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Multiple Recombinational Events in Primate Immunoglobulin Epsilon and Alpha Genes Suggest Closer Relationship of Humans to Chimpanzees than to Gorillas

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Summary. Immunoglobulin epsilon and alpha genes of chimpanzee and gorilla were isolated and their structures were compared with their human counterparts. Multiple deletions and duplications seem to have happened in both genes during hominoid evolution; the chimpanzee had deleted the entire $C_{\epsilon 2}$ gene after its divergence. In addition, the length of the $C_{\alpha 1}$ hinge region of gorilla is distinct from those of chimpanzee and humans. Structural homology of the epsilon and alpha genes suggests that humans are evolutionarily closer to chimpanzees than to gorillas.

Key words: Immunoglobulin gene — Hominoid evolution — Multiple recombination

Introduction

The heavy-chain constant region (C_H) genes of the human immunoglobulin are located on chromosome 14 in the order 5′-C_μ-C_δ-$C_{\gamma 3}$-$C_{\gamma 1}$-$C_{\epsilon 2}$-$C_{\alpha 1}$ . . . ψC_γ . . . $C_{\gamma 2}$-$C_{\gamma 4}$-$C_{\epsilon 1}$-$C_{\alpha 2}$ . . . 3′ (Ellison and Hood 1982; Flanagan and Rabbitts 1982; Krawinkel and Rabbitts 1982; Lefranc et al. 1982; Max et al. 1982; Takahashi et al. 1982; Bech-Hansen et al. 1983; Hisajima et al. 1983; Flanagan et al. 1984; Migone et al. 1984). In addition to this cluster, the human genome contains a processed C_ϵ pseudogene ($C_{\epsilon 3}$) on chromosome 9 (Battey et al. 1982; Ueda et al. 1982). The human immunoglobulin C_ϵ gene family thus consists of three members; the $C_{\epsilon 1}$ gene (active), the $C_{\epsilon 2}$ gene (truncated pseudogene), and the $C_{\epsilon 3}$ gene (processed pseudogene), which are located on 2.7-kb, 5.9-kb, and 8.0-kb *Bam*HI fragments, respectively (Nishida et al. 1982; Ueda et al. 1982; Hisajima et al. 1983). Since mouse contains only one C_ϵ gene (Shimizu et al. 1982), the addition of two C_ϵ pseudogenes in the human genome seems to have taken place after mammalian radiation. Using a human C_ϵ probe specific to each family member, we have recently found that Old World monkeys have two C_ϵ genes, one of which is processed. Among the hominoids, only the gorilla and the human genomes contained the three C_ϵ genes, whereas other hominoids, including chimpanzee, had the $C_{\epsilon 1}$ and $C_{\epsilon 3}$ genes but not the $C_{\epsilon 2}$ gene (Ueda et al. 1985). The results indicated two alternative possibilities: (1) gorilla is more closely related to human than is chimpanzee, or (2) chimpanzee has lost the $C_{\epsilon 2}$ gene after the divergence of this species. To distinguish between these possibilities we have isolated DNA fragments containing the $C_{\epsilon 1}$, $C_{\epsilon 2}$, $C_{\alpha 1}$, and $C_{\alpha 2}$ genes from gorilla and chimpanzee DNAs and analyzed characteristics of these DNA segments by nucleotide sequence determination.

Materials and Methods

Materials. Restriction endonucleases, T4 DNA ligase, bacterial alkaline phosphatase, and M13 sequencing kit were purchased from Takara Shuzo Co. [α-^{32}P]dCTP (ca. 3000 Ci/mmol) was from New England Nuclear.

Cloning and Characterization of the Epsilon and Alpha Genes. High molecular weight DNAs of chimpanzee and gorilla were prepared as previously described (Ueda et al. 1985). The *Hin*dIII

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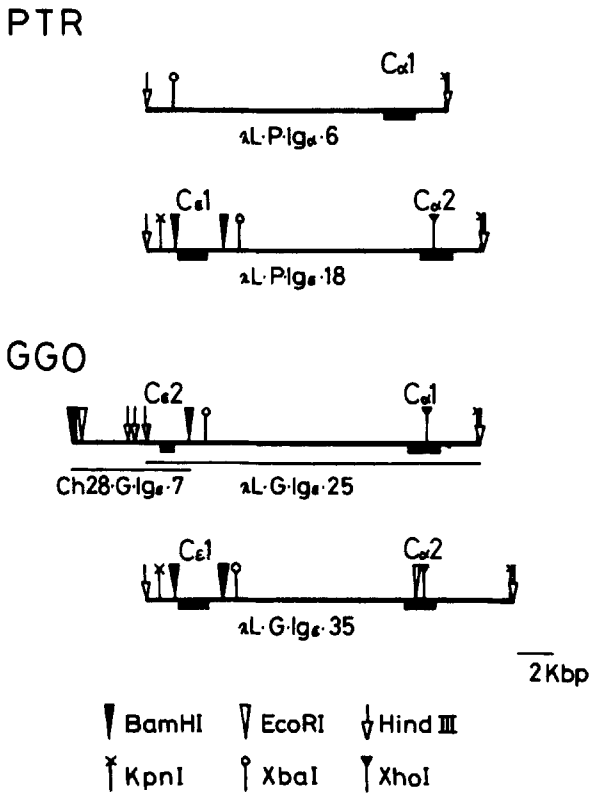

Fig. 1. Linkage maps of the C_ϵ and C_α genes in the chimpanzee (PTR) and the gorilla (GGO) genomes. Closed boxes represent the exons and introns of each gene. The subclasses of C_ϵ and C_α genes were identified as mentioned in the text. The position of the chimpanzee $C_{\alpha 1}$ gene is tentative because there are no appropriate six-base restriction enzyme cleavage sites. The restriction map of the segment linking the $C_{\epsilon 2}$ and $C_{\alpha 1}$ genes in the gorilla genome was constructed from those of the overlapping clones and the Southern hybridization data of the gorilla genomic DNA.

and *Bam*HI complete digests of each DNA were fractionated by agarose gel electrophoresis and cloned using bacteriophage λL47.1 and Charon 28, respectively, as vectors. The recombinant clones containing C_ϵ and C_α genes were isolated using the human $C_{\epsilon 1}$ and $C_{\alpha 1}$ gene fragments, respectively, as probes as previously described (Takahashi et al. 1982). Restriction fragments of the phage clones were subcloned into M13mp10, mp11, or pUC18 and the nucleotide sequences were determined by the dideoxynucleotide chain-termination method (Messing 1983).

Results

Cloning of Epsilon and Alpha Genes from Chimpanzee and Gorilla DNAs

The C_ϵ and C_α genes were cloned from chimpanzee and gorilla DNAs using the human $C_{\epsilon 1}$ and $C_{\alpha 1}$ gene fragments, respectively, as probes. In the human genome the $C_{\epsilon 2}$–$C_{\alpha 1}$ genes and the $C_{\epsilon 1}$–$C_{\alpha 2}$ genes are located on the 20.3-kb and 21.7-kb *Hin*dIII fragments, respectively (Flanagan and Rabbitts 1982; Hisajima et al. 1983). Southern blot hybridization of *Hin*dIII digests of chimpanzee DNA revealed 19-kb and 16-kb C_α-hybridizing fragments, whereas the C_ϵ probe detected 19-kb and 7.7-kb *Hin*dIII fragments (data not shown). The 7.7-kb *Hin*dIII fragment was shown to contain the $C_{\epsilon 3}$ gene by the $C_{\epsilon 3}$-specific probe. Similar studies on the *Hin*dIII-digested gorilla DNA showed 19-kb doublet fragments of the C_α genes, and 19-kb and 8.5-kb fragments of the C_ϵ genes. The 8.5-kb *Hin*dIII fragment was shown to contain the $C_{\epsilon 3}$ gene by the $C_{\epsilon 3}$-specific probe.

We have fractionated 19-kb and 16-kb C_α fragments of *Hin*dIII-digested chimpanzee DNA and the 19-kb C_α fragments of *Hin*dIII-digested gorilla DNA by agarose gel electrophoresis, and cloned them into λL47.1 vector (Loenen and Brammar 1980). A clone ($\lambda L \cdot P \cdot Ig_\alpha \cdot 6$) containing only a C_α gene (16-kb *Hin*dIII fragment) and a clone ($\lambda L \cdot P \cdot Ig_\epsilon \cdot 18$) containing a C_ϵ and a C_α gene (19-kb *Hin*dIII fragment) were obtained from chimpanzee DNA as shown in Fig. 1. On the other hand, two independent clones ($\lambda L \cdot G \cdot Ig_\epsilon \cdot 25$ and $\lambda L \cdot G \cdot Ig_\epsilon \cdot 35$), each containing a C_ϵ and a C_α gene (19-kb *Hin*dIII fragment), were obtained from gorilla DNA (Fig. 1). The 6.9-kb *Bam*HI fragment of gorilla DNA was previously shown to contain the $C_{\epsilon 2}$ (truncated) gene (Ueda et al. 1985). The fragment was purified, cloned into Charon 28 vector, and designated as $Ch28 \cdot Ig_\epsilon \cdot 7$. The C_ϵ genes in the chimpanzee clone $\lambda L \cdot P \cdot Ig_\epsilon \cdot 18$ and in the gorilla clone $\lambda L \cdot G \cdot Ig_\epsilon \cdot 35$ seem to be the $C_{\epsilon 1}$ gene, as both are located on the 2.7-kb *Bam*HI fragments (Ueda et al. 1985). Another gorilla clone ($\lambda L \cdot G \cdot Ig_\epsilon \cdot 25$), carrying a C_ϵ gene, overlapped with the clone $Ch28 \cdot Ig_\epsilon \cdot 7$ containing the 6.9-kb *Bam*HI fragment of the gorilla $C_{\epsilon 2}$ gene.

Characteristics and Linkage of the Epsilon and Alpha Genes

To confirm the assignment of the C_α and C_ϵ genes and to analyze their detailed structures, we determined nucleotide sequences of the regions hybridized with C_α and C_ϵ probes. The hinge region of the human $\alpha 1$ chain is 13 amino acid residues longer than that of the human $\alpha 2$ chain, whereas the remaining portions of the two chains can be aligned without any insertions and deletions (Torano and Putnam 1978). Comparison of the hinge region is therefore most appropriate to distinguish the $C_{\alpha 1}$ gene from the $C_{\alpha 2}$ gene. The hinge regions of both human and mouse C_α genes are encoded by the 5′ portion of the CH2 exon, whereas the C_δ and C_γ genes have one or more small separate hinge-encoding exons (Tucker et al. 1981; Flanagan et al. 1984).

As shown in Fig. 2, the hinge region of the human $C_{\alpha 1}$ gene consisted of two tandem 30-bp repeats with

a 6-bp overlap. The 30-bp units were further divided into two tandem 15-bp units. The hinge region of the human $C_{\alpha 2}$ gene consisted of one 15-bp unit. The C_α genes in the chimpanzee clone λL·P·Ig$_\epsilon$·18 and the gorilla clone λL·G·Ig$_\epsilon$·35 had short hinge regions (15 bp) that are almost identical to that of the human $C_{\alpha 2}$ gene. The C_α gene in the chimpanzee clone λL·P·Ig$_\alpha$·6 had a long hinge region (54 bp) like the human $C_{\alpha 1}$ gene. The C_α gene of the gorilla clone λL·G·Ig$_\epsilon$·25 had a hinge region of intermediate length (30 bp). Although the hinge region of this C_α gene is shorter than that of the human $C_{\alpha 1}$ gene, this C_α gene should be classified as the $C_{\alpha 1}$ gene for two reasons: (1) the C_α gene in λL·G·Ig$_\epsilon$·35 is identified as the $C_{\alpha 2}$ gene and (2) the C_α gene in λL·G·Ig$_\epsilon$·25 is linked to the $C_{\epsilon 2}$ gene described below.

Since the $C_{\epsilon 2}$ gene is linked to the $C_{\alpha 1}$ gene in the human genome, we have determined the nucleotide sequence of the 5′ portion (1.2-kb *Hin*dIII/*Xba*I fragment) of λL·P·Ig$_\alpha$·6 to test whether the $C_{\epsilon 2}$ gene was deleted from the chimpanzee DNA. We have also determined the nucleotide sequence of the gorilla $C_{\epsilon 2}$ gene in CH28·G·Ig$_\epsilon$·7 and compared it with that of the human $C_{\epsilon 2}$ gene. As shown in Fig. 3, the coding sequence of the $C_{\epsilon 2}$ gene was completely deleted from the chimpanzee genome. The gorilla $C_{\epsilon 2}$ gene has the 5′ portion deleted and the 3′ half of the CH2 exon retained, and has the entire CH3 and CH4 exons. The deletion of the $C_{\epsilon 2}$ gene began approximately 2.1 kb upstream of the CH1 exon in both species, and ended approximately 1 kb downstream to the CH4 exon in the chimpanzee $C_{\epsilon 2}$ gene and at the 64th nucleotide of the CH2 exon in the gorilla $C_{\epsilon 2}$ gene. In the human $C_{\epsilon 2}$ gene (Max et al. 1982; Hisajima et al. 1983) the deletion began at a similar position as above but ended at the second nucleotide of the CH3 exon. The results showed, as expected, that the $C_{\epsilon 1}$ and $C_{\epsilon 2}$ genes were linked to the $C_{\alpha 2}$ and $C_{\alpha 1}$ genes, respectively, in the gorilla genome. The $C_{\epsilon 2}$ exons and introns were completely deleted from the chimpanzee genome, confirming our previous results (Ueda et al. 1985).

The nucleotide sequence upstream from the deletion points of the human $C_{\epsilon 2}$ gene matched well those of the corresponding regions of the chimpanzee and the gorilla $C_{\epsilon 2}$ DNA. It is worth noting that the 35-bp unit sequence is tandemly duplicated several times at the border of the deletion in the $C_{\epsilon 2}$ genes of the two species as well as in the human $C_{\epsilon 2}$ gene. It is reasonable to find tandem repetition of unit sequences in this region, which corresponds to the S region mediating class switching of the immunoglobulin heavy chain (Shimizu and Honjo 1984). The repeating units of the S$_\epsilon$ region are less homologous with each other (Nikaido et al. 1982), but typical short sequences (TGGG, AGCT), common to S regions, are seen in this region. There was

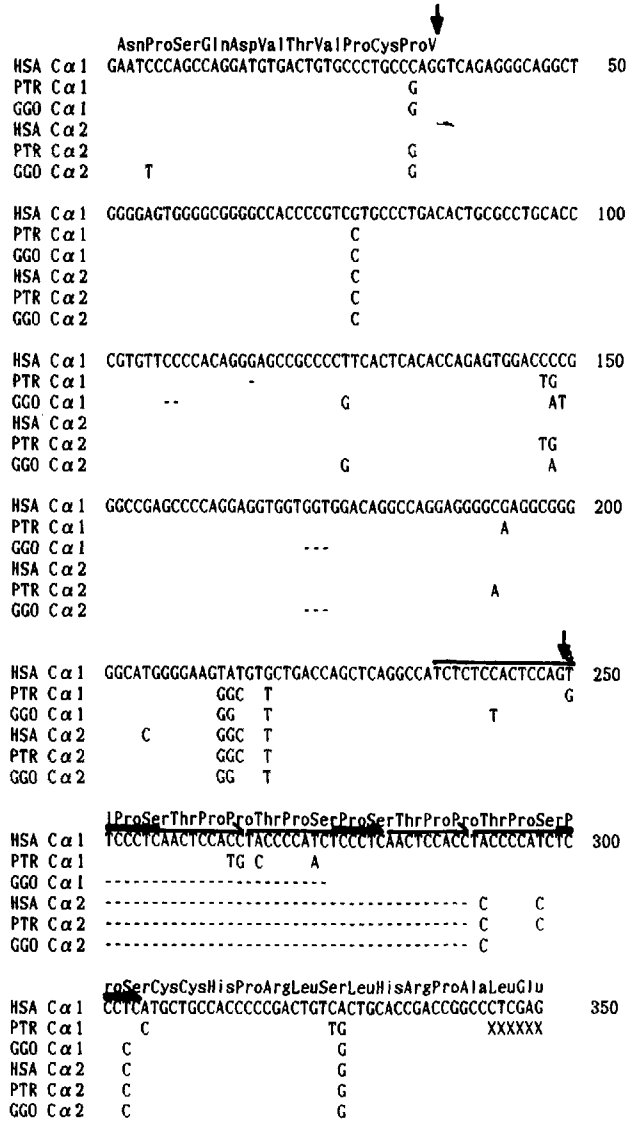

Fig. 2. Comparison among nucleotide sequences of the human (HSA), the chimpanzee (PTR), and the gorilla (GGO) C_α genes. The sequences between the 3′ end of the CH1 exon to the 5′ end of the CH2 exon. The vertical arrows mark RNA splicing sites. Only nucleotides different from the human $C_{\alpha 1}$ gene sequence (Flanagan et al. 1984) are shown in the other C_α genes. Deleted nucleotides are shown by hyphens. Horizontal arrows indicate the 15-bp repeats of the hinge region. Thicker underlines show conserved 6-bp overlaps between repeats and their homologs.

no strict homology between the nucleotide sequences of the deletion points.

Discussion

In summary, humans, chimpanzees, and gorillas have two C_α genes and so the duplication of the C_γ-C_γ-C_ϵ-C_α genes is likely to have taken place in their common ancestor. The $C_{\alpha 2}$ genes of the three species have homologous hinge regions (Fig. 2). On the other hand, the hinge regions of the human and the

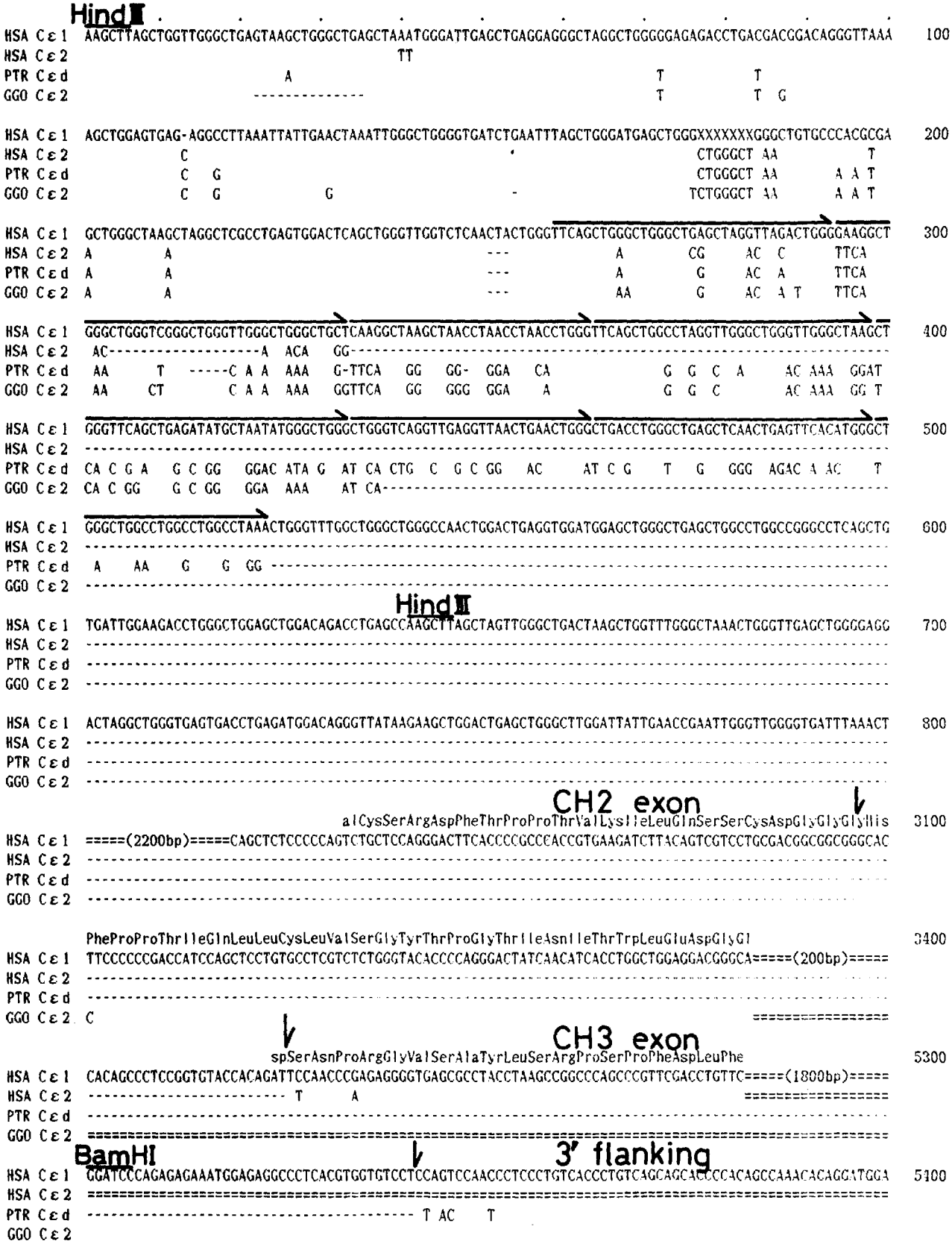

Fig. 3. Nucleotide sequences of the border regions of deletion in the human (HSA), chimpanzee (PTR), and gorilla (GGO) $C_{\varepsilon 2}$ genes. The nucleotide sequences shown are starting from the *Hin*dIII site located at the 5′ ends of both the clone λL·P·Ig$_{\varepsilon}$·6 and the clone λL·G·Ig$_{\varepsilon}$·25 (left ends in Fig. 1). PTR C_{ε}d represents the completely deleted $C_{\varepsilon 2}$ gene in the chimpanzee genome. Only nucleotides different from the human $C_{\varepsilon 1}$ sequence are shown. We used the nucleotide sequences of the human $C_{\varepsilon 1}$ and $C_{\varepsilon 2}$ genes that were previously determined (Ueda et al. 1982; Hisajima et al. 1983). The nucleotide sequences approximately 1 kb downstream from the CH4 exon

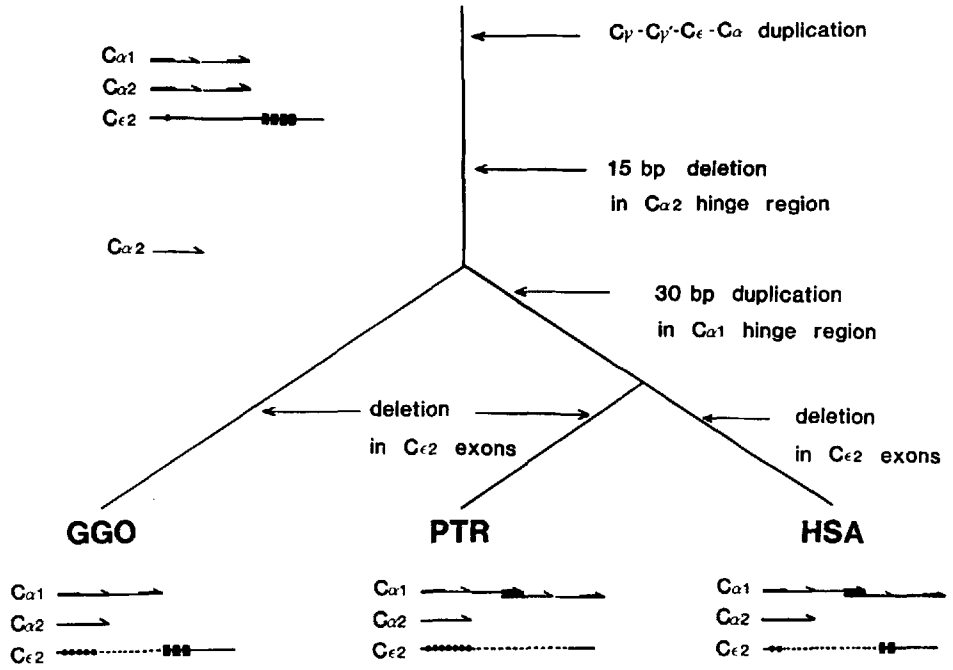

Fig. 4. Evolutionary order of rearrangements in the C_ϵ and C_α genes of the human, chimpanzee, and gorilla. Genetic events in the C_ϵ and C_α genes are ordered according to the phylogenetic tree of the human, chimpanzee, and gorilla as mentioned in the text. Schematic structures of the hinge regions of the C_α genes are shown by horizontal arrows representing 15-bp units. Conserved 6-bp sequences are indicated by thicker lines. Structures of the $C_{\epsilon 2}$ genes are shown by horizontal lines with closed rectangles and circles, which indicate exons and 35-bp repeating units, respectively. Broken lines show deletions in the $C_{\epsilon 2}$ genes and their flanking regions. HSA, human; PTR, chimpanzee; and GGO, gorilla.

chimpanzee $C_{\alpha 1}$ genes are almost identical to each other but strikingly different in length from that of the gorilla $C_{\alpha 1}$ gene. The result is consistent with the phylogeny depicting humans as being more closely related to chimpanzees than to gorillas.

Since two repeating units (30 bp) of the hinge region of the human $C_{\alpha 1}$ gene are identical, this duplication must have taken place very recently. It is likely that a common ancestor of humans and chimpanzee duplicated the 30-bp unit of the hinge region after divergence from gorilla. Alternatively, duplication of the 30-bp unit may have taken place in the common ancestor of the three species, and then the $C_{\alpha 1}$ gene of gorilla might have deleted one 30-bp unit from the hinge region after divergence of this species. We prefer the former hypothesis because the number of genetic events required to produce this result is smaller and a single C_α gene in the mouse genome has the 30-bp hinge region. It will be necessary to determine the structure of the $C_{\alpha 1}$ genes of other hominoids in order to distinguish between the above possibilities definitively.

The above question is related to which of the $C_{\alpha 1}$ and $C_{\alpha 2}$ genes is closer to the ancestor C_α gene. The mouse genome contains only one C_α gene, which might be related to the ancestor of the primate C_α genes. We have therefore compared the nucleotide sequences of the hinge region of the mouse C_α gene with those of the $C_{\alpha 1}$ and $C_{\alpha 2}$ genes of the three primates. Assuming that the gorilla–mouse type $C_{\alpha 1}$ gene containing the 30-bp hinge region is closer to the ancestor of the primate C_α and that the $C_{\alpha 2}$ genes were created by deletion of the 15-bp unit of the $C_{\alpha 1}$ hinge region, the following observations are expected: (1) homologies between the first (or third) and second (or fourth) 15-bp units of the $C_{\alpha 1}$ hinge regions of the same species' genes should be similar to each other, (2) homology between the first (or second) 15-bp units of the $C_{\alpha 1}$ hinge regions of different species should be higher than that between the first (or third) and second (or fourth) 15-bp units of the same species, and (3) homologies between the 15-bp units of the $C_{\alpha 1}$ and $C_{\alpha 2}$ hinge regions should be higher than those between the first and second

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of the human C_ϵ gene were determined in this study, using the cloned DNA (Ch4A-H-Ig$_\epsilon$-12) previously obtained by Ueda et al. (1982). Deleted nucleotides are indicated by hyphens. The double hyphens indicate nucleotide sequences determined but not shown. The vertical arrows show the ends of deletion. The horizontal arrows show the 35-bp sequences that are duplicated tandemly at the deletion points.

Table 1. Comparison of the 15-bp units of $C_{\alpha 1}$ hinge regions

Comparison	Species	Positions of 15-bp units	Matched bases/15 bases
Different pairs of the same species	Mouse	1st/2nd	11
	Human	1st (or 3rd)/ 2nd (or 4th)	10
	Chimpanzee	1st/2nd	12
		3rd/4th	10
	Gorilla	1st/2nd	11
Homologous pairs of different species	Human/chimpanzee	1st	13
		2nd	13
		3rd	15
		4th	15
	Human/gorilla	1st	15
		2nd	14
	Chimpanzee/gorilla	1st	13
		2nd	12
		3rd/1st	15
		4th/2nd	14

Sequence of the murine C_{α} gene was taken from Tucker et al. (1981). Positions of 15-bp units were numbered from 5′ to 3′. Six-bp overlapped sequences were used in both units. Note that the first and the second 15-bp units of the human $C_{\alpha 1}$ gene are identical to the third and the fourth 15-bp units, respectively

15-bp units of the $C_{\alpha 1}$ genes. By contrast, the alternative hypothesis, that the $C_{\alpha 2}$ gene is closer to the ancestor of the primate $C_{\alpha 1}$ genes, predicts the contrary to (1), (2), and (3).

As shown in Table 1, comparison of the 15-bp units of the hinge regions of the primate and murine C_{α} genes fulfills the predictions (1) and (2) described above. The 15-bp units of $C_{\alpha 2}$ genes of all the species are homologous in at least 12 out of 15 bases when compared with any 15-bp units of the $C_{\alpha 1}$ genes except for the first 15-bp unit of the chimpanzee $C_{\alpha 1}$ gene. This is consistent with prediction (3). We therefore conclude that the $C_{\alpha 2}$ gene was derived from the prototype $C_{\alpha 1}$ gene by the 15-bp deletion in the hinge region, which took place before divergence of the three species.

The branching patterns of the lineages and the datings of the divergence nodes among humans and the African apes are still in dispute (Koop et al. 1986). One of the difficulties in resolving these problems from nucleotide sequence data is that the three species are so closely related that a large amount of sequence data is required to draw a statistically significant conclusion. The present sequence data are not large enough to clarify the relationships among the three species. Counting each gap as one substitution regardless of its length, there were 46 substitutions among 945 nucleotides of the $C_{\alpha 1}$ and $C_{\alpha 2}$ genes, and the 5′ flanking region of the $C_{\epsilon 2}$ gene in total; 17 were common between human and chimpanzee, 17 between chimpanzee and gorilla, and 9 between human and gorilla, respectively.

However, the recent studies based on nucleotide sequence of the $C_{\epsilon 3}$ pseudogene support the idea that chimpanzee is more closely related to human than is gorilla (Ueda et al. 1986). The structural comparison of the $C_{\alpha 1}$ hinge regions of the three species also supports this conclusion (Fig. 2). Assuming that this is the case, the most simple evolutionary steps for generation of the immunoglobulin C_{ϵ} and C_{α} genes is as shown in Fig. 4. The order of human heavy-chain C_H genes suggests that duplication of DNA containing the C_{γ}, C_{ϵ}, and C_{α} genes took place after divergence from mouse, probably in a common ancestor of the three species. Then, deletion of a part of the hinge region (15-bp unit) took place to yield the $C_{\alpha 2}$ gene. After divergence of gorilla, there was duplication of the hinge-coding region (30-bp unit) in the $C_{\alpha 1}$ gene of the common ancestor of human and chimpanzee. Moreover, deletion of the $C_{\epsilon 2}$ gene exons independently in the individual lineages. The present study has shown that DNA rearrangement is a good marker for tracing evolutionary lineage, as we proposed previously (Ueda et al. 1985).

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