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Evolution

Evolutionarily different alphoid repeat DNA on homologous chromosomes in human and chimpanzee

(molecular drive/satellite DNA/speciation/primates)

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ABSTRACT **Centromeric alphoid DNA in primates represents a class of evolving repeat DNA. In humans, chromosomes 13 and 21 share one subfamily of alphoid DNA while chromosomes 14 and 22 share another subfamily. We show that similar pairwise homogenizations occur in the chimpanzee (*Pan troglodytes*), where chromosomes 14 and 22, homologous to human chromosomes 13 and 21, share one partially homogenized alphoid DNA subfamily and chromosomes 15 and 23, homologous to human chromosomes 14 and 22, share another extensively homogenized subfamily. Such a pattern of homogenization presumably predates speciation 3–10 million years ago. However, the alphoid DNA on these human and chimpanzee chromosomes is not orthologous but originates from two evolutionarily different repeat families. It follows that dramatic sequence evolution has occurred in a concerted fashion among the chromosomes in one or both species during or after separation.**

Molecular data support a view concerning human origins that groups chimpanzees (*Pan*) and gorillas (*Gorilla*) with humans rather than with orangutans (*Pongo*) (1). Orthologous nonrepetitive DNAs share a high overall identity (98%) between humans and the great African apes, whereas analyses of pseudogene DNA sequences and protein structure suggest that humans and chimpanzees are more closely related and diverged from a common ancestor 3–10 million years ago (1–5). High-resolution banding patterns of prophase chromosomes also link humans with chimpanzees (6), but these conclusions have been challenged by recent studies on gene structure (7). Compared to chimpanzee, the human lineage has experienced rapid phenotypic evolution (8) and at the same time has had the lowest mutation rate yet found, 0.1% per million years (3–5, 9). The dramatic morphological changes may therefore be more easily explained by increased frequency of rearrangement of preexisting DNA sequences rather than by fixation of mutations (10).

A class of noncoding DNA in primates, known to undergo frequent rearrangements, is the family of centromeric alphoid repetitive DNA (α-repeat). From extensive studies in humans, it is well established that the α-repeat is subject to genetic mechanisms (molecular drive; see ref. 11) that generate chromosome-specific subfamilies, which consist of homogenized long tandem arrays of higher-order amplification units, each constructed as a distinct succession of either a basic 171-base-pair (bp) monomer or 340-bp dimer units (ref. 12 and references therein). The different subfamilies are further classified into three evolutionarily distinct suprafamilies (13). The α-repeat shows genetic instability. Consistent with theories of the evolution of tandem arrays (14), α-repeat undergoes frequent recombinatorial events, which is also reflected in a high frequency of array-length polymorphism among haplotypes (15, 16) and in the presence of α-repeat sequences as extrachromosomal covalently closed circular DNA (17). We characterized the α-repeat on the human acrocentric chromosomes 13, 14, 21, and 22 that organize nucleoli (NOR-bearing chromosomes) (18, 19). The α-repeat shows a peculiar pattern being homogenized between nonhomologous chromosomes, whereby chromosomes 13 and 21 share one subfamily and chromosomes 14 and 22 share another (the 13/21 and 14/22 subfamilies of the α-repeat). The α-repeat on the fifth acrocentric NOR-bearing chromosome 15 apparently is not cross-homogenized to any of the other four acrocentric chromosomes (19, 20).

Exchange of centromeric DNA by unequal crossing-over between nonhomologous chromosomes can be tolerated for acrocentric chromosomes as the p arms carry no genes other than the rRNA genes. The chimpanzee, but not the gorilla (21), possesses NOR-bearing acrocentric chromosomes 14, 15, 22, and 23, which are homologous to human chromosomes 13, 14, 21, and 22, respectively. We demonstrate here that the α-repeat on chimpanzee chromosomes 14 and 22 is partially homogenized and that on chimpanzee chromosomes 15 and 23 it is extensively pairwise homogenized. All the chimpanzee repeats are shown to belong to a suprafamily that is evolutionarily different from the suprafamily to which the α-repeat on the human chromosomes belongs.

MATERIALS AND METHODS

Hybrid Cells and Molecular Techniques. Chimpanzee chromosome-specific DNAs originate from Chinese hamster/chimpanzee hybrid cells R93-8, R133-6A5, and R48-40A, which contain, respectively, chimpanzee chromosomes 14, 15, and 23 as the only detectable chimpanzee chromosome or subchromosomal fragment. Other hybrids used were R48-16B, which contains chimpanzee chromosomes 8, 20, 23, and X, and R237-4A, which contains chimpanzee chromosomes 3, 15, and Y (22, 23). Standard procedures, as described in ref. 19, were used for DNA extraction, Southern blotting, cloning, and DNA sequencing.

Computer Analysis of DNA Sequences. The different sets of α-repeat dimers analyzed are the sequences in Figs. 1 and 2 and in refs. 18, 19, and 25. For identity calculations, these sequences were analyzed with the GAP program from the Genetics Computer Group (Madison, WI) DNA software package, version 6.2 (26). GAP makes an optimal alignment between two complete sequences by inserting gaps to maximize the number of matches. A dichotomous tree for chimpanzee dimeric sequences was calculated by using a maximum likelihood program (DNAML) (see legend to Fig. 3 and refs. 28 and 29). As DNAML is primarily designed to deal with

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Abbreviations: α-repeat, alphoid repetitive DNA; NOR-bearing chromosome, nucleoli-organizing chromosome.

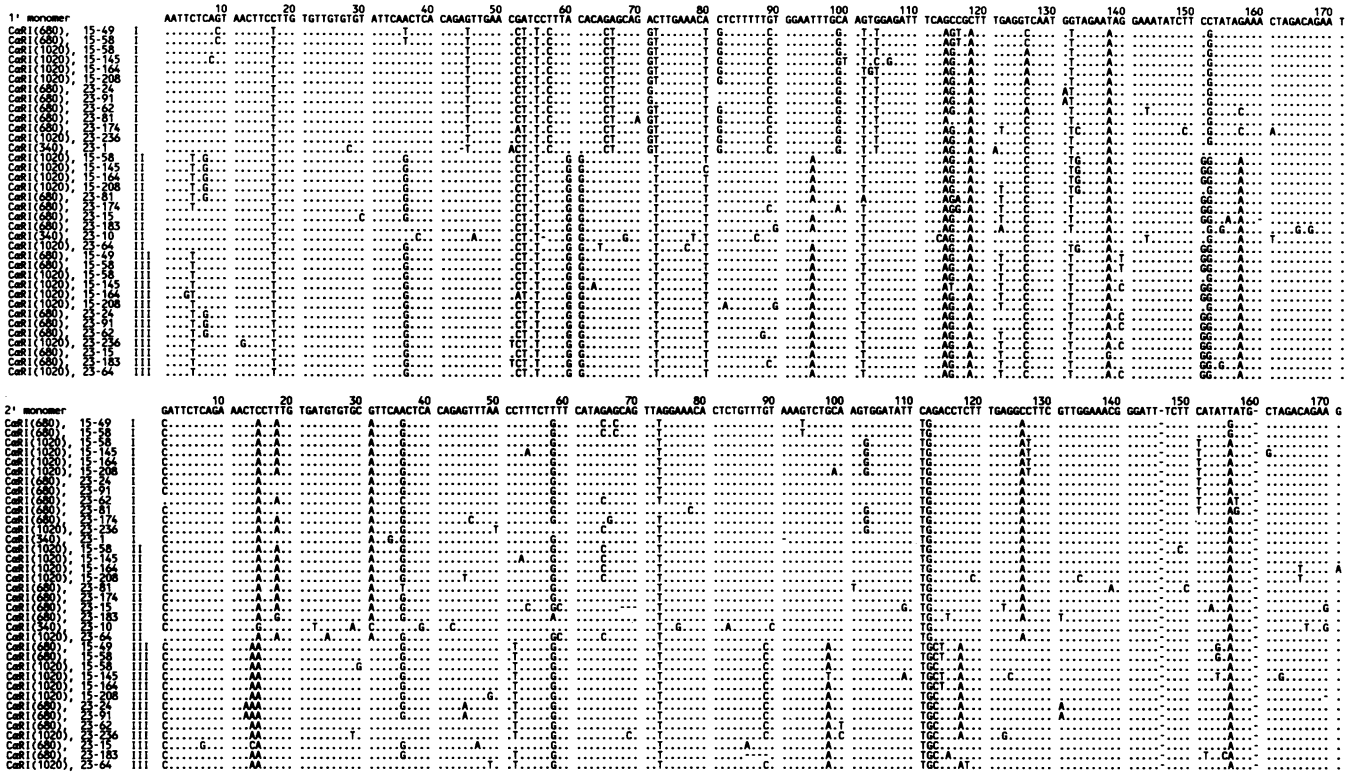

FIG. 1. Nucleotide sequences of clones of α-repeat DNA from chimpanzee chromosomes 15 and 23. Dots indicate identical nucleotides and dashes indicate nucleotide deletions relative to a human alphoid reference sequence (24). All clones studied were obtained from isolated *Eco*RI fragments in DNA from hybrid cells containing either chromosome 15 (R133-6A5) or chromosome 23 (R48-40A) as the only chimpanzee chromosome. Southern blot analyses (data not shown) at low stringency using probe αRI-6 (25) identified restriction bands representative of the majority of α-repeat DNA on the two chromosomes, at high stringency, a regular ladder of 340-bp multimers with the heaviest band at 1020 bp using clones from chromosomes 15 and 23 as probes [CαRI(1020), 15-58 and CαRI(680), 23-24]. The same results were obtained with DNA from hybrid cell R237-4A, which contains chimpanzee chromosomes 3, 15, and Y, and hybrid cell R48-16B, which contains chimpanzee chromosomes 8, 20, 23, and X. The clones were labeled conventionally (18, 19, 25)—e.g., in CαRI(680), 15-49, C designates chimpanzee, αRI is *Eco*RI digestible α-repeat, (680) indicates a 680-bp fragment, 15 indicates that the cloned fragment originates from chromosome 15, and 49 is the individual clone number. Roman numerals designate the corresponding dimers. Dimer I of the clones (680) 15-49 and 15-58 share specific changes (e.g., C at position 9 and T at position 36 in the 1′ monomer) and represent a subset designated C15 IA in Fig. 3. Dimer I of the rest of the clones is designated C15 IB.

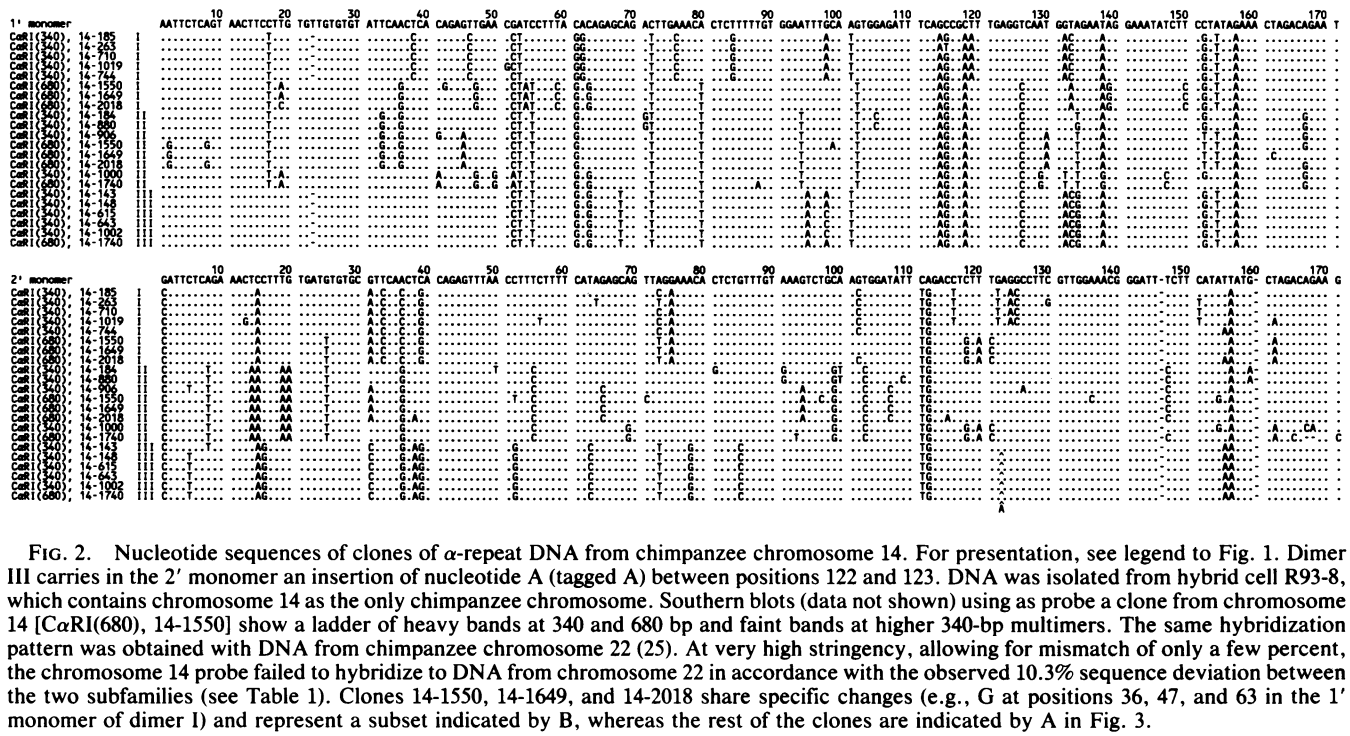

FIG. 2. Nucleotide sequences of clones of α-repeat DNA from chimpanzee chromosome 14. For presentation, see legend to Fig. 1. Dimer III carries in the 2′ monomer an insertion of nucleotide A (tagged A) between positions 122 and 123. DNA was isolated from hybrid cell R93-8, which contains chromosome 14 as the only chimpanzee chromosome. Southern blots (data not shown) using as probe a clone from chromosome 14 [CαRI(680), 14-1550] show a ladder of heavy bands at 340 and 680 bp and faint bands at higher 340-bp multimers. The same hybridization pattern was obtained with DNA from chimpanzee chromosome 22 (25). At very high stringency, allowing for mismatch of only a few percent, the chromosome 14 probe failed to hybridize to DNA from chromosome 22 in accordance with the observed 10.3% sequence deviation between the two subfamilies (see Table 1). Clones 14-1550, 14-1649, and 14-2018 share specific changes (e.g., G at positions 36, 47, and 63 in the 1′ monomer of dimer I) and represent a subset indicated by B, whereas the rest of the clones are indicated by A in Fig. 3.

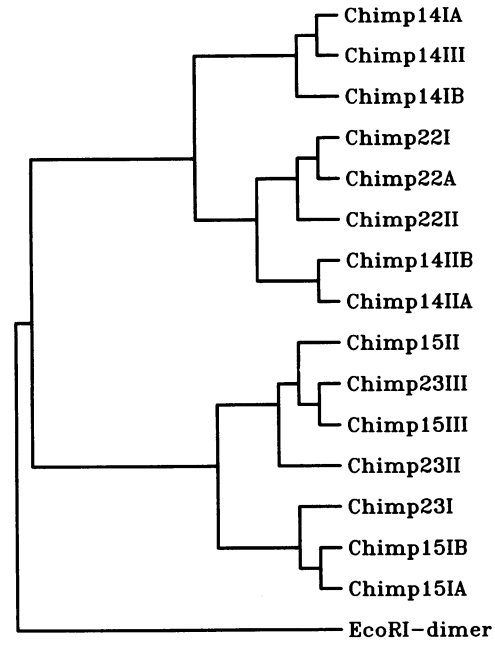

FIG. 3. Relationship between consensus dimeric sequences from pairwise homogenized α-repeat subfamilies on chimpanzee chromosome pairs 14/22 and 15/23. Ambiguity positions in consensus sequences were denoted by using the nucleic acid codes described (27). Roman numerals designate corresponding dimers. In case of subsets within a given subfamily, these were subdivided—e.g., chimp (C)15IA includes clones 15-49 and 15-58, C15IB includes the rest of the clones; C14IB includes clones 14-1550, 14-1649, and 14-2018 and C14IA includes the rest of the clones (see Figs. 1 and 2), C22A includes all the 340-bp fragments from this subfamily (25). Ln likelihood for the tree was −1532.84. The C14/22 sequences branch out into three clusters, reflecting partial homogenization of the chimpanzee 14/22 subfamily.

base substitutions, a transition/transversion ratio of 2 is normally used. However, a majority of subfamily-specific changes within α-repeat DNA are caused by recombinatorial events (unequal crossing-over). Changes present in at least two sequences within a given set were listed as subfamily-specific changes. The tree was therefore also constructed by using transition/transversion ratios of 1 and 0.5, which have been found for satellite DNA in *Drosophila* (30). The different approaches gave visually indistinguishable trees and significance values. The results were graphically visualized by using the DRAWGRAM program (29).

RESULTS

The α-Repeat Subfamily on Chimpanzee Chromosomes 15 and 23. Sequences representative of the α-repeat from chimpanzee chromosomes 15 and 23 are shown in Fig. 1. As shown for the homologous human 14/22 subfamily (19), the α-repeat sequences from chimpanzee chromosomes 15 and 23 are principally indistinguishable and form the chimpanzee 15/23 subfamily. The sequences of the individual repeat units of the subfamily deviate by <1% from each other. The higher-order repeat unit is a hexamer with dimers I, II, and III. The greater similarity between 1′ monomers II and III and 2′ monomers I and II suggests that the hexamer may originate from a tetramer brought about by duplication of one dimer.

The α-Repeat Subfamilies on Chimpanzee Chromosomes 14 and 22. Sequences representing the majority of α-repeat DNA on chimpanzee chromosome 14 are shown in Fig. 2. This subfamily consists of distinct subsets within a collection of otherwise well-defined hexameric repeat units. The overall sequence deviation between these subsets and the more homogeneous subfamily on chimpanzee chromosome 22 (25) is ≈10% (Table 1). The higher-order structure (a hexamer on chromosome 14 and a tetramer on chromosome 22; Fig. 3) is also different. Despite this difference, the chimpanzee 14 and 22 subfamilies are more related to each other than either of them is to the chimpanzee 15/23 subfamily. Thus, 19 specific nucleotide changes in the subfamily on chromosome 22 are also found in the subfamily on chromosome 14 but not in the 15/23 subfamily—e.g., dimer I 1′ monomer has a deletion at position 23 and AC at positions 132 and 133. In contrast, only one such change is found in both the chromosome 22 subfamily and in the 15/23 subfamily and not in the chromosome 14 subfamily. Three such changes are present in both the 14 and 15/23 subfamilies and not in the 22 subfamily.

Table 1. Sequence comparison between acrocentric α-repeat subfamilies

α-Repeat subfamilies	Identity, %	±SD	*n*
C14 vs. C22	89.7	1.4	396
C14 vs. C15/23	88.4	1.5	792
C22 vs. C15/23	88.4	1.6	648
H13/21 vs. H14/22	84.1	1.6	256
H13/21 vs. C14	74.8	1.9	352
H13/21 vs. C22	74.8	1.7	288
H13/21 vs. C15/23	75.7	1.6	576
H14/22 vs. C14	76.0	1.8	352
H14/22 vs. C22	75.9	1.2	288
H14/22 vs. C15/23	76.7	1.4	576

Average identity with SD and number (*n*) of comparisons between all possible combinations of the sequences in Figs. 1 and 2 and refs. 18, 19, and 25. For calculations, see *Materials and Methods*. C, chimpanzee; H, human.

Divergence of the Human and Chimpanzee α-Repeat Subfamilies. Human chromosome-specific subfamilies may have evolved from a smaller number of ancestral sequences since the subfamilies can be classified into three evolutionarily distinct suprafamilies (13). A major proportion of the α-repeat in higher primates and humans is represented by *Eco*RI dimer (340 bp) (suprafamily I) and *Xba* I dimer (suprafamily II) sequences that do not appear to be present in lower primates (31, 32). We analyzed (Table 2) the deviations of the two human acrocentric subfamilies and the corresponding chimpanzee subfamilies relative to a representative set of *Eco*RI and *Xba* I dimers from published nonacrocentric subfamilies (see legend to Table 2). The results clearly demonstrate that

Table 2. Evolutionary origin of human and chimpanzee acrocentric subfamilies

Subfamily	Suprafamily (*Eco*RI dimers)	Suprafamily (*Xba* I dimers)
H13/21	74.5 ± 1.4 (*n* = 40)	83.9 ± 2.2 (*n* = 84)
H14/22	75.7 ± 1.6 (*n* = 80)	83.5 ± 1.6 (*n* = 168)
C14/22	85.8 ± 1.6 (*n* = 80)	75.7 ± 2.1 (*n* = 168)
C15/23	86.8 ± 1.6 (*n* = 70)	77.3 ± 1.9 (*n* = 147)

Average percentage identity with SD and number (*n*) of comparisons between consensus sequences (see legend to Fig. 3) from human and chimpanzee acrocentric dimers and individual human *Eco*RI (suprafamily I) and *Xba* I (suprafamily II) reference dimers. Chromosome-specific *Eco*RI sequences are from A. Baldini, D. I. Smith, M. Rocchi, O. J. Miller, and D. A. Miller [GenBank (EMBL) accession nos. M16087 and M16101]; A. Baldini, M. Rocchi, N. Archidiacono, O. J. Miller, and D. A. Miller (accession no. M28221); and ref. 33 (accession no. M58446). Chromosome-specific *Xba* I sequences are from ref. 32 (accession nos. X14299–14303), ref. 34 (accession nos. X03692 and X03693), ref. 35 (accession nos. M38466 and M38467), and ref. 36 (accession no. X01750). For calculations, see *Materials and Methods*. H, human; C, chimpanzee.

the human acrocentric subfamilies belong to suprafamily II, while the corresponding subfamilies in the chimpanzee belong to suprafamily I. This finding is consistent with the 25% deviation observed when repeats from the two species are compared (Table 1). A similar degree of divergence (≈25%) is found when the chimpanzee repeats (and also the human *Eco*RI and *Xba* I repeats) are compared with the monomeric human suprafamily III (data not shown). Thus, the subfamilies in the two species are not orthologous, which means that the interspecies divergence observed does not reflect evolution from a common ancestral sequence. The calculations in Table 3 show the same degree of deviation (≈15%) for both acrocentric and nonacrocentric human *Xba* I dimers. Table 3 also shows that the degree of deviations between dimers within subfamilies is the same as between dimers belonging to different subfamilies. An exception is the smaller deviation (8.5% vs. 11.5%) between dimers within the chimpanzee 15/23 subfamily. This presumably results from the described recent duplication of a dimer within this subfamily.

The proven nonorthology precludes direct comparison of sequence deviations between the human acrocentric *Xba* I dimers with deviations between the chimpanzee acrocentric *Eco*RI dimers. The human *Xba* I and *Eco*RI dimer families appear to be of equal ancient origin, as the monomers within the dimers show equal low identity; this monomer deviation of ≈30% is also found in the chimpanzee dimers (Table 3). Furthermore, the degree of deviation is the same for human nonacrocentric and acrocentric *Xba* I dimers and nonacrocentric *Eco*RI dimers. It may therefore be relevant to compare the deviation between the chimpanzee acrocentric *Eco*RI dimers with the deviation between the human nonacrocentric *Eco*RI dimers. The difference between the listed deviations (Table 3) is statistically significant ($P < 0.001$).

Dichotomous Tree. Comparison of dimers of the chimpanzee subfamilies is shown in Fig. 3. An arbitrarily chosen *Eco*RI dimer, representing suprafamily I, was used to root the tree containing chimpanzee dimers. Other arbitrarily chosen *Eco*RI dimers gave the same result (data not shown). The branching of the chimpanzee chromosome 14/22 subfamily is complex. While the tree clearly groups the dimers together, separate from the 15/23 subfamily, the heterogeneous sequences of the chromosome 14/22 subfamily branch out into three clusters. The subpopulations of chimpanzee chromosome 14 dimer II are grouped together with chromosome 22 dimers, which indicates a partial homogenization between the α-repeat on these chromosomes.

DISCUSSION

The main conclusion of the present study is that the patterns of homogenization of α-repeat have changed in closely related species and with time. Human NOR-bearing chromosomes 13 and 21 share one α-repeat subfamily, while chromosomes 14 and 22 share a different subfamily (18, 19). We discovered similar distribution on NOR-bearing acrocentric chromosomes in the chimpanzee. Chromosomes 15 and 23 in the chimpanzee share a subfamily that has been extensively homogenized within and between the chromosomes (Fig. 1). Although the subfamilies on chimpanzee chromosomes 14 and 22 (Figs. 2 and 3; ref. 25) share a set of specific nucleotide changes, we are less certain whether this also reflects a paired homogenization between these chromosomes. Alternatively, the fact that these nucleotide changes might also be found on other chimpanzee chromosomes cannot be excluded. However, the pairwise distribution (at least for the chimpanzee 15/23 subfamily) is principally analogous to that found in humans, which strongly suggests that it was present in the ancestral species. This study and extensive sequence analyses by others (13, 32, 37) demonstrate that it is possible to classify α-repeat subfamilies into three evolutionarily distinct suprafamilies. The acrocentric subfamilies in the two species can be classified accordingly. Table 2 shows the surprising finding that the human subfamilies belong to one suprafamily (II), whereas the chimpanzee subfamilies belong to a different suprafamily (I). It follows that the subfamilies, although residing on homologous chromosomes, are not orthologous in the two species.

The origin and physiological role of the paired homogenization is unknown, but it might be an indication that some acrocentric chromosomes of humans and chimpanzees have arisen by centric fission of metacentric chromosomes (ref. 21, p. 70). Also, the existence of evolutionarily different repeats on homologous chromosomes in the two species is intriguing. We have no answers to these questions but our results clearly demonstrate that dramatic changes in centromeric DNA can occur on homologous chromosomes in closely related species. Recent *in vitro* (38) and *in vivo* (39) experiments have implicated the α-repeat in centromere structure and function. It is conceivable that specific interaction between centromeric DNA and components of the kinetochore protein complex is a prerequisite for proper meiotic and mitotic segregation. Such interaction (40) may acquire species specificity as a result of concerted evolution of the α-repeat. New variants of this DNA may therefore increase the probability that new species emerge. In the process (molecular drive; see ref. 11) leading to concerted evolution, it is expected that stages of transition will exist during the spread of a variant repeat. Such stages of incomplete homogenization of variants have been found in tandem DNA families from closely related *Drosophila* species, and analyses of sequence variation at each nucleotide position considered independently showed that the species that had diverged most had a higher number of fully homogenized variants. The study suggested that the homogenization process is faster and independent of the mutation rate and occurs at species-specific rates (30, 40). We detect similar transitional stages—e.g., G at position 90 in the

Table 3. Sequence identity within human and chimpanzee α-repeat subfamilies

	Human nonacrocentric		Acrocentric			
	Xba I	*Eco*RI	Human (*Xba* I)		Chimpanzee (*Eco*RI)	
Monomer vs. monomer (within dimers)	71.6 ± 2.9 (n = 17)	71.6 ± 4.1 (n = 54)	73.5 ± 2.4 (n = 32)		70.4 ± 2.2 (n = 76)	
Dimer vs. dimer (between subfamilies)	84.7 ± 2.6 (n = 115)	85.7 ± 1.5 (n = 37)	84.1 ± 1.6 (n = 256)		88.4 ± 1.6 (n = 1440)	
Dimer vs. dimer (within subfamilies)	86.3 ± 3.8 (n = 11)	86.8 ± 1.5 (n = 9)	13/21	84.7 ± 0.5 (n = 32)	14/22	88.5 ± 2.6 (n = 196)
			14/22	83.7 ± 1.1 (n = 48)	15/23	91.6 ± 2.1 (n = 217)

Average percentage identity with SD and number (n) of comparisons for all possible combinations of repeat units (monomers and dimers). Human nonacrocentric *Xba* I and *Eco*RI sequences are the reference subfamilies described in the legend to Table 2. Human (13/21 and 14/22) and chimpanzee (14/22 and 15/23) acrocentric subfamilies are the sequences in Figs. 1 and 2 and refs. 18, 19, and 25. For calculations, see *Materials and Methods*.

1′ monomer of dimer I on both chromosomes sharing the human 13/21 subfamily (18), and T at position 51 in the 2′ monomer of dimer I on both chromosomes that share the chimpanzee 15/23 subfamily (Fig. 1). Overall, there seems to be a higher number of partially homogenized variants in the chimpanzee subfamilies.

Interesting features are revealed about sequence deviation between and within α-repeat subfamilies by the calculations shown in Tables 1 and 3. We find the same (≈15%) deviation between the two human acrocentric subfamilies as between several human nonacrocentric *Xba* I subfamilies taken from the literature. This presumably means that all these human subfamilies have evolved for approximately equal lengths of time. We also included in the calculations all known *Eco*RI dimers assigned to specific human chromosomes and found the same (≈15%) deviation. Thus, all human dimeric subfamilies appear to deviate equally from one another. Despite a lack of orthology, it may therefore be relevant to compare the deviation between the chimpanzee acrocentric *Eco*RI subfamilies with the deviation between the human acrocentric *Xba* I subfamilies. The two chimpanzee subfamilies deviate ≈12% from each other, which is significantly ($P < 0.001$) less than the 15% deviation found in humans for both *Xba* I and *Eco*RI subfamilies. Less deviation in the chimpanzee is also seen when dimers within subfamilies are compared. In each species, deviations between dimers are thus about the same both within and between subfamilies. This may indicate that the evolution and chromosome specificity of α-repeat subfamilies are determined mainly by intrachromosomal (within and between homologues) mechanisms leading to more well-defined higher-order structures. From studies in humans and the great apes of X chromosome-specific α-repeats, which are clearly orthologous, we find a considerably increased rate of evolution of the human α-repeat (to be published elsewhere). The higher number of partially homogenized nucleotide changes and the smaller deviation between dimers in the chimpanzee repeats described here could possibly also be explained by such rate differences.

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