

The evolutionary history of human chromosome 7[☆]

Stefan Müller,^{a,*} Palma Finelli,^b Michaela Neusser,^a and Johannes Wienberg^{a,c}

^a*Institute for Anthropology and Human Genetics, Department of Biology II, Ludwig-Maximilians University, Richard-Wagner-Strasse 10, D-80333 Munich, Germany*

^b*Cytogenetics and Molecular Genetics Laboratory, Auxological Institute, Via Ariosto 13, 20145 Milan, Italy*

^c*Institute of Human Genetics, GSF–National Research Center for Environment and Health, Ingolstaedter Landstrasse 1, 85764 Munich, Germany*

Received 4 February 2004; accepted 7 May 2004

Available online 8 July 2004

Abstract

We report on a comparative molecular cytogenetic and in silico study on evolutionary changes in human chromosome 7 homologs in all major primate lineages. The ancestral mammalian homologs comprise two chromosomes (7a and 7b/16p) and are conserved in carnivores. The subchromosomal organization of the ancestral primate segment 7a shared by a lemur and higher Old World monkeys is the result of a paracentric inversion. The ancestral higher primate chromosome form was then derived by a fission of 7b/16p, followed by a centric fusion of 7a/7b as observed in the orangutan. In hominoids two further inversions with four distinct breakpoints were described in detail: the pericentric inversion in the human/African ape ancestor and the paracentric inversion in the common ancestor of human and chimpanzee. FISH analysis employing BAC probes confined the 7p22.1 breakpoint of the pericentric inversion to 6.8 Mb on the human reference sequence map and the 7q22.1 breakpoint to 97.1 Mb. For the paracentric inversion the breakpoints were found in 7q11.23 between 76.1 and 76.3 Mb and in 7q22.1 at 101.9 Mb. All four breakpoints were flanked by large segmental duplications. Hybridization patterns of breakpoint-flanking BACs and the distribution of duplicons suggest their presence before the origin of both inversions. We propose a scenario by which segmental duplications may have been the cause rather than the result of these chromosome rearrangements.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Chromosome 7 evolution; Primates; Inversion breakpoint; Segmental duplication

The reconstruction of the evolutionary history of human chromosome 7 in primates and in other mammals has been the subject of several comparative cytogenetic studies for more than 20 years. Early G- and R-banding of human and great ape chromosomes already suggested that human and chimpanzee homologs share a similar banding pattern, which differs from the gorilla homolog by a paracentric inversion. It was further suggested that the chromosome morphology found in the gorilla represents an evolutionary intermediate state, which could be derived by a pericentric inversion from the ancestral hominoid chromosome form conserved in the orangutan ([1–3] and references therein). This particular succession of evolutionary chromosome rearrangements was one of the very few characteristics

based on cytogenetic data that seemed to be informative for the phylogeny of human and great apes. The pericentric inversion provided an argument for a last common ancestor of all African great apes and human to the exclusion of the orangutan. Furthermore, the paracentric inversion would indicate an exclusive last common ancestor of human and chimpanzee. Yunis and Prakash [3] assigned the breakpoints of the pericentric inversion to bands 7p22 and 7q11.23–q21 and those of the paracentric inversions to 7q11.23–q21 and 7q22. However, high-resolution G-banding analysis was not able to determine whether the 7q11.23–q21 breakpoint was reused during evolution. DeSilva et al. [4] performed a comparative FISH analysis with bacterial artificial chromosome (BAC) clones that were derived from the Williams–Beuren syndrome (WBS) region in 7q11.23 and contained low-copy repeats, including NCF1 (p47-phox) sequences. FISH analysis revealed the presence of duplicated segments in the 7q11.23 homologous region of chimpanzee, gorilla, orangutan, and a gibbon. Moreover, as in human, cross-hybridization was observed in the inversion breakpoint

[☆] Supplementary data for this article may be found on ScienceDirect.

* Corresponding author. Fax: +49-89-21806719.

E-mail address: s.mueller@lrz.uni-muenchen.de (S. Müller).

regions at 7q22 and 7p22 in African apes, but not in the homologous chromosome regions in orangutan and gibbon. Since a detailed analysis of the WBS orthologous region on mouse chromosome 5 provided no evidence of duplicated segments, the authors concluded that these segmental duplications are of recent evolutionary origin. Their data indicated that a p47-phox-containing segment first duplicated locally and then some copies were distributed to three locations on chromosome 7 by inversion events. This would imply that the inversions that occurred during hominoid evolution and the complex rearrangements that led to WBS in humans are mediated by the same duplicated sequences.

Attempts to reconstruct chromosome 7 homologies between more distant related primates or in nonprimate mammals on the basis of chromosome banding patterns led to controversial interpretations. Most reports agreed, however, that in the putative ancestral primate homolog segments are found on at least two ancestral chromosomes. Further, complex evolutionary intra- and/or interchromosomal rearrangements have to be assumed to derive the extant human chromosome form [1,2,5]. Recently, Richard et al. [6] revisited this problem by performing cross-species chromosome painting and reviewing previous R-banding data from over 40 primates and nonprimate mammals. Their results indicated that the ancestral chromosome forms found in Eutherian mammals may have comprised a small submetacentric chromosome showing association of part of human chromosome 7 (7b) and 16p homologous material and a large acrocentric chromosome showing homology to part of human chromosome 7 (7a) only. The subregional organization of the putative ancestral mammalian chromosome 7a, however, remained elusive. Since these chromosome forms were also found in prosimians, the authors concluded that separate chromosomes 7a and 7b/16p should be included in the putative ancestral primate karyotype. Thus, a fission of the ancestral 7b/16p homolog may have occurred before the prosimian/simian split, followed by a fusion of 7b/5 in the lineage leading to New World primates and fusion of 7a/7b present in all higher Old World primates.

Here we report on a detailed comparative molecular cytogenetic study of the human chromosome 7 homologs in primates and other nonprimate mammals to shed light on important yet unresolved aspects concerning the evolution of this chromosome: (i) the subregional organization of the ancestral mammalian and primate chromosome form 7a and (ii) the presence of three or four breakpoints in the two inversions in hominoid primates. We integrated FISH and “in silico chromosome painting” data to reconstruct the putative ancestral mammalian chromosome form and the succession of rearrangements including those within chromosomes in all major primate lineages. We used FISH with chromosome painting probes from species with disrupted synteny of the human chromosome 7 homologs (concolor gibbon and African green monkey) as well as microdissection probes that would

delineate breakpoints of intrachromosomal rearrangements. The same principle was applied in the in silico approach: the human chromosome 7 sequence map was compared with homologous chromosome segments in mouse and rat, both species that show highly disrupted synteny. Further, we employed a panel of BAC clones to search for the breakpoints of the inversions that occurred during hominoid evolution. Finally, to define breakpoints better we performed a detailed FISH analysis with BAC contigs spanning all four inversion breakpoints.

Results

Delineation of primate chromosome 7 evolutionary rearrangements

To date, the succession of evolutionary intrachromosomal rearrangements that shaped human chromosome 7 has been investigated only by comparative banding analysis. To delineate the subchromosomal organization of human chromosome 7 homologs in nonhuman primates and to reconstruct the sequence of evolutionary intrachromosomal rearrangements in greater detail, we used a series of subregional DNA probes for cross-species FISH (Figs. 1A–1D). Hybridization patterns observed with these probes on human chromosome 7 are illustrated in Fig. 1E, their precise chromosomal assignments in Fig. 1G. When hybridized to chromosomes of other primates and nonprimate mammals, African green monkey and concolor gibbon chromosome paint probes showed a reproducible hybridization signal in all species analyzed, whereas the usefulness of human microdissection probes was restricted to higher primates. The reason for this limitation was most presumably their low complexity, because very few copies of microdissected chromosome fragments were used as template DNA in the primary amplification reaction, compared to several hundred chromosomes for paint probes established by flow sorting.

With African green monkey chromosome 21 and 28 probes, the simplest hybridization pattern, with one signal each on the same chromosome, was observed in the orangutan, the Lar gibbon, and the macaque (Fig. 1E). Single signals with both probes, however on two separate chromosomes, were detected in the New World monkey, the lemur, and the carnivore. Simple split signals on a single chromosome were observed in the langur and the gorilla, while both chimpanzees and human shared a more complex hybridization pattern (Fig. 1E, top row).

The paint probes derived from the concolor gibbon chromosomes 11, 13, and 17 with homology to human chromosome 7 subregions showed three conserved homologous segments on a single chromosome pair in the orangutan, the Lar gibbon, the macaque, and the langur. The homologs of the New World monkey and the lemur share the same pattern, but with a translocated segment homologous to HCO 17. Split signals indicating intrachromosomal

rearrangements were noticed in the carnivore, human, chimpanzee, and gorilla homologs (Fig. 1E, middle row).

The paint set composed of differentially labeled microdissection probes 7p, 7q, 7pter, and 7qter showed a similar hybridization pattern in human, gorilla, and chimpanzee, however with a gap in the 7q homolog of the chimpanzees. Orangutan, gibbon, macaque, langur, and marmoset share a split 7pter signal. In addition, the langur showed a split signal of the 7p probe and the New World monkey a split signal of the 7q probe, which highlight additional peri- and paracentric inversions, respectively (Fig. 1E, lower row).

Comparative FISH analysis with a set of 19 sequence-tagged BAC clones (BAC 1–19) that are part of the clone set described in [13] was performed to determine the orientation of conserved segment homologs in hominoid primates. These clones map to human chromosome 7 in 5- to 10-Mb intervals (Supplementary Table 1). They were mapped to chromosomes of human, chimpanzee, gorilla, orangutan, and Lar gibbon in sets of two to seven differentially labeled clones (Fig. 1D). Human and chimpanzee shared the same order of BACs. In gorilla, the order of BACs 11–13 was inverted, indicating a paracentric inversion with breakpoints in intervals between BAC pairs 10/11 and 13/14. Compared to the gorilla homolog, in orangutan and Lar gibbon the sequential order of BACs 3–13 was inverted, narrowing down the pericentric inversion breakpoints to regions between BAC pairs 2/3 and 12/13 (Fig. 1G).

Fig. 2 summarizes the evolutionary chromosomal rearrangements detected with subregional paint probes in the various primate species and the mink, serving as outgroup for primates.

Characterization of hominoid inversion breakpoints

For the characterization of the breakpoints of the pericentric inversion in 7p22.1 and 7q22.1 that distinguishes the homologs of orangutan and gorilla, two BAC contigs including clones A/B/C/D (6.39–6.86 Mb) and O/P/Q (97.01–97.32 Mb) were chosen (Supplementary Table 1 for clone identity). The hybridization patterns observed on all species investigated are illustrated in Figs. 3A and 3B.

To determine the breakpoint regions of the paracentric inversion in 7q11.23 and 7q22.1 by which the human/chimpanzee homologs are derived from that found in the gorilla, BAC clones E–N, R, and S were hybridized to great ape and macaque chromosomes (Figs. 3C and 3D). Clone E maps at 73.2 Mb on the human sequence map within the Williams–Beuren syndrome region, which previously was suggested to be involved in the formation of both the peri- and the paracentric inversion [4]. Clone F maps distal to the WBS region to a nonduplicated DNA segment at 74.5 Mb. Our comparative FISH analysis clearly showed that both clones map proximal to the 7q11.23 breakpoint (Fig. 3C).

BACs G/H/I/K form a contig between 75.59 and 76.13 Mb of the human reference sequence in an approx 800-kb region comprising mainly segmental duplications (DI352–365; www.chr7.org). Clones G, H, and I mapped proximal to the 7q11.23 breakpoint. BAC K showed no hybridization signal in the orangutan and macaque chromosome 7 homologs, while in human, chimpanzee, and gorilla it mapped to the 7q11.23 homologous region and therefore proximal to the breakpoint. BAC L repeatedly produced no signal on human chromosome 7, but hybridized to other human chromosomes and their great ape homologs. BAC clones M and N show an inverted orientation in gorilla, orangutan, and macaque compared to human and chimpanzee, indicating that these probes flank the breakpoint on the distal side. The 7q11.23 breakpoint could therefore be assigned to the interval between 76.13 and 76.35 Mb on the human sequence map. The overlapping BACs R and S (101.78–102.03 Mb) pinpoint the 7q22.1 breakpoint of the paracentric inversion to approx 101.91 Mb on the human sequence map (Fig. 3D).

Discussion

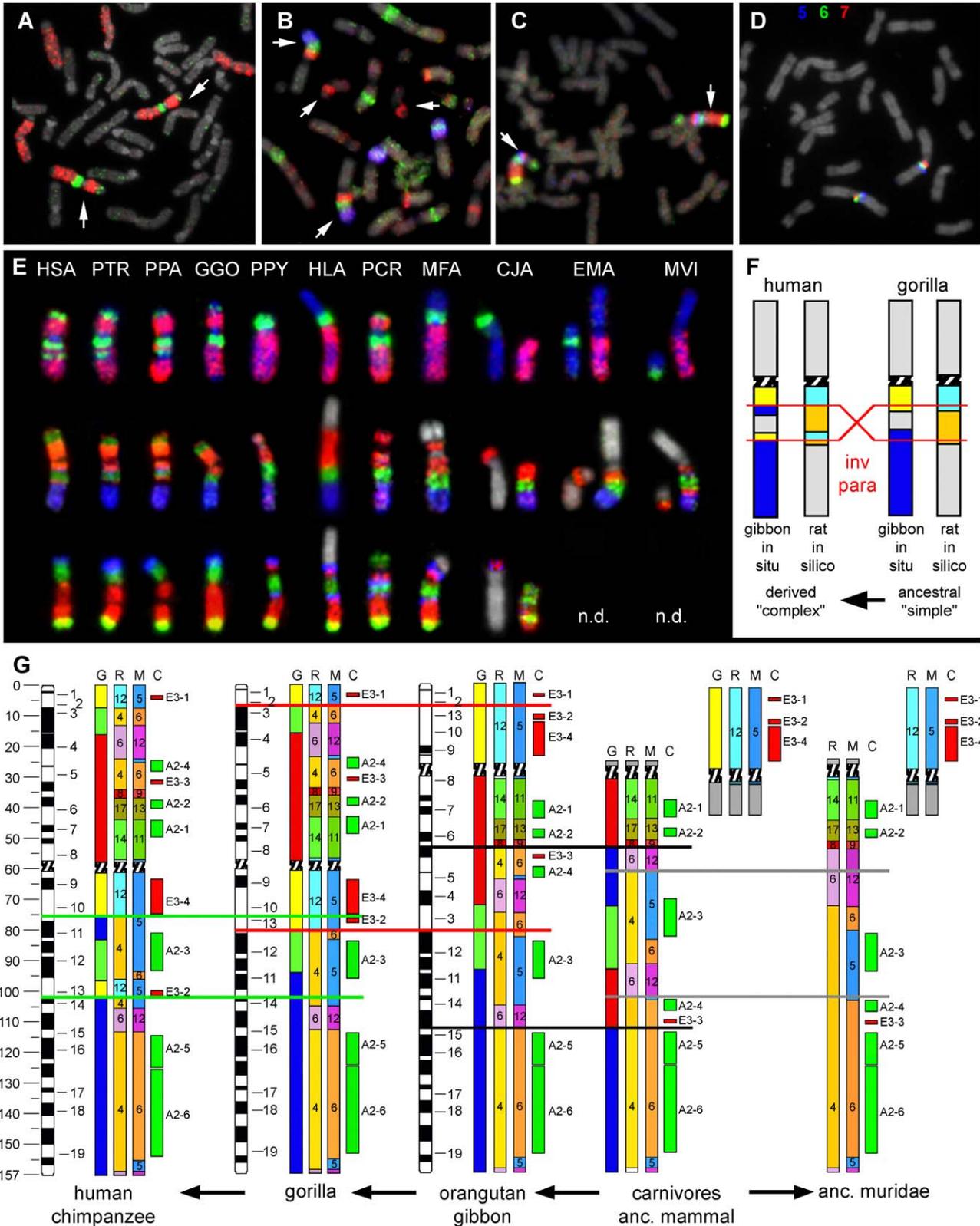
Evolutionary rearrangements of primate chromosome 7 homologs

The information extracted from cross-species FISH experiments with chromosome 7 subregional DNA probes provided a comprehensive overview defining the landmarks of inter- and intrachromosomal rearrangements that occurred

Fig. 1. (A–D) Illustrations of representative FISH experiments on primate metaphases using subregional paint probes and BAC clones. (A) Gorilla metaphase hybridized with African green monkey chromosomes 21 (red) and 28 (green); (B) concolor gibbon chromosomes 11 (green), 13 (blue), and 17 (red) on black lemur chromosomes; (C) microdissection-derived chromosome 7p- (green), 7q- (red), 7pter- (blue), and 7qter- (yellow) specific probes to silvered-leaf monkey chromosomes; and (D) BAC clones 5 (blue), 6 (green), and 7 (red) to a white-handed gibbon metaphase. (E) Summary of the hybridization pattern observed with African green monkey (top row), gibbon (middle row), and microdissection probes (bottom row) on human (HSA) chromosome 7 and homologs of chimpanzee (PTR), bonobo (PPA), gorilla (GGO), orangutan (PPY), white-handed gibbon (HLA), silvered-leaf monkey (PCR), crab eating macaque (MFA), marmoset (CJA), black lemur (EMA), and American mink (MVI). (F) Both in silico and in situ painting results visualize inversion breakpoints as well as their evolutionary direction by an increasingly simple pattern when tracing the rearrangements in the evolutionary reverse direction. As an example the paracentric inversion between human and gorilla is shown (see Discussion for details). (G) Summary of the mapping positions of BAC clones 1–19 in human and great apes together with the cross-species FISH data derived from gibbon and African green monkey (G) paint probes. The FISH data were aligned with the comparative sequence maps of mouse (M) and rat (R) and the comparative gene map of the cat (C). From left to right the reconstruction of the ancestral mammalian organization of conserved segment homologs and the evolutionary succession of chromosome 7 rearrangements are presented. Colored horizontal bars indicate inversion breakpoints.

during primate evolution, as well as their evolutionary direction (Fig. 2). As recently summarized [6], chromosome painting studies in a variety of nonprimate mammals already demonstrated that the ancestral condition for primates and various nonprimate mammals is represented by two separate

chromosome 7 homologs, the smaller one being associated with the human chromosome 16p homolog (7a and 7b/16p). This condition is still conserved in lemurs. In addition, our present results indicate that the subchromosomal organization of the 7a homolog is conserved between the lemur and



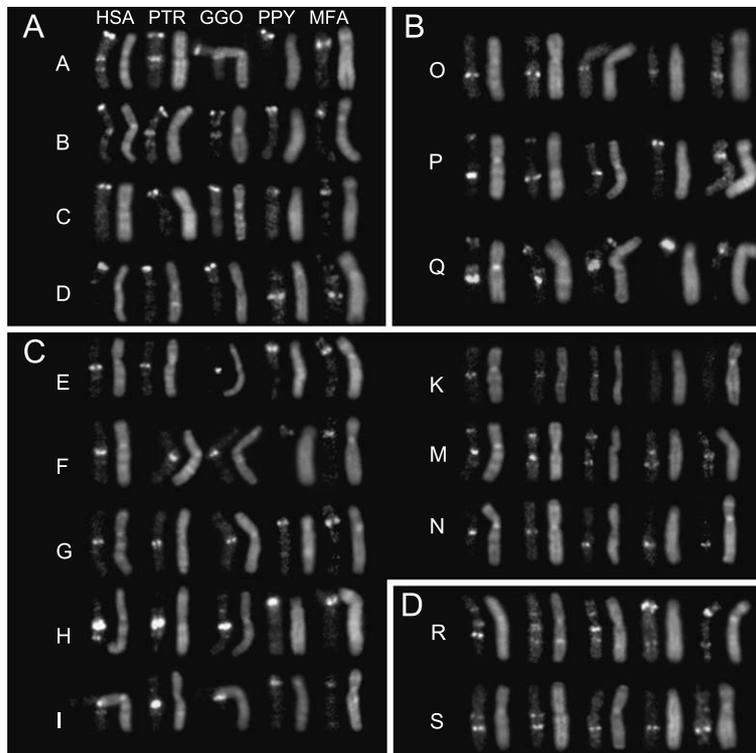


Fig. 3. Hybridization patterns of breakpoint flanking BAC clones A–S on human chromosome 7 (HSA) and on the homologs of chimpanzee (PTR), gorilla (GGO), orangutan (PPY), and macaque (MFA) (each row from left to right). All BACs are derived from chromosomal regions rich in segmental duplications. (A and B) Flanking clones of the pericentric inversion, (C and D) those of the paracentric inversion. (A) Contiguous clones A/B/C/D map to human 7p22.1, and (B) clones O/P/Q map to 7q22.1. The breakpoints of the pericentric inversion are located between BACs C/D and O/P, because in orangutan and macaque BACs C and P map to the p arm, while D and O hybridize to the q arm. (C and D) Clones E/F/G/H/I/K/M/N map to human chromosome 7q11.23 and R/S to 7q22.1. The 7q11.23 breakpoint of the paracentric inversion is located between BAC I and BAC M. It is associated with a large insertion derived from the human chromosome 1 homolog defined by BAC K and is specific for human and African apes. The 7q22.1 breakpoint is located between BAC R and BAC S.

The evolutionary origin of primate chromosome 7 homologs

On the basis of comparative banding studies, it has been hypothesized that the ancestral mammalian chromosome 7a was a large acrocentric chromosome with a banding pattern similar to that found in lemur, in cat, and in some other mammals [6]. We verified this hypothesis by the integration of *in silico* chromosome painting data extracted from interspecies comparative sequence and gene maps with our *in situ* subregional painting data in the various primate species and the carnivore (Figs. 1F and 1G). In a first step we aligned the human/cat comparative gene map [18] with the human/mouse/rat comparative sequence map (NCBI Human Build 34, NCBI Mouse Build 32, and RGSC Rat Build 3.1; www.ensembl.org) and the hybridization pattern observed with gibbon and African green monkey painting data probes on human chromosomes. Then the hybridization patterns observed with concolor gibbon and African green monkey painting probes on human chromosomes were aligned with the human/mouse/rat comparative sequence maps (NCBI Human Build 34, NCBI Mouse Build 30/32, and RGSC Rat Build 3.1; www.ensembl.org) (Fig. 1G, left).

Most notably, on human chromosome 7 the location of all four hominoid inversion breakpoints delineated by *in situ*

subregional painting showed a near-perfect correlation with the *in silico* segment borders of mouse chromosomes 5 and 6 as well as rat chromosomes 4 and 12 (Figs. 1F and 1G). The presence of multiple disrupted syntenic mouse/rat segments (mouse chromosomes 5/6 and rat chromosomes 4/12) reflects the highly derived state of human chromosome 7 by inversions. Muridae (mouse and rat) homologs were also derived, but by multiple translocations. These translocations occurred prior to the above-mentioned primate-specific intrachromosomal rearrangements and show different breakpoints. The mouse/rat *in silico* painting data therefore can be read like the *in situ* data derived from gibbon and African green monkey paint probes (see above). For example, both breakpoints of the paracentric inversion, by which the human and gorilla homologs are distinguished, correspond to disrupted syntenies that formed gibbon chromosomes 13 and 17 as well as rat chromosomes 4 and 12. When the paracentric inversion is traced back in evolution, both the FISH and the inferred rat/mouse segment homolog arrangement observed in the gorilla became less complex, reflecting its more ancestral state (Fig. 1F).

Further information came from the comparison of gene order in human and domestic cat. Genes mapped to cat

chromosome A2 [18], which is homologous to the ancestral primate chromosome 7a, were located on the orangutan q arm. They were separated from genes that map to cat chromosome E3, which is homologous to the ancestral primate 7b/16p homolog. The only exception is a single gene (c7orf16, ENSG10634, cat segment homolog E3-3, Fig. 1G), which may have been wrongly assigned or transposed by other mechanisms. When further comparing the present hybridization pattern in the American mink with gene mapping data obtained in the domestic cat, the segment order of both human chromosome 7 homologs appears identical in the two carnivores.

When going farther in the evolutionary reverse direction, we could identify the rearrangement by which the ancestral primate differs from that of the cat. A large paracentric inversion in the 7a homolog has to be assumed, which inverts cat segment homologs A2-4 and A2-3, to derive the ancestral carnivore chromosome form from that of the ancestral primate. The inferred breakpoints again would fuse homologous segments on mouse chromosome 6 and rat chromosome 4, respectively (Fig. 1G). This rearrangement results in the ordered sequence of cat chromosome markers A2-1 to A2-6 as described by Menotti-Raymond et al. [18].

Recent molecular data placed primates and rodents in the same clade, Euarchontoglires, while carnivores are grouped into the sister clade Laurasiatheria [19]. Thus, the most parsimonious conclusion from the observed mouse/rat, primate, and carnivore patterns of human chromosome 7a homologs is that the two carnivores have conserved the ancestral Boreo-eutherian chromosome form, from which the ancestral muridae and the ancestral primate 7a homologs were derived each by one distinctly different inversion. This is also in agreement with the *in situ* hybridization pattern observed with concolor gibbon paint probes on human chromosome 7 homologs of the mink, but differs from previous interpretations of comparative banding analysis [6].

In summary, the following sequence of chromosome 7 rearrangements during primate evolution is the most probable: a paracentric inversion in the putative Boreo-eutherian ancestor (breakpoints at approx 33 and 112 Mb of the human reference sequence; www.ncbi.org) created the ancestral primate homolog 7a, followed by a centromeric fusion of 7a and 7b in higher Old World primates. In hominoids, a pericentric inversion led to the chromosome form in the African ape ancestor (breakpoints at approx 7 and 96 Mb), followed by a paracentric inversion in the human/chimpanzee ancestor (breakpoints at approx 75 and 101 Mb) (Figs. 1G and 2).

The results with BAC clones 1–19 in hominoid primates (see Supplementary Table 1 for clone identity) revealed no intrachromosomal rearrangements other than those already delineated by region-specific paint probes. The results (Fig. 1G) confirmed that the breakpoints of the pericentric inversion map between 5.7 and 9.7 Mb (BACs

2 and 3) and between 90.0 and 99.9 Mb (BACs 12 and 13). The breakpoints of the paracentric inversion lie between 72.8 and 81.6 Mb (BACs 10 and 11) and between 99.9 and 104.7 Mb (BACs 14 and 15). All four breakpoints were localized in regions where we already predicted that the borders of mouse chromosome 5/6 and rat chromosome 4/12 segment homologs would correspond with hominoid inversion breakpoints (see above). Interestingly, the human/mouse/rat comparative sequence map shows gaps for the breakpoint regions in mouse and rat, in which inter- and intrachromosomal segmental duplications have been identified in the human genome [20]. Under the assumption that all four breakpoints would colocalize with the respective junctions, we performed a comparative FISH study with tile path BAC clones named A to S (see Supplementary Table 1 for clone identity), which spanned these chromosomal regions in the human genome.

Hominoid chromosome 7 pericentric inversion breakpoints

In human, chimpanzee, gorilla, and orangutan, clones A/B/C/D (7p22.1, 6.39–6.86 Mb) and O/P/Q (7q22.1, 97.01–97.32 Mb) showed extensive cross-hybridization due to the presence of both inter- and intrachromosomal segmental duplications DI143–149 and DI370–378 (in total approx 300 kb each) in both regions ([20]; www.chr.7.org). However, under the hybridization conditions used, in the macaque homolog the breakpoints could be securely localized between BAC pairs C/D and O/P, respectively. Since neither BAC C nor BAC D showed a split signal, the 7p inversion breakpoint has to be localized near the proximal end of BAC C or the distal end of BAC D at 6.81 Mb on the human sequence map. BAC P produced a weak split signal in orangutan and macaque and therefore may be a breakpoint-spanning clone in 7q. Alternatively, the split signal could be the result of an intrachromosomal duplication already present in these two species. The latter hypothesis may be favorable, since the same intensity ratio of the split signal was observed in inverted orientation in human, chimpanzee, and gorilla (Fig. 3B). In any case, the proximally overlapping BAC Q entirely mapped to the other side of the 7q breakpoint, which confined it to approx 97.08 Mb on the human sequence map. Both breakpoints are located in regions with predominantly interchromosomal duplications, which were already present in the orangutan, but spread to a variety of additional chromosomes in the gorilla (Supplementary Fig. 2). Notably, the breakpoint flanking the 32-kb segmental duplications DI145 and DI376 share 10- and 6-kb DNA stretches of 95–96% sequence identity on the human sequence map as well as the same transchromosomal duplicons on human chromosomes 2, 3, 4, 8, 9, 10, 11, 12, 13, 14, and 15. The analysis of the gene content in both regions (Supplementary Table 2) revealed two putative members of the olfactory gene superfamily within 100–200 kb of the breakpoints.

Hominoid chromosome 7 paracentric inversion breakpoints

The 7q11.23 breakpoint of the paracentric inversion was assigned by BACs mapping to the interval 76.13 and 76.35 Mb, while the 7q22.1 breakpoint mapped to 101.78–102.03 Mb on the human sequence map (Fig. 3D). The 7q11.23 breakpoint is located in close proximity to a 200-kb or even larger insertion (DI357–361; www.chr7.org) of chromosome 1 material, highlighted by clone K (Fig. 3C, Supplementary Fig. 2B). Clone K hybridized to chromosome 1 in all higher primates analyzed. The signal on the chromosome 7 homologs was restricted to gorilla, chimpanzee, and humans. This insertion may therefore be dated back to the African ape ancestor. Intrachromosomal duplicons DI364 and 365 (76.3 Mb) and DI400 and 401 (101.9 Mb) mark an at least 110-kb stretch of nearly identical DNA sequence, which is most probably directly flanking the proximal and the distal breakpoint at approx 101.91 Mb. This assumption gets support from the hybridization pattern of BACs M, R, and S, which show split signals in human and great apes, including the orangutan (Fig. 3, Supplementary Figs. 2A, 2C, and 2D). Thus, it seems very much likely that the duplicated segment should have been already present before the origin of this inversion.

The analysis of the gene content in these breakpoint regions revealed a number of putative duplicated genes (Supplementary Table 2), which were duplicated close to one of the two breakpoints (Deltex *Drosophila* homolog 2 in 7q11.23 and “similar to WBSCR 19” in 7q22.1). In addition MGC35361 and “similar to WBSCR 19” were duplicated near both breakpoints. Finally, ZP3 formed a second fusion gene (POMZP3) in 7q11.23.

Segmental duplications and evolutionary chromosome 7 inversions

Segmental duplications or low-copy repeats (LCRs) have been demonstrated to be involved in several genomic disorders ([21] for a recent review). They have also been shown to be associated with a number of chromosomal rearrangements that occurred during higher primate evolution, for example with the fusion of human chromosome 2 [22], translocation t(5;17) in the gorilla [23], the emergence of neocentromeres on chromosomes 6 and 15 [24,25], and the inversions of chromosomes 3, 15, and 18 [26–28]. The breakpoints associated with chromosome 7 inversions provide another example of the association of segmental duplications with intrachromosomal rearrangements that took place in hominoid primates.

All four breakpoints are located in chromosome regions without any sequence homology to mouse or rat, suggesting that the amplification and transposition of LCRs occurred after the split of the primate/rodent lineages. In fact, in our FISH experiments the majority of duplicons in proximity to chromosome 7 breakpoints were also barely or not detectable in the macaque. These findings indicate, but do not

necessarily prove, a more recent intra- or transchromosomal expansion in the hominoid lineage. Alternatively, it can be argued that these duplicated sequences were already present in the macaque genome, but have diverged too far to be detected by FISH under the stringency conditions used in our study. A more accurate answer to this question would necessitate a comparative sequence analysis of the homologous regions in a variety of primates.

Within hominoid primates, the occurrence and chromosomal distribution of LCRs again showed drastic differences, in particular comparing the orangutan with African apes and human. LCRs present in BACs C and P flanking the pericentric inversion were detected on only three different chromosomes in the orangutan, but in at least eight in human, chimpanzee, and gorilla (Supplementary Figs. 1B and 1D). In addition, the insertion of chromosome 1 homologous material (Supplementary Figs. 2A and 2B) and the intrachromosomal duplication close to the proximal breakpoint of the paracentric inversion in 7q11.23 (Supplementary Fig. 2C) were not detected in the orangutan and may also be dated back to the ancestor of African apes.

The distribution of LCRs raises the question whether the segmental duplications flanking the breakpoints were present prior to the rearrangements or alternatively were spread in the course of the rearrangements. A definite answer may be given only by comparative sequence analysis in species representing the ancestral state, although the results presented here provide some suggestions. First, different types of duplicons appear to flank the peri- and paracentric inversion breakpoints, indicating that they may have emerged in different waves, predominantly interchromosomal duplications in the pericentric inversion, while a transposition from chromosome 1 in combination with mainly intrachromosomal duplications flanks the paracentric inversion. In addition, the transchromosomal duplicons were already present in the orangutan, while the inversion occurred in the ancestor of African apes. Further, considering that both the duplicated sequences within the chromosome and the chromosome 1 transposition were already present in the gorilla homolog, segmental duplications may have been the cause and not the result of both rearrangements.

The large-scale organization of the 7q11.23 breakpoint in hominoid primates could still not be fully resolved, which may be partly due to the fact that the human sequence alignment in this region was still in flux during releases 31–34 of the human genome assembly (www.ncbi.nlm.nih.gov). This region, however, may hold the key to a better understanding of the molecular mechanisms that shaped human chromosome 7 during evolution. Most intriguing in this respect are previous findings on the involvement of the WBS region in these evolutionary rearrangements [4]. Duplicons DI334, 335, and 345–348 from the WBS region represent two 1.5-Mb spaced blocks of more than 500 kb in size each (www.chr7.org). They are also found within a short distance of the actual 7q11.23 inversion breakpoint

(less than 200 kb distance and 1.5 Mb distal of the WBS region), near the 7p22.1 and the 7q22.1 breakpoints (500 kb and less than 100 kb distal, respectively). Interestingly, both in 7p22 and in 7q22.1 a second copy of the same duplicated segment is observed in approximately 1.5 Mb distance. It could be speculated that this strikingly similar long-range organization in three of the actual breakpoint regions and the WBS region, which all can be traced back to a contiguous, roughly 5-Mb segment in the evolutionary ancestral state, originated from local duplications. These larger segments were then dispersed to other regions on the human chromosome 7 homologs, which can be associated with both the WBS and the observed evolutionary rearrangements.

Materials and methods

Cell samples, tissue culture, and chromosome preparation

We used lymphoblastoid cell lines from human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), bonobo (*Pan paniscus*), gorilla (*Gorilla gorilla*), orangutan (*Pongo pygmaeus*), white-handed gibbon (*Hylobates lar*), silvered-leaf monkey (*Presbytis cristata*), and marmoset (*Callithrix jacchus*). Fibroblast cell lines were used from the crab-eating macaque (*Macaca fascicularis*), African green monkey (*Cercopithecus aethiops*), black lemur (*Eulemur macaco*), and a carnivore (American mink, *Mustela vison*). The cell lines are the same as described [7–9], except for the mink cell line Mv.1.Lu, which was obtained from the European Collection of Cell Cultures (ECACC No. 88050503) and had a normal diploid karyotype of $2n = 30$. Metaphase preparations for FISH experiments were obtained by standard techniques.

DNA probes for FISH

Chromosome 7 subregional painting probes were employed in a variety of primates and a carnivore. These probes were derived from flow-sorted concolor gibbon chromosomes 11, 13, and 17 and African green monkey chromosomes 21 and 28 [7,10]. Further microdissection-derived human chromosome 7p-, 7q-, 7pter-, and 7qter-specific probes [11,12] were hybridized. BAC clones were used for a detailed analysis of the subregional organization and evolutionary breakpoints in chromosome 7 homologs of hominoid primates. A set of 19 clones (BAC 1–19), which mapped in 5- to 10-Mb intervals along chromosome 7 were kindly provided by Dr. T. Ried, National Cancer Institute (Bethesda, MD, USA; www.ncbi.nih.gov/genome/cyto [13]). Further, for a detailed breakpoint characterization a set of 18 “tile path” clones (BAC A–S) was obtained from BACPAC Resources at CHORI (www.bacpac.chori.org). Supplementary Table 1 summarizes the mapping positions of all BAC clones used in the experiments.

Probe labeling, in situ hybridization, and detection

The African green monkey chromosome 21 and 28 probes were DOP-PCR labeled [14] with biotin–dUTP and digoxigenin–dUTP (Fig. 1A). The concolor gibbon chromosome 11, 13, and 17 (Fig. 1B) and the microdissection probes (Fig. 1C) were labeled with biotin–dUTP, digoxigenin–dUTP, and TAMRA–dUTP, respectively. BAC clones were labeled with biotin–dUTP, digoxigenin–dUTP, and/or TAMRA–dUTP either by DOP-PCR or by nick-translation and were hybridized in sets of two or three (Fig. 1D) clones. For a single FISH experiment, 200–500 ng chromosome paint probe or 500 ng–1 µg BAC DNA was mixed with 10–50 µg human Cot-1 DNA, ethanol precipitated, and dissolved in hybridization buffer (50% deionized formamide, 10% dextran sulfate, $2\times$ SSC). After hybridization for 24–72 h at 37°C, the slides were washed for 3×5 min in $0.1\times$ SSC at 62°C, except for the hybridizations to lemur and mink. For the latter species washings were performed for 2×5 min in 50% formamide/ $2\times$ SSC and 2×5 min in $2\times$ SSC (37°C). Biotinylated probes were detected with either avidin–Cy3 or avidin–Cy5, digoxigenin labeled probes with sheep anti-digoxigenin–FITC-coupled antibody. Chromosomes were counterstained with DAPI.

Microscopy and image analysis

Metaphase images were captured with a cooled CCD camera (Photometrics C250/A equipped with a Kodak KAF1400 chip) coupled to a Zeiss Axiophot microscope. Camera control and digital image acquisition were performed using SmartCapture VP software (Digital Scientific, Cambridge, UK).

Acknowledgments

This study was supported by DFG Grant Wi 970/6-1. We thank T. Cremer and T. Meitinger for continuous support and T. Ried for providing the DNA of various BAC clones.

References

- [1] I.C. Clemente, M. Ponsa, M. Garcia, J. Egozcue, Evolution of the Simiiformes and the phylogeny of human chromosomes, *Hum. Genet.* 84 (6) (1990) 493–506.
- [2] B. Dutrillaux, Chromosomal evolution in primates: tentative phylogeny from *Microcebus murinus* (Prosimian) to man, *Hum. Genet.* 48 (3) (1979) 251–314.
- [3] J.J. Yunis, O. Prakash, The origin of man: a chromosomal pictorial legacy, *Science* 215 (4539) (1982) 1525–1530.
- [4] U. DeSilva, H. Massa, B.J. Trask, E.D. Green, Comparative mapping of the region of human chromosome 7 deleted in Williams syndrome, *Genome Res.* 9 (5) (1999) 428–436.

- [5] B. Dutrillaux, Y. Rumpler, Chromosome banding analogies between a prosimian (*Microcebus murinus*), a platyrrhine (*Cebus capucinus*), and man, *Am. J. Phys. Anthropol.* 52 (1) (1980) 133–137.
- [6] F. Richard, M. Lombard, B. Dutrillaux, Phylogenetic origin of human chromosomes 7, 16, and 19 and their homologs in placental mammals, *Genome Res.* 10 (5) (2000) 644–651.
- [7] P. Finelli, R. Stanyon, R. Plesker, M.A. Ferguson-Smith, P.C. O'Brien, J. Wienberg, Reciprocal chromosome painting shows that the great difference in diploid number between human and African green monkey is mostly due to non-Robertsonian fissions, *Mamm. Genome* 10 (7) (1999) 713–718.
- [8] S. Müller, R. Stanyon, P. Finelli, N. Archidiacono, J. Wienberg, Molecular cytogenetic dissection of human chromosomes 3 and 21 evolution, *Proc. Natl. Acad. Sci. USA* 97 (1) (2000) 206–211.
- [9] S. Müller, M. Neusser, J. Wienberg, Towards unlimited colors for fluorescence in-situ hybridization (FISH), *Chromosome Res.* 10 (3) (2002) 223–232.
- [10] S. Müller, P.C. O'Brien, M.A. Ferguson-Smith, J. Wienberg, Cross-species colour segmenting: a novel tool in human karyotype analysis, *Cytometry* 33 (4) (1998) 445–452.
- [11] X.Y. Guan, H. Zhang, M. Bittner, Y. Jiang, P. Meltzer, J. Trent, Chromosome arm painting probes, *Nat. Genet.* 12 (1) (1996) 10–11.
- [12] L. Hu, J.S. Sham, W.M. Tjia, Y. Tan, G. Lu, X.Y. Guan, Generation of a complete set of human telomeric band painting probes by chromosome microdissection, *Genomics* 83 (2) (2004) 298–302.
- [13] V.G. Cheung, N. Nowak, W. Jang, I.R. Kirsch, S. Zhao, X.N. Chen, et al., Integration of cytogenetic landmarks into the draft sequence of the human genome, *Nature* 409 (6822) (2001) 953–958.
- [14] H. Telenius, A.H. Pelmear, A. Tunnacliffe, N.P. Carter, A. Behmel, M.A. Ferguson-Smith, et al., Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes, *Genes Chromosomes Cancer* 4 (3) (1992) 257–263.
- [15] A. Jauch, J. Wienberg, R. Stanyon, N. Arnold, S. Tofanelli, T. Ishida, et al., Reconstruction of genomic rearrangements in great apes and gibbons by chromosome painting, *Proc. Natl. Acad. Sci. USA* 89 (18) (1992) 8611–8615.
- [16] J. Wienberg, A. Jauch, R. Stanyon, T. Cremer, Molecular cytogenomics of primates by chromosomal in situ suppression hybridization, *Genomics* 8 (2) (1990) 347–350.
- [17] U. Koehler, F. Bigoni, J. Wienberg, R. Stanyon, Genomic reorganization in the concolor gibbon (*Hylobates concolor*) revealed by chromosome painting, *Genomics* 30 (2) (1995) 287–292.
- [18] M. Menotti-Raymond, V.A. David, Z.Q. Chen, K.A. Menotti, S. Sun, A.A. Schaffer, et al., Second-generation integrated genetic linkage/radiation hybrid maps of the domestic cat (*Felis catus*), *J. Hered.* 94 (1) (2003) 95–106.
- [19] M.S. Springer, W.J. Murphy, E. Eizirik, S.J. O'Brien, Placental mammal diversification and the Cretaceous–Tertiary boundary, *Proc. Natl. Acad. Sci. USA* 100 (2003) 1056–1061.
- [20] L.W. Hillier, R.S. Fulton, L.A. Fulton, T.A. Graves, K.H. Pepin, C. Wagner-McPherson, et al., The DNA sequence of human chromosome 7, *Nature* 424 (6945) (2003) 157–164.
- [21] R.V. Samonte, E.E. Eichler, Segmental duplications and the evolution of the primate genome, *Nat. Rev. Genet.* 3 (1) (2002) 65–72.
- [22] Y. Fan, T. Newman, E. Linardopoulou, B.J. Trask, Gene content and function of the ancestral chromosome fusion site in human chromosome 2q13–2q14.1 and paralogous regions, *Genome Res.* 12 (11) (2002) 1663–1672.
- [23] P. Stankiewicz, S.S. Park, K. Inoue, J.R. Lupski, The evolutionary chromosome translocation 4;19 in *Gorilla gorilla* is associated with microduplication of the chromosome fragment syntenic to sequences surrounding the human proximal CMT1A-REP, *Genome Res.* 11 (7) (2001) 1205–1210.
- [24] V. Eder, M. Ventura, M. Ianigro, M. Teti, M. Rocchi, N. Archidiacono, Chromosome 6 phylogeny in primates and centromere repositioning, *Mol. Biol. Evol.* 20 (9) (2003) 1506–1512.
- [25] M. Ventura, J.M. Mudge, V. Palumbo, S. Burn, E. Blennow, M. Pierluigi, et al., Neocentromeres in 15q24–26 map to duplicons which flanked an ancestral centromere in 15q25, *Genome Res.* 13 (9) (2003) 2059–2068.
- [26] E. Tsend-Ayush, F. Grutzner, Y. Yue, B. Grossmann, U. Hansel, R. Sudbrak, et al., Plasticity of human chromosome 3 during primate evolution, *Genomics* 83 (2) (2004) 193–202.
- [27] D.P. Locke, N. Archidiacono, D. Misceo, M.F. Cardone, S. Deschamps, B. Roe, et al., Refinement of a chimpanzee pericentric inversion breakpoint to a segmental duplication cluster, *Genome Biol.* 4 (8) (2003) R50.
- [28] B.K. Dennehey, D.G. Gutches, E.H. McConkey, K.S. Krauter, Inversion, duplication, and changes in gene context are associated with human chromosome 18 evolution, *Genomics* 83 (3) (2004) 493–501.