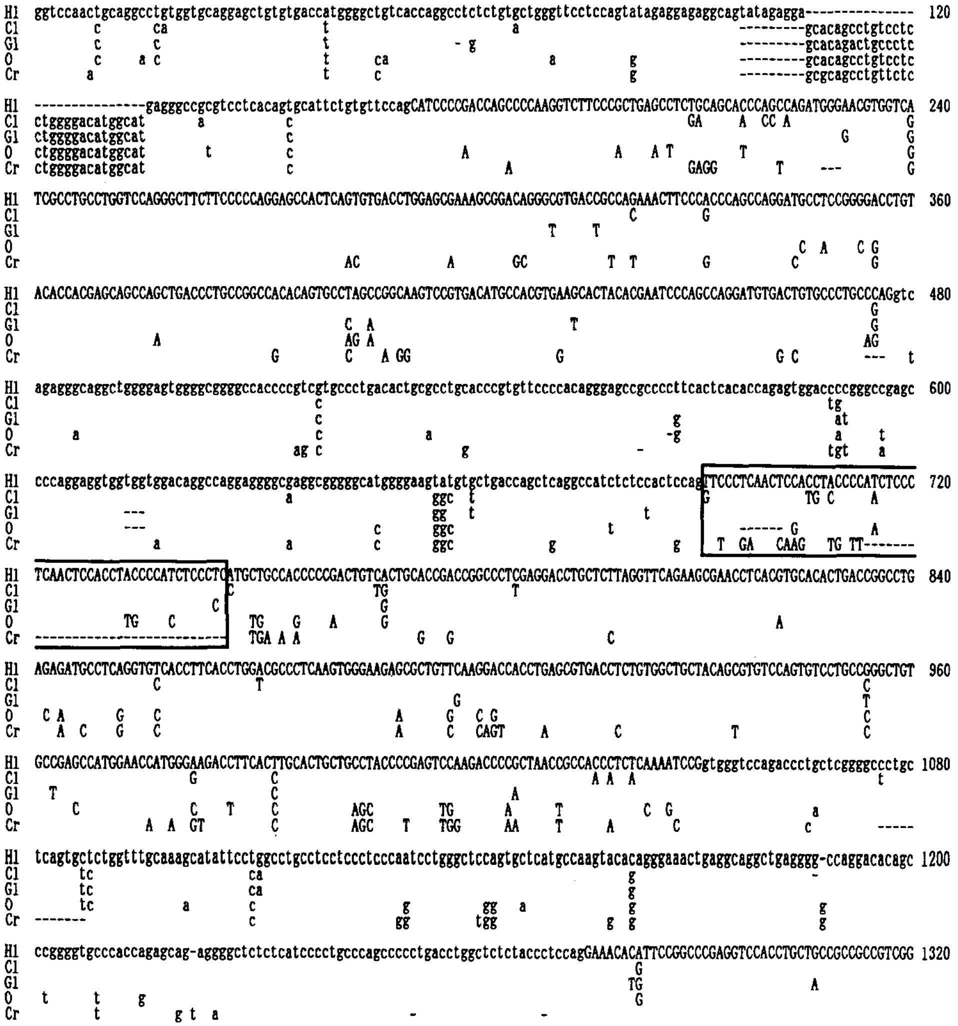

FIG. 1—Aligned nucleotide sequences of human (H1), chimpanzee (C1), and gorilla (G1) Cα 1 and of orangutan (O) and crab-eating macaque (Cr) Cα genes. The nucleotide sequence of the human Cα 1 gene is from Flanagan et al. (1984). Only nucleotides different from that of the human Cα 1 sequence are shown. Gaps are designated by hyphens. Noncoding and coding sequences are denoted by lowercase and capital letters, respectively. Hinge regions are boxed.

$\phi\eta$-globin gene region. DNA/DNA hybridization studies did not support the hypothesis of a hominoid slowdown (Sibley and Ahlquist 1984, 1987; Caccone and Powell 1989).

Especially in the case of a noncoding region or synonymous sites, rate variation is often attributed to the effect of generation time (Laird et al. 1969), to the number of cell divisions in the germ line (Wu and Li 1985), or to changes in DNA repair or replication mechanisms (Britten 1986) rather than to population structure or selection. According to the generation-time hypothesis, taxa with shorter generation times are supposed to have a faster rate of molecular evolution than do those with longer generation times.

In the present study we determined the nucleotide sequences of the immunoglobulin alpha 1 (Cα 1) genes of chimpanzee and gorilla, as well as single-copy Cα genes of orangutan and crab-eating macaque, and compared them with that of the

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H1  AGGAGCTGGCCCTGAACGAGCTGGTGACGCTGACGTGCCTGGCACGCGGCTTCAGCCCCAAGGACGTGCTGGTTCGCTGGCTGCAGGGGTCACAGGAGCTGCCCCGCGAGAAGTACCTGA 1440
C1
G1                                                T                                                           T
0
Cr                                                             G    T                  A    AA  G AC          T  C

H1  CTTGGGCATCCCGGCAGGAGCCCAGCCAGGGCACCACCACCTTCGCTGTGACCAGCATACTGCGCGTGGCAGCCGAGGACTGGAAGAAGGGGGACACCTTCTCCTGCATGGTGGGCCACG 1560
C1                                                                           T              A
G1                                                                                                                        T
0                                             A
Cr        A       A                               C

H1  AGGCCCTGCCGCTGGCCTTCACACAGAAGACCATCGACCGCTTGGCGGGTAAACCCACCCATGTCAATGTGTCTGTTGTCATGGCGGAGGTGGACGGCACCTGCTACTGAgccgcccgcc 1680
C1                                           C                                           A                           a
G1                                           C                   C
0                                            C                   C              G
Cr                                           C                   C              G           A

H1  tgtccccacccctgaataaactccatgctcccccaagcagccccacgcttccatccggcgcctgtctgtccatcctcagggtctcagcacttgggaaagggccagggcatggacagggaa 1800
C1                          g                                                                             t ----------
G1                                                                                                             a
0           g                        t                     t                                  a                  c a a
Cr                               t                                --                          g                  c   g

H1  gaatacccccctgccctgagcctcggggggcccctggcacccccatgagactttccaccctggtgtgagtgtgagttgtgagtgtgagagtgtgtggtgcaggaggcctcgctggtgtgag 1920
C1                                          t       c                         c
G1                                                  c
0                               -                   c                                                                      a
Cr     c     -              t       -          g    c                                   c                  a               a

H1  atcttaggtctgccaaggcaggcacagcccaggatgggttctgagagacgcacatgccccggacagttctgagtgagcagtggcatggccgtttgtccctgagagagccgcctctggctg 2040
C1                g                  g                             g                     c
G1                g                  g                             g                     c
0                 g                  g                             g           g
Cr                g                  g                             g           g    c                           ----------

H1  tagctgggagggaatagggagggtaaaaggagcaggctagccaagaaaggcgcaggtagtggcaggagcggcgagggagtgaggggctggactccagggccccactgggaggacaagctc 2160
C1  c                                             c               g
G1  c                                                             g                            t
0   c                       g g           g  gg      ag           g
Cr  c               --   t  g g a         g   g      ag        c  g      a                                 c

H1  caggagggccccaccaccctagtgggtgggcctcaggacgtccc-actgacgcatgcaggaaggggcacctccccttaaccacactgctctgt---------------acggggcacgtg 2280
C1                                                                                             c                        t
G1                                                                            -------------------               ------------
0   tg             c             -                       g     a              gc                 accacactgctctgt
Cr                         a              t     c     t    c   a                                                         ac

H1  ggcacaggtgcacactcacactcacatatatgcctgagccctgcaggagcggaacgttcacagcccagacccagttccagaaaagccagggggagtcccctcccaagcccccaa------- 2400
C1        ct                      c
G1  -----------              g    c                   a   t                                                   a
0         -       a               c                   a                   at  c        g a                          gcgggcat
Cr   a    c             gc        c                   a                       c                                     gcgggcat

H1  ------gctcagcctgctcccctaggcccctctggcttccctgtgtttccactgtgcacagatcaggcaccaactccacagacccc-tcccaggcagcccctgctccctgcctggccaag 2520
C1                        c                                      c
G1                        c                                      c     g
0   tcccag                c             c                        c     g t
Cr  tcccag            g   c             c                a       c     g                  c                               --

H1  tctcccatcccttcctaagcccaactaggacccaaagcatagacagggaggggccacgtggggtggcatcagaag 2595
C1                                 a                       g
G1
0                                                       a  g a
Cr  -------                         gt                    tg        a
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FIG. 1 (*Continued*)

human Cα 1 gene. We show that the evolutionary rate of the Cα noncoding region is greater in hominoids than in Old World monkeys and discuss possible causes for the evolutionary-rates difference between the two lineages of higher primates.

Table 1
Estimated Numbers ± SEs of Nucleotide Substitutions of Primate Cα Noncoding Regions

	Human	Chimpanzee	Gorilla	Orangutan
Chimpanzee	2.77 ± 0.46			
Gorilla	2.46 ± 0.43	1.86 ± 0.37		
Orangutan	5.64 ± 0.66	5.17 ± 0.63	4.62 ± 0.60	
Crab-eating macaque	6.36 ± 0.71	5.96 ± 0.68	5.88 ± 0.68	5.80 ± 0.67

NOTE.—A total of 1,362 nucleotides were used for comparison. Values are $K^c \times 100$.

Material and Methods

Chimpanzee (*Pan troglodytes*) and gorilla (*Gorilla gorilla*) Cα 1 genes were isolated from their genome libraries, as were orangutan (*Pongo pygmaeus*) and crab-eating macaque (*Macaca fascicularis*) Cα genes (Ueda et al. 1988; Kawamura et al. 1990). DNA manipulations were performed using standard procedures (Sambrook et al. 1989). All sequencing was performed in both sense and antisense strands by using the dideoxynucleotide chain-termination method. The number of nucleotide substitutions per site (K^c) was estimated by using the formula $K^c = -(3/4)\ln[1-(4/3)K]$, where K is the number of nucleotide differences per site (Jukes and Cantor 1969). In this computation, gaps were excluded. The standard errors of branching points were calculated by using Nei et al.'s (1985) method. To test rate constancy within hominoid lineages, Wu and Li's (1985) relative-rate test was applied.

Results and Discussion

Human, chimpanzee, gorilla, and gibbon have two Cα genes (Cα 1 and Cα 2), while orangutan and Old World monkeys have a single Cα gene in their genomes (Flanagan et al. 1984; Ueda et al. 1988; Kawamura et al. 1990). The orangutan Cα gene is considered to be orthologous to the Cα 1 genes in other hominoids, because its hinge-coding region consisting of two 30-bp repeats with a 6-bp overlap (boxed in fig. 1) is characteristic of Cα 1 genes (Kawamura et al. 1990). In the present study we added further nucleotide sequence to our previous data of the gorilla Cα 1 gene (Kawamura et al. 1989) and newly determined those of the chimpanzee Cα 1 gene and of the orangutan and crab-eating macaque Cα genes (fig. 1). We analyzed the nucleotide sequences of noncoding regions (table 1), most of which are considered to be effectively neutral. Therefore, substitution rates estimated in the present study would be expected to be equal to the mutation rate per year (Kimura 1983). We first evaluated the rate variation among hominoid lineages by carrying out the relative-rate test by using the crab-eating macaque gene as an outgroup reference. There was no statistically significant variation among the evolutionary rates of hominoids, as shown in table 2. Therefore, we reconstructed a phylogenetic tree by using the unweighted pair-group method of analysis (UPGMA), which assumes rate constancy (Sokal and Sneath 1963). The branching pattern of the resulting tree (fig. 2) where the orangutan diverged first among the four hominoids was in agreement with a generally accepted phylogenetic tree (Saitou 1991, and references therein). Chimpanzee clusters not with human but with gorilla in

Table 2
Evolutionary-Distance Differences between Old World Monkey and Hominoids

Species 1/Species 2	$K_1 - K_2$[a]	SE	P[b]
Human/chimpanzee	0.40	0.48	>0.2
Human/gorilla	0.48	0.45	>0.2
Human/orangutan	0.56	0.68	>0.2
Chimpanzee/gorilla	0.08	0.39	>0.5
Chimpanzee/orangutan	0.16	0.65	>0.5
Gorilla/orangutan	0.08	0.61	>0.5

[a] K_i (i = 1 or 2) is the number of nucleotide substitutions in the Cα noncoding region, per 100 sites, between species i and an Old World monkey (crab-eating macaque).

[b] Probability that there is no difference between K_1 and K_2, which is evaluated using a two-tailed t-test with degree of freedom = ∞.

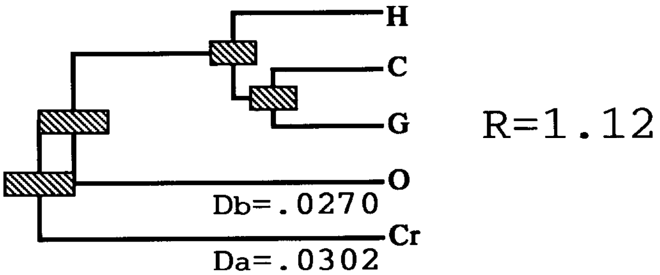

FIG. 2—Phylogenetic trees of primate immunoglobulin Cα genes, constructed by UPGMA. H = human; C = chimpanzee; G = gorilla; O = orangutan; and Cr = crab-eating macaque. Branch lengths are proportional to the numbers of nucleotide substitutions per site. For each branch, a shaded box shows the standard error. Da and Db are the branch lengths leading to Old World monkey and orangutan, respectively. R = Da/Db.

this tree, though this branching order is not statistically significant. Other estimation methods (Kimura 1980; Tajima and Nei 1984) gave essentially the same K^c values as did the Jukes-Cantor method used in the present study.

In this tree, the branch length (Da = 0.0302) leading to Old World monkey was not much longer than that (Db = 0.0270) leading to orangutan (fig. 2). The ratio (R) of Da to Db was 1.12. We compared these values (Da, Db, and R) with corresponding values for other nuclear DNA sequences available (fig. 3). All these sequences are from the noncoding regions of β-globin-family genes (γ1-, γ2-, ϕη-, and δ-globin genes; see table 3) (Fitch et al. 1988; Maeda et al. 1988; Miyamoto et al. 1988; Slightom et al. 1988; Koop et al. 1989*a*). As these data included the nucleotide sequences of New World monkeys, it enabled us to conduct a relative-rate test between hominoids and Old World monkeys by using New World monkey as an outgroup. Significantly lower substitution rates in hominoids than in Old World monkeys were observed in the ϕη-globin region of all the hominoids (0.1% level) and in the γ2-globin region of chimpanzee (5% level). This is consistent with the result that, compared with those in the Old World–monkey lineage, the β-globin-family genes in the hominoid lineage show a slowdown in substitution rates (Koop et al. 1986; Li and Tanimura 1987; Li et al. 1987; Easteal 1991). However, rate variation among hominoids was not detected in these four DNA regions. We also used the neighbor-joining method, which does not assume rate constancy (Saitou and Nei 1987). The resulting trees (not shown) had the same branching patterns as those of figures 2 and 3, and their branch lengths were more or less the same, though the branch length for the human lineage was slightly longer than those for other hominoid lineages in the Cα tree. The Db value for the Cα gene was significantly greater than that for the γ2- and δ-globin genes [at the 5% level (t = 1.965 and 2.553, respectively, with degrees of freedom = ∞ when a two-tailed t-test is used)] and than that for the ϕη-globin gene [at the 0.1% level (t = 4.581)]. The Db for the γ1-globin gene was smaller than that for the Cα gene, though there was no statistical significance (t = 1.630). In contrast, Da values for these genes were close to each other.

From data obtained from fossil records, it is generally accepted that the orangutan diverged from the lineage of man-chimpanzee-gorilla 12–16 Mya and that Old World monkeys diverged from hominoids 22–28 Mya (Gingerich 1984; Fleagle 1986; Pilbeam 1986). Therefore, the R value would be expected to be in the range of 1.38–2.33, if the evolutionary rate is assumed to be constant in all phylogenetic lineages. As shown in figure 4, however, there was a great heterogeneity among the R values observed; the R values of the γ1-, γ2-, and δ-globin genes were within the expected range, whereas

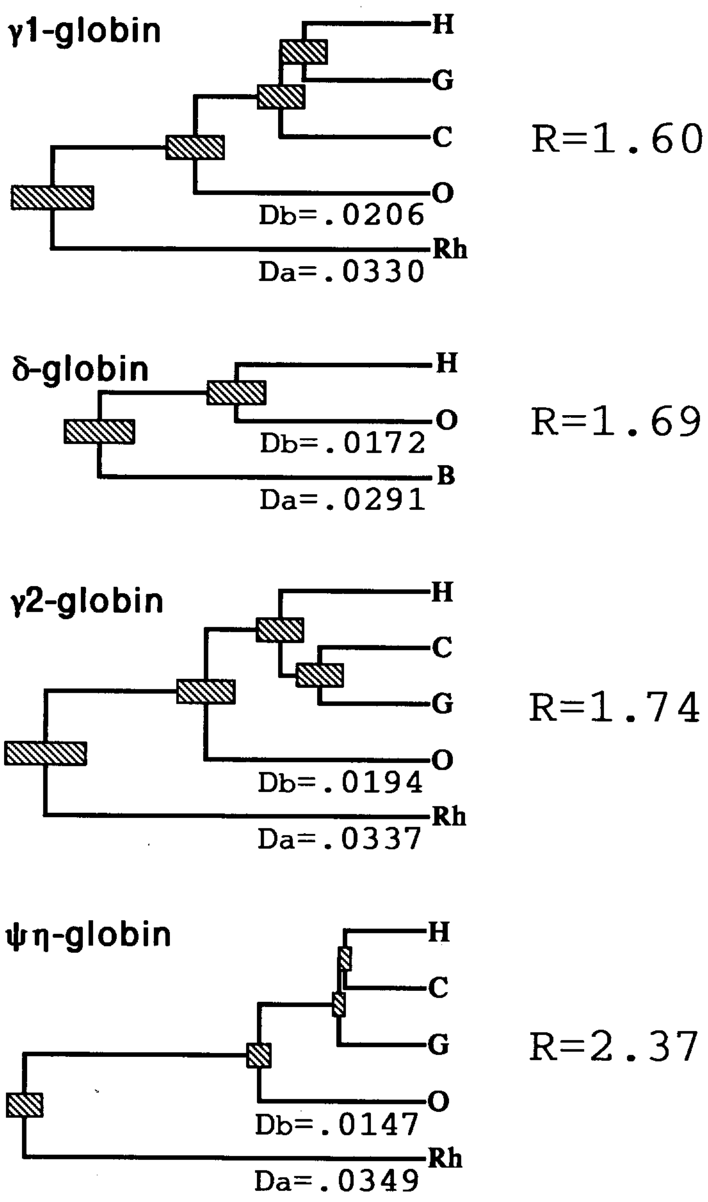

FIG. 3—Phylogenetic trees of primate β-globin family genes, constructed by UPGMA. Rh = rhesus monkey; B = baboon; all other abbreviations are as in fig. 2. Branch lengths are denoted by shaded boxes. Da, Db, and R are as in fig. 2.

that of the Cα gene was smaller than expected and that of the ϕη-globin gene was larger than expected. Contrary to a former claim that a slowdown in evolutionary rates occurred in hominoids, these results indicate that the evolutionary rate in the noncoding region—namely, the mutation rate per year—of the Cα gene is greater in hominoids than in Old World monkeys. These results suggest that the mutation rate for the Cα gene has increased in the hominoid lineage.

The evolutionary distances between the Old World monkey Cα gene and each hominoid Cα gene, including the Cα 2 gene, have been shown to be similar to each

Table 3
Estimated Numbers ± SEs of Nucleotide Substitutions of Primate γ1-, γ2-, φη-, and δ-Globin Noncoding Regions

	Human	Chimpanzee	Gorilla	Orangutan	Rhesus Monkey
γ1-Globin region:[a]					
Chimpanzee	2.17 ± 0.41				
Gorilla	2.17 ± 0.41	2.96 ± 0.48			
Orangutan	3.83 ± 0.55	4.00 ± 0.56	4.56 ± 0.60		
Rhesus monkey	6.12 ± 0.71	6.61 ± 0.74	6.95 ± 0.75	6.70 ± 0.74	
Spider monkey	11.88 ± 1.01	11.79 ± 1.01	12.42 ± 1.04	11.79 ± 1.01	13.23 ± 1.08
γ2-Globin region:[b]					
Chimpanzee	2.41 ± 0.44				
Gorilla	2.89 ± 0.48	1.86 ± 0.38			
Orangutan	4.09 ± 0.57	3.69 ± 0.54	4.25 ± 0.58		
Rhesus monkey	6.55 ± 0.73	6.64 ± 0.74	7.39 ± 0.78	7.06 ± 0.76	
Spider monkey	12.37 ± 1.04	11.92 ± 1.02	12.46 ± 1.04	12.37 ± 1.04	13.92 ± 1.11
φη-Globin region:[c]					
Chimpanzee	1.44 ± 0.13				
Gorilla	1.47 ± 0.13	1.58 ± 0.13			
Orangutan	2.88 ± 0.18	2.96 ± 0.18	2.98 ± 0.18		
Rhesus monkey	6.96 ± 0.29	7.09 ± 0.29	6.92 ± 0.28	6.94 ± 0.28	
Spider monkey	10.32 ± 0.35	10.47 ± 0.36	10.30 ± 0.35	10.47 ± 0.36	11.90 ± 0.38
	Human	Orangutan	Baboon		
δ-Globin region:[d]					
Orangutan	3.43 ± 0.50				
Baboon	5.58 ± 0.64	6.04 ± 0.67			
Spider monkey	8.58 ± 0.81	9.05 ± 0.83	9.60 ± 0.86		

NOTE.—Values are $K^c \times 100$.

[a] A total of 1,311 nucleotides were used.

[b] A total of 1,307 nucleotides were used.

[c] A total of 9.204 nucleotides were used.

[d] A total of 1,431 nucleotides were used.

other (S. Kawamura, N. Saitov, and S. Ueda, unpublished data). This suggests that changes introduced/erased by gene conversion are canceled out, so that gene conversion has little effect on evolutionary distances between Old World monkeys and hominoids. If the nucleotides of Cα 1 and Cα 2 genes were considerably different before orangutan diverged, and if gene conversions of Cα 1 genes by Cα 2 genes occurred after the divergence, the evolutionary distance between the orangutan Cα and the human-chimpanzee-gorilla Cα 1 genes would be longer than it would be in the absence of such a conversion. We applied the site-by-site reconstruction method of gene conversion (Fitch et al. 1990) to examine this possibility, but such events were not detected (S. Kawamura, N. Saitov, and S. Ueda, unpublished data). Sequence data from New World monkeys would provide more insight into the evolution of the Cα gene.

For each gene examined in the present study, base composition was similar among different species. The G+C content of the Cα genes of higher primates was much higher (average 64%) than that of the remaining genes (average 45%, 44%, 39%, and 37% for γ1-, γ2-, φη-, and δ-globin genes, respectively), but there was no association between the R values and G+C contents.

Various mechanisms have been proposed to explain the variation in substitution

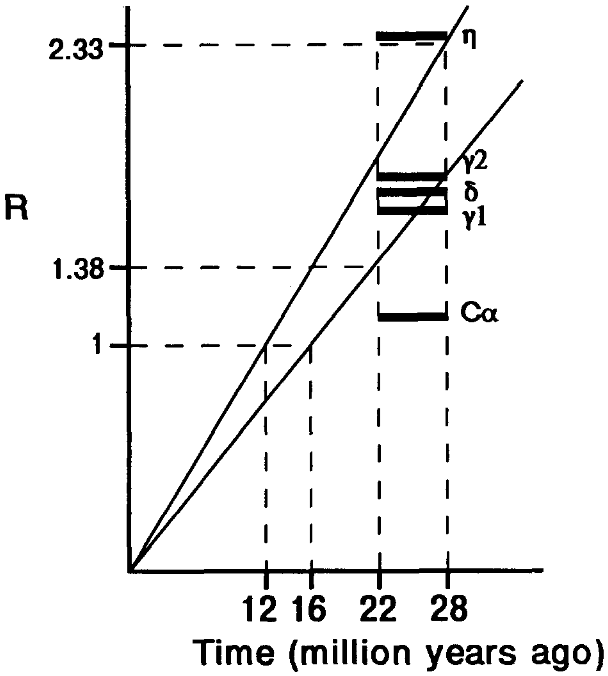

FIG. 4—Heterogeneity among observed R values. From fossil records, the divergence time between orangutan and man-chimpanzee-gorilla and that between Old World monkeys and hominoids have been estimated to be 12–16 and 22–28 Mya, respectively. Therefore, the range of the expected R value would be 1.38–2.33, if constancy of evolutionary rate is assumed.

rate. These include selection pressure, population size (Kimura 1983), generation time (Laird et al. 1969), number of germ-line replications (Wu and Li 1985), and DNA repair mechanisms (Britten 1986). The nucleotide sequences compared in the present study are noncoding regions, for which the substitution rate is considered to be affected neither by selection nor by population size (Kimura 1983). The generation-time-effect hypothesis and the germ-line-replication hypothesis are unlikely to be correct, because they (1) require a hominoid-directed slowdown of the evolutionary rate during primate evolution and (2) are incompatible with the coexistence of slowdown and speedup in DNA regions in the genome, a coexistence that was found in the present study.

A recent analysis suggests that mutation rate varies according to region of the mammalian genome, possibly because of regional differences in DNA repair or replication mechanisms (Wolfe et al. 1989). However, if we assume only regional differences in mutation rate, the R values of the different DNA regions shown in figures 2 and 3 would have to be similar to each other. Alternatively, if only a lineage (taxonomic) difference in mutation rate exists (Britten 1986), the presence of similar Da values in different DNA regions would be incompatible with the great variation in Db values. Thus, variation in evolutionary rate must be caused not by a single factor but by multiple factors.

Sequence Availability

Nucleotide sequence data reported in the present paper have been deposited in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession numbers X53702 (chimpanzee Cα 1), X53703 (gorilla Cα 1), X53704 (orangutan Cα), and X53705 (crab-eating macaque Cα).

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