Human chromosome 16 conservation in primates

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Abstract

A study was made of the organization of the chromosome orthologous to HSA16 in primates using a panel of 8 BAC probes spanning human chromosome 16. The probes were used in FISH experiments on great apes and on representatives of the Old World monkeys, New World monkeys, and lemurs. The domestic cat was used as an outgroup. The results indicate that 16p and 16q were separate chromosomes in a primate ancestor. They fused in a Catarrhini ancestor giving rise to the present day form found in HSA, great apes, and Old World monkeys. Several rearrangements were found in New World monkeys.

Introduction

Whole chromosome paints (WCP) have been widely exploited to track karyotype evolution of primates. Their use has the advantage of rapidly delineating the conservation of entire chromosomes but, conversely, their resolution is relatively low because no information is given on markers orientated along the chromosomes. A more analytical approach has been made possible by the availability of well-characterized BAC/PAC probe contigs covering almost entirely the human genome, generated by the Human Genome Sequencing Project. In this paper, we report a detailed study of the organization of chromosomes orthologous to HSA16 in primates using BAC probes spanning the human chromosome 16.

Materials and methods

A panel of BAC probes (from RP11 de Jong library, http://www.chori.org/bacpac/, see Figure 1) was used in FISH experiments on great apes: common chimpanzee (*Pan troglodytes*, PTR), gorilla (*Gorilla gorilla*, GGO), orangutan (*Pongo pygmaeus*, PPY); Old World monkeys (OWM): vervet monkey (*Cercopithecus aetyops*, CAE), silvered leaf monkey (*Presbytis cristata*, PCR), rhesus monkey (*Macaca mulatta*, MMU), sacred baboon (*Papio hamadryas*, PHA); New World monkeys (NWM): common marmoset (*Callitrix jaccus*, CJA), squirrel monkey (*Saimiri sciureus*, SSC), dusky titi monkey (*Callicebus moloch*, CMO). Additional experiments were also performed on the following OWM species: celebes
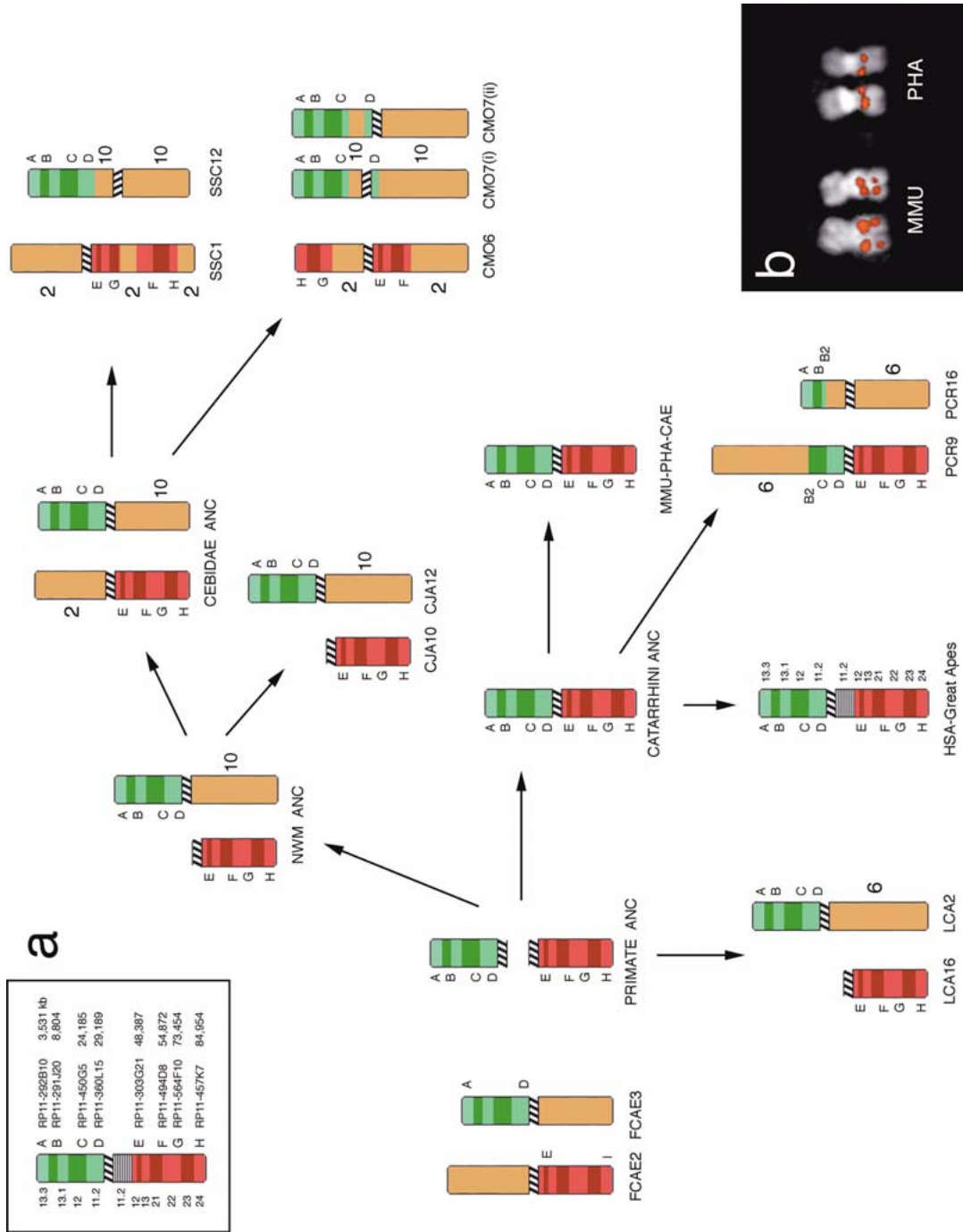
Figure 1. (**a**) A summary of the results obtained using the BAC probes reported in the box in the upper-left corner, where their positions in the UCSC database are also reported. (**b**) The FISH signals obtained using the BAC 497D8 in MMU (double signal) and in PHA. The ideogram of the SSC12 chromosome has been reported upside down to facilitate comparison. For details, see text.

macaque (*Macaca nigra*, MNI), stumptailed macaque (*Macaca arctoides*, MAR), long-tailed macaque (*Macaca fascicularis*, MFA); and on ring tailed lemur (*Lemur catta*, LCA). The cat (*Felis catus*, FCA) was used as an outgroup. Cohybridization experiments were used to establish marker order with certainty.

Results and discussion

The results are summarized in Figure 1, where also the used FISH probes along with their precise map position in the UCSC human genome sequence database (http://genome.ucsc.edu, November 2002 release) are reported (box in the upper-left corner). The karyotype organization of the species under study has been described by Yunis & Prakash 1982 (for great apes); Wienberg *et al.* 1992 (MMU); Sherlock *et al.* 1996 (CJA); Finelli *et al.* 1999 (CAE); Stanyon *et al.* 2000 (CMO and SSC).

The HSA16 marker order was found to be identical in PTR, GGO, PPY phylogenetic XVI, MMU (chromosome 20), CAE (chromosome 5), and PHA (chromosome 20). This arrangement was therefore considered as the ancestral form of Catarrhinae. In PCR, this chromosome underwent a reciprocal translocation with a chromosome corresponding to HSA6, giving rise to PCR chromosomes 9 and 16, as reported in Figure 1. These data are in agreement with those reported in the literature (Bigoni *et al.* 1997). The breakpoint was found to fall between BACs 291J20 (B) and 450G5 (C), that are 15 Mb apart. To refine the breakpoint region, additional BACs were used. The most informative one was BAC 715P22 (B2 in Figure 1a), mapped at 18 136−18 270 kb in UCSC, which gave a splitting signal (data not shown).

The karyotypes of macaque and papio genera have been supposed to be almost identical. Indeed, G-banding and spectral karyotyping analyses of metaphases of a hybrid resulting from a mating between a female baboon (PHA) and a male rhesus macaque (MMU) failed to reveal any chromosomal difference (Moore *et al.* 1999). BAC probe 497D8 (F) gave a double signal on MMU (chr. 20) (Figure 1b), suggestive of a paracentric inversion or a segmental duplication. The double signal, however, was not found in PHA (Figure 1b), CAE, PCR, or in three additional macaque species examined with

this probe: MNI, MAR, and MFA (data not shown). Additional appropriate BAC probes (46D6, 315K4, 357N13, 419C5, 432I24, and 566K11, internal to the hypothetical inverted region) were cohybridized with BAC 497D8 in MMU metaphases. The experiments clarified that the double signal was not due to a paracentric inversion (data not shown). Our data, therefore, strongly suggest that the unusual behavior of BAC 497D8 on MMU is due to a segmental duplication. Segmental duplication in primates is a phenomenon that recently gained special attention for its evolutionary implications (Johnson *et al.* 2001, Bailey *et al.* 2002). The finding that the duplication is homozygous suggests that it is a common feature of the MMU population. If this is the case, it could represent a unique identification marker for MMU.

In all the NWM species we have investigated in the present study, HSA16p sequences were always found associated with the HSA10 synteny group. It was therefore assumed that this association, as found in CJA (Callitrichidae), was ancestral to NWM and can be supposed to be conserved also in the Cebidae ancestor. We have previously described that the metacentric morphology of SSC12 is the consequence of a centromere repositioning (Carbone *et al.* 2002). A pericentric inversion with the breakpoint between BACs 450G5 (C) and 360L15 (D) leads to the CMO7 form (i). Interestingly, the position of BAC 360L15 (D) in CMO7 is polymorphic: signals appear on the opposite side of the centromere in the two homologs (50 metaphases analyzed). This polymorphism can be explained assuming an additional pericentric inversion leading to the form (ii), and which exists in the heterozygous state, as we have already described tracking the evolutionary history of human chromosome 10 (Carbone *et al.* 2002). We do not know which is the most common form and its frequency in the population.

The evolutionary history of HSA16q in NWM was more complex. In CJA, it is a separate acrocentric chromosome (CJA20), with HSA16q conserved marker order. In SSC and CMO, HSA16q is fused with sequences corresponding to a portion of HSA2 (CMO6; SSC1). A pericentric inversion disrupted the 16q marker order generating the present-day form of CMO6, the breakpoint being located between 497D8 (F) and

564F10 (G). The complex rearrangement of HSA16q in SSC1 can be derived from the ancestral form assuming a paracentric inversion with breakpoints between markers E/F and G/H, followed by two pericentric inversions and a fusion with HSA5 sequences.

HSA16q is a single chromosome in LCA, while 16p is fused with HSA6 sequences (Cardone *et al.* 2002). HSA16p and HSA16q marker order is conserved. The findings that both HSA16p and HSA16q appear as separate chromosomes or fused with different partners in OWM, NWM, and in LCA strongly support the hypothesis that 16p and 16q were organized as separate chromosomes in the primate ancestor (Murphy *et al.* 2001), as reported in Figure 1. The panel of BAC probes was also hybridized to metaphases of the cat, chosen as outgroup. Only probes A, D, and E gave satisfactory FISH signals. A pool of probes 533D19, 309G16, and 443M9 (I in Figure 1a) was used as an alternative to probe H. They map very close to the 16q telomere (interval 86−91 Mb) and yielded a distinct FISH signal close to the FCAE2 telomere (Figure 1a). These results suggest that, in cat, HSA16q and HSA16p constitute the entire and uninterrupted long arm and short arm of FCAE2 and FCAE3 chromosomes, respectively, and reinforce the conclusion that 16p and 16q were separate in the primate ancestor.

Acknowledgements

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