Chromosome 6 Phylogeny in Primates and Centromere Repositioning

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A panel of 15 human BAC/PAC probes, covering the entire chromosome 6, was used in FISH experiments on great apes and on representatives of Old World monkeys, New World monkeys, and lemurs to delineate the chromosome 6 phylogeny in primates. The domestic cat was used as an outgroup. The analysis showed a high marker order conservation, with few rearrangements required to reconcile the hypothesized chromosome 6 organization in primate ancestor with marker arrangement in all the examined species. Contrary to this simple evolutionary scenario, however, the centromere was found to be located in three distinct regions, without any evidence of chromosomal rearrangement that would account for its movement. One of the two centromere repositioning events occurred in great apes ancestor. The centromere moved from 6p22.1 to the present day location after the inversion event that differentiated marker order of the primate ancestor from the ancestor of Catarrhini. A cluster of intrachromosomal segmental duplications was found at 6p22.1, scattered in a region of about 9 Mb, which we interpret as remains of duplicons that flanked the ancestral centromere. Our data, therefore, suggest that some duplicon clusters found in noncentromeric/nontelomeric locations may represent traces of evolutionary silenced centromeres that inactivated after the occurrence of a centromere repositioning. In addition, the neocentromere emergence we have documented in Old World monkeys at 6q24.3 appears to have arisen and progressed without affecting the displaced flanking sequences.

Introduction

Chromosomal phylogeny studies in primates were initiated using the chromosome banding technique (Dutrillaux 1979; Yunis and Prakash 1982). In this respect, fluorescence in situ hybridization (FISH) introduced more powerful tools, and several papers have contributed in the elucidation of primate karyotype architecture. In most cases, whole or partial chromosome paint libraries have been utilized (Wienberg et al. 1992; Murphy, Stanyon, and O'Brien 2001). Painting libraries alone, however, are not effective in precisely defining marker order along chromosomes. We have shown that marker order assessment is crucial to disclose unpredicted phenomena such as centromere repositioning (Montefalcone et al. 1999; Ventura, Archidiacono, and Rocchi 2001). For this reason, we undertook a systematic investigation of chromosome evolution in primates using panels of appropriate BAC/PAC probes to reveal evolutionary cytogenetic events that led to the architectural organization of chromosomes of extant primates (Carbone et al. 2002; Cardone et al. 2002). In the present paper, we used a panel of 15 BAC/PAC probes mapping along human chromosome 6 to delineate in detail the phylogeny of this chromosome. The results have shown that marker order of this chromosome is relatively conserved, whereas the centromere underwent two repositioning movements.

Methods

Human metaphase spreads were obtained from PHA-stimulated peripheral lymphocytes of a normal donor by standard procedures. Metaphase preparations from primates were obtained from lymphoblastoid or fibroblast cell lines of the following species—great apes: common chimpanzee (*Pan troglodytes* [PTR]), pygmy chimpanzee (*Pan paniscus* [PPA]), gorilla (*Gorilla gorilla* [GGO]), and Borneo orangutan (*Pongo pygmaeus pygmaeus*, [PPY]); Old World monkeys (OWM): long-tailed macaque (*Macaca fascicularis* [MFA, Cercopithecinae]) and silvered-leaf monkey (*Presbytis cristata* [PCR, Colobinae]); New World monkeys (NWM): common marmoset (*Callithrix jacchus* [CJA]) and wooly monkey (*Lagothrix lagothricha* [LLA]); prosimians: black lemur (*Eulemur macaco* [EMA]). The domestic cat (*Felis catus* [FCA]) was also investigated as an outgroup.

All human BAC/PAC probes belong to the RP de Jong libraries (http://www.chori.org/bacpac/). They are reported in table 1. Their position on the human genome sequence is derived from the University of California Santa Cruz database (http://genome.ucsc.edu, November 2002 release) and confirmed by FISH. The cat BAC probe RP86-11M10 was obtained by screening high-density filters of the cat RP86/segment 1 library. The screening was carried out using human PCR products of primers SHGC-102104. We initially used a panel of 15 BAC/PAC probes (table 1). Several additional probes (about 100) were used to refine the precise location of breakpoints and of centromeric regions. Only the most informative(s) are reported.

FISH experiments were performed essentially as described by Lichter et al. (1990). DNA probes were directly labeled with Cy3-dUTP (Perkin-Elmer) or FluorX-dCTP by nick-translation. Two hundred nanograms of labeled probe were used for the FISH experiments. Hybridization was performed at 37°C in 2 × SSC, 50% (v/v) formamide, 10% (w/v) dextran sulphate, 5μg COT1 DNA (Roche), and 3 μg sonicated salmon sperm DNA, in a volume of 10 μl. Posthybridization washing was at 60°C in 0.1 × SSC (three times, high stringency). Washes of FISH experiments using human probes on primates were performed at lower stringency: 37°C in 2 × SSC, 50% formamide (×3), followed by washes at 42°C in 2 × SSC (×3). Digital images were obtained using a Leica DMRXA

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Table 1
Bacterial and P1 Artificial Chromosome Probes (BAC/PAC) Used in the Study

Code	Probe	Accession Number	UCSC (kb)[a]	Map
A	RP11-328C17	AL365272	253 kb	6p25.3
B	RP11-391F23	AL589203	969	6p25.3
C	RP11-4A24	AL137221	12,187	6p24.1
D	RP1-59B16	AL032822	23,959	6p22.3
E	RP1-139D8	AL096814	42,097	6p21.1
F	RP11-346L9	Ends	57,239	6p12.3
Centromere			58,5–62,0	
G	RP11-346M3	Ends	62,374	6q11.1
H	RP5-1046G13	AL035633	72,961	6q13
I	RP3-494K13	AL136312	85,650	6q14.3
J	RP11-437I16	AL450340	106,171	6q21
K	RP11-117A20	AL589920	119,805	6q22.31
L	RP11-472E5	AL138828	136,269	6q23.3
M	RP11-64M7	AL589705	149,095	6q25.1
N	RP1-230L10	AL137005	163,992	6q27
O	RP11-302L19	AL596442	170,038	6q27
6qter			170,670	
B2	RP11-147C6	Ends	10,572	6p24.2
C2	RP11-100C22	Ends	13,602	6p23
D2	RP11-59N15	Ends	25,965	6p22.2
D3	RP11-104P20	Ends	26,248	6p22.2
E3	RP11-297M4	Ends	28,866	6p22.1
E2	RP11-261L19	Ends	29,108	6p22.1
H2	RP11-460K15	Ends	77,000	6q14.1
H3	RP1-974F1	AL133460	77,698	6q13
J2	RP11-815N24	Ends	108,355	6q21
K2	RP11-732K1	Ends	127,736	6q22.33
L2	RP11-474A9	Ends	145,457	6q24.3
M2	RP11-507C10	Ends	158,931	
P	RP86-11M10	PRP4 gene	Cat library	FCAB2

NOTE.—Probes A to O are used in all species. Probes B2 through P are additional probes.

[a] Only the beginning is reported.

epifluorescence microscope equipped with a cooled CCD camera (Princeton Instruments, NJ). Cy3 (red), FluorX (green), and DAPI (blue) fluorescence signals, detected with specific filters, were recorded separately as gray scale images. Pseudocoloring and merging of images were performed using Adobe Photoshop™ software.

Segmental duplications on chromosome 6p were searched using GenAlyzer program (an improved version of Reputer software), performed according to authors' instructions (Kurtz et al. 2001 [http://www.genomes.de/]) on a masked sequence, downloaded from the UCSC FTP site.

The percentage of similarity among different duplicons were analyzed using MegAlign software in DNAstar package (www.dnastar.com). The alignment was performed using ClustalW method and default parameters for this method (15.00 gap penalty and 6.60 gap length penalty).

Results

A set of 15 human probes (see table 1) was used in FISH experiments on nine primate species (see *Methods*). Probes in telomeric and pericentromeric regions were chosen among those located very close to the telomere or centromere, respectively, while still yielding a single FISH signal. Almost all couples of contiguous markers were cohybridized in each species to confirm with certainty their reciprocal order. The overall results are summarized in figure 1*a*. Marker order was found perfectly colinear in all great apes (HSA, PTR, PPA, GGO, and PPY). The MFA showed an inverted order of markers K-L-M, with the centromere located between markers L and M. Several additional BAC probes were used to better define the human region corresponding to the MFA centromere and to characterize the two breakpoints encompassing the inverted K-L-M chromosomal segment. BAC RP11-474A9 (L2 in figure 1*a* and in table 1) gave a split signal around MFA6 centromere, and the two very close BACs RP11-846G18 and RP11-646L14 were found on opposite sides. Similarly, the two breakpoints encompassing the inverted segment K-L-M were identified inside BACs RP11-815N24 (J2), and RP11-507C10 (M2). The two pairs of BACs RP11-347A10/RP11-315K22 and RP11-330C14/RP11-483G1, closely flanking markers J2 and M2 on opposite sides, respectively, yielded FISH signals in agreement with J2 and M2 being the breakpoints of the inversion involving markers K-L-M. Results of probes closely flanking markers L2, J2, and M2 are reported to reject the alternative interpretation that splitting signals were due to the presence of duplicons. For this reason flanking markers were also used to validate the splitting results reported below.

PCR marker arrangement was found to be relatively complex. Sequences corresponding to HSA6 underwent a balanced reciprocal translocation with sequences of chromosome 16, giving rise to PCR chromosomes 9 and 16. The chromosome 6 translocation breakpoint falls between markers H and I. The marker arrangement in PCR16 can be resolved assuming a pericentric inversion in this species, involving the segment defined by markers I-J-M-L (arranged as in MFA). Breakpoints of translocation and inversion were further refined. As far as the translocation is concerned, the most informative clones were BAC RP11-460K15 (code H2, at 76,900 kb in UCSC), found on PCR9q, and RP1-974F1(code H3, at 77,698 kb), mapping on PCR16p (data not shown). Probe RP11-662B13, mapping between H2 and H3, did not give any FISH signal. The two clones are approximately 500 kb apart. The most informative marker in respect to the inversion was RP11-732K1 (code K2, at 127,636 kb). This probe gave a split signal on PCR16q and PCR16p (fig. 1*c*). Probes RP11-662E15 and RP11-432I16, closely flanking the K2 probes, validated the splitting (see above). BAC RP11-474A9 (L2) gave split signal as in MFA.

In both NWM species (CJA and LLA), HSA6 is a unique chromosome (CJA4 and LLA1) (Sherlock et al. 1996; Stanyon et al. 2001). These two chromosomes showed a perfectly matching marker arrangement, differing from the marker order found in great apes for an inversion of markers A-B-C-D. The centromere is between markers A and E. Markers F and G, flanking the human centromere, were cohybridized on CJA. They gave almost overlapping signals (data not shown). Several human probes were used to restrict the breakpoint of the NWM inversion delimited by markers D and E. The most informative BACs were RP11-261L19 (29,108 kb in UCSC, E2 in the table 1) and RP11-297M4 (28,866 kb,

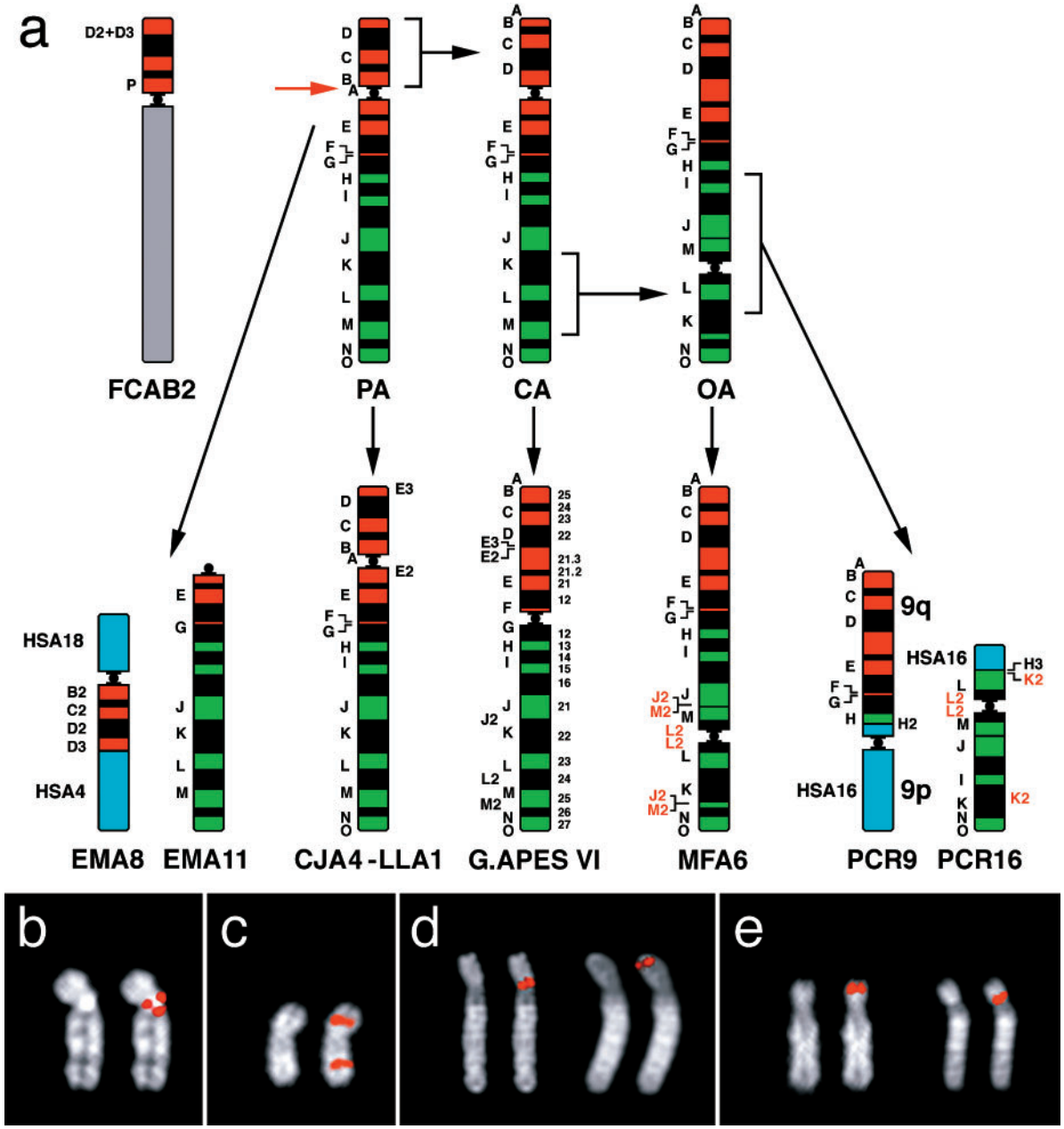

FIG. 1.—(*a*) Reconstruction of the chromosome 6 phylogeny in primates. MFA6 and PCR9 chromosomes are upside down to facilitate comparison. PA = primate ancestor; CA = Catarrhini ancestor; OA = OWM ancestor. (*b–e*) Examples of FISH experiments. (*b*) Marker L2 yielded FISH signals encompassing the MFA6 centromere. (*c*) The split signal of marker K in PCR16 chromosome. (*d*) CJA4 chromosomes showing FISH signals of markers E2 (left), localized adjacent to the centromere, and E3 (right), mapping close to the telomere. (*e*) Markers D2+D3 (left) and marker P (right) on cat chromosome B2. In each panel, the DAPI is separately shown on the left to allow a better morphological identification of the centromere position. For details see text.

E3) at 6p22.1. E2 was localized adjacent to the centromere on the CJA4/LLA1 long arm side, and E3 yielded a signal on the tip of the CJA4/LLA1 short arm (fig. 1*d*). Probe RP11-258N15, mapping between these two markers, did not yield any appreciable FISH signal.

All the HSA6p probes failed to give detectable FISH signals in EMA, as well as most of HSA6q probes, with the exception of I, N, and O. Additional HSA6p BAC clones (B2, C2, D2, and D3 [see table 1]) were chosen in highly conserved regions as suggested by the ''Human/Mouse Evolutionary Conservation'' track in the UCSC database. EMA11 marker order appeared identical to CJA4q and LLA1q. The centromere of this acrocentric chromosome is close to the marker E, as in CJA4 and LLA1. The additional probes B2, C2, D2, and D3 were found localized on EMA8, arranged as shown in figure 1*a*. EMA8 is fused with regions corresponding to HSA4 and HSA18, as already described (Muller et al. 1997; Cardone et al. 2002).

To better define marker order of chromosome 6 short arm in the primate ancestor (PA), the cat (*Felis catus* [FCA]) was used as an outgroup. Chromosome 6 in cat has

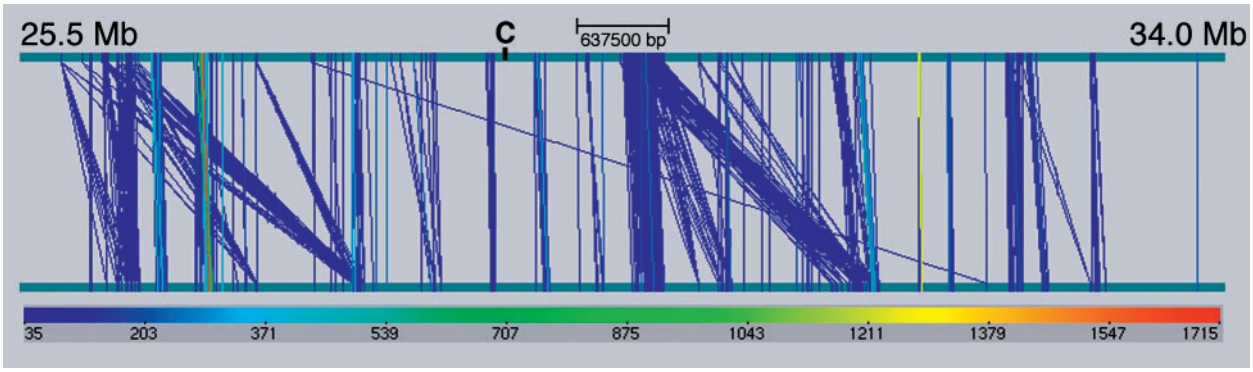

FIG. 2.—Wide-view graphic results of segmental duplication analysis of 8.5 Mb masked sequence around 6p22.1 (for detail see text), starting at 25.5 and ending at 34.0 Mb (UCSC database) obtained with the GenAlyzer software (see *Methods*), which performed a self-comparison of the sequence. The region was delimited by a previous analysis performed on a larger region (20 Mb). The starting positions of the first instance of the repeat on the upper strand and the starting position of the second instance of the repeat on the lower strand are connected by a diagonal line. Either forward or reverse complemented (palindromic) repeats are displayed. The color key (bottom line) associates a color to the range of repeat size. The length of the shortest (chosen at setup) and longest (found) repeat are the starting and ending values of the color key scale. A few isolated clusters of repeats shorter than 200 bp were eliminated from the picture for a better overall inspection. Parameters used: seedlength 14 bp; minimum size 35 bp; edit distance 3. To avoid undesirable background the sequence has been masked.

been shown to constitute the entire FCAB2 (Murphy et al. 2000). All the 6p human probes failed to reveal any FISH signal on cat chromosomes, with the exception of a combination of probes D2 and D3, located at HSA6p22.2, which gave a signal close to the telomere of FCAB2 short arm (fig. 1*e*). To collect additional data on the organization of the FCAB2 short arm, we screened the cat RP86 BAC library with PCR amplification products of STS SHGC-102104, using human DNA as template. In human, this STS is located inside the conserved PRP4 gene (pre-mRNA processing factor 4 homolog B, yeast), at 6p25.2 (4,006,594 to 4,045,963 bp on UCSC database), close to the 6p telomere. One strong positive signal was identified. The probe (RP86-11M10) gave a FISH signal close to the centromere on FCAB2, on the short arm side (fig. 1*e*).

As it will be discussed below, the centromere position showed some inconsistencies with marker arrangement in the different species, suggesting the occurrence of two centromere repositionings. In one instance, the centromere moved from 6p21 to the present position in great apes. The second repositioning occurred in OWM ancestor (OA). We searched for remains of centromeric-pericentromeric sequences in the region around markers E2 to E3, where the centromere of the Catarrhini ancestor (CA) was located. Sequences around markers E2 to E3 (from 20 to 40 Mb in UCSC) were analyzed using the GenAlyzer software in search of intrachromosomal duplications, which are typical of pericentromeric regions (Bailey et al. 2002). A clear cluster of duplicons was found in the 25.5 to 33.2Mb region (UCSC) (fig. 2). This region was then searched for duplicons against the entire human genome, using the same software. The results are reported in table 2 and, graphically, in figure 3. Sequence homology among these duplicons was investigated by MegAlign software (DNAstar package). The results are also reported in table 2 (last column).

Discussion

In this paper we have delineated the phylogeny of human chromosome 6 in primates using a panel of appropriate BAC/PAC probes distributed along the chromosome. The marker order was found to be perfectly matching in all the great apes. In macaque (Cercopithecinae) the marker arrangement was found different from great apes for an inversion of the chromosomal segment encompassing markers K, L, and M (fig. 1*a*). A splitting BAC was identified for both breakpoints of the inversion. In PCR (Colobinae), chromosome 6 underwent a balanced reciprocal translocation with the chromosome homologous to HSA16 (Bigoni et al. 1997*a*; Misceo et al. 2003). The HSA6 portion of PCR9 was colinear with the corresponding region of MFA. The translocation breakpoint was defined as occurring between probes H2 and H3, mapping about 500 kb apart. We have already delimited the breakpoint on the chromosome corresponding to HSA16 as lying inside the BAC RP11-715P22, mapped at 18,136 to18,270 kb in UCSC (Misceo et al. 2003). A pericentric inversion of markers I-J-M-L differentiated PCR16 from the corresponding region of MFA. The chromosomal regions involved in the two rearrangements (translocation and inversion) are not overlapping; therefore we could not determine their reciprocal timing. The PCR centromere was located between markers L and M, as in MFA. Bigoni et al. (1997*b*) have shown, using whole-chromosome paints, that chromosome 3 in guereza (*Colobus guereza* [CGU, Colobinae]) is an entire chromosome homologous to HSA6. CGU3 banding pattern and arm ratio strongly suggest that this chromosome has the identical marker order of MFA6. It can be reasonably concluded that the chromosome 6 form in Old World monkeys ancestor (OA in figure 1*a*) was identical to the form of MFA6/CGU3, with the centromere lying between markers L and M, and that both chromosomal rearrangements that we have detected in PCR are apomorphic (derived).

CJA4 and LLA1 showed a perfectly matching marker order, differing from the marker arrangement found in great apes for an inversion involving the region delimited by markers A to D. The centromere is located in a region delimited by markers A and E. The sequence divergence

Table 2
Details of Mapping, Length, and Position 0n UCSC Database (November 2002 Release) of Duplicons Detected by the Ancestral Centromere Domain at 6p22.1

Map	kb	Start	End	Chromosome 6 Start	Chromosome 6 End	Similarity (%)
2q12.3	3 127	106 035 006	106 038 133	32 840 845	32 843 595	61
3q21.2	3 293	125 708 186	125 711 479	29 772 305	29 875 573	81
3q24	3 163	143 723 708	143 726 871	32 173 541	32 178 819	72
5p14.3	36 459	20 935 805	20 972 264	26 862 981	26 901 971	93
5p14.3	37 323	21 537 789	21 575 112	26 988 641	27 027 726	93
5p14.3	119 524	21 661 559	21 781 083	26 902 210	27 027 057	80
5p14.3	4 421	21 858 470	21 862 891	26 929 488	26 933 814	94
5p14.3	26 356	21 921 991	21 948 347	26 902 201	26 929 430	93
5p13.3	195 067	34 589 254	34 784 321	26 862 682	26 990 748	73
5p13.3	196 302	34 891 964	35 088 266	26 902 201	27 007 730	82
5q13.2	124 266	70 691 222	70 815 488	26 902 210	27 027 818	43
5q21.1	18 287	99 288 921	99 307 208	26 909 189	26 929 244	84
5q21.1	9 177	99 827 682	99 836 859	26 909 198	26 916 360	53
5q21.1	21 376	100 142 190	100 163 566	26 910 282	26 929 430	86
6p11.2	129 769	57 998 763	58 128 532	26 725 342	26 929 430	68
7p11.2	6 402	55 118 576	55 124 978	33 132 357	33 140 663	66
7q11.21	7 953	62 213 273	62 221 226	26 903 573	26 914 493	22
7q11.21	13 079	64 067 942	64 081 021	26 902 915	26 914 526	40
9q22.33	4 785	91 701 175	91 705 960	26 692 164	26 717 408	56
10q21.1	117 618	53 427 990	53 545 608	32 365 897	32 563 800	32
12q24.33	3 786	132 739 192	132 742 978	158 239 192	158 242 978	99
21q22.12	3 206	33 919 740	33 922 946	32 844 279	32 840 797	80
22q13.2	5 706	36 323 422	36 329 128	31 303 603	31 302 546	10

NOTE.—Only duplicons larger than 3 kb are reported. The similarity reported in the seventh column (see *Methods*) was calculated comparing the duplicon of chromosome 6 (fifth and sixth columns) and the corresponding one reported in the first to the fourth columns.

between HSA and EMA prevented FISH signal detection of some human markers in EMA. The results obtained with 6 probes (fig. 1*a*), however, suggest that the marker order of CJA11q, LLA1q, and great apes 6q is consistent with the corresponding region of EMA11, therefore defining with high confidence the organization of the long arm of chromosome 6 in primate ancestor (PA in fig. 1*a*). This conclusion was reinforced by the analysis of radiation hybrids data of the long arm cat chromosome B2 (see http://rex.nci.nih.gov/lgd/cat/catgenome.htm). This chromosome corresponds to human HSA6 (Murphy et al. 2000). We chose the cat as an outgroup because it is well known that its karyotype is highly conserved and closely resembles the ancestral karyotype of mammals (Murphy et al. 2000; Yang et al. 2000). The complex organization of EMA8, containing the region corresponding to the PA short arm, was not conclusive in defining the marker arrangement of the chromosome 6 short arm of PA. To solve this question, we compared our results with available data on cat B2 chromosome. The radiation data on the organization of FCAB2 short arm are in agreement with the marker order we have found in CJA4 and LLA1. To further substantiate this conclusion, we used in FISH experiments a mixture of probes D2 and D3, that map on human 6p22.2, and cat marker P (BAC RP86-11M10), which was obtained by screening the cat RP86 BAC library with PCR products of a human STS mapping at 6p25.2 as a probe. The D2+D3 FISH signals were localized close to the FCAB2 telomere, while marker P mapped close to the centromere, on the short arm side. Data on the organization of FCAB2 short arm, CJA4/LLA1 short arm, and EMA11, indicate that the chromosome 6 in PA was arranged as in CJA4/LLA1, with the centromere located between markers E and A.

In summary, the overall data strongly support the reconstruction of the evolutionary history of HSA6 depicted in figure 1*a*. Few rearrangements are necessary to reconcile the present day organization of chromosome 6

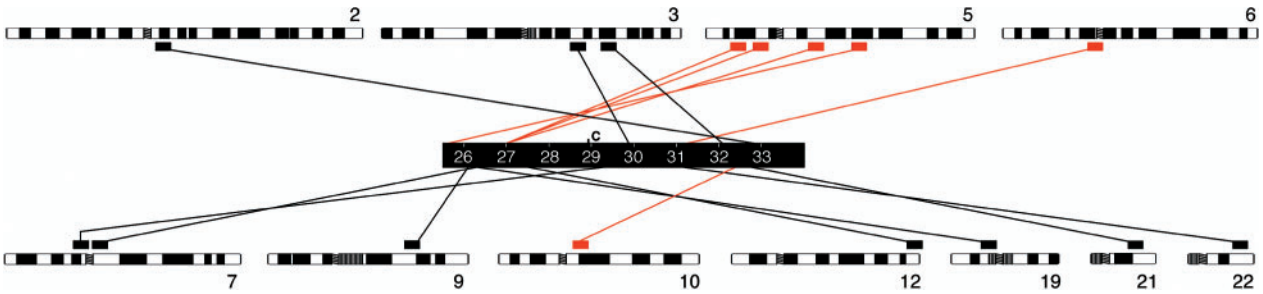

FIG. 3.—Graphic representation of duplicons found by GenAlyzer using the ancestral centromere sequence (25.5 to 34 Mb, represented by the central black bar) against all human chromosomes. Only duplicons larger than 3 kb are reported. Those larger than 100 kb are in red. Some close duplicons could not be resolved in the figure. For details see table 2.

in the species we have examined with the proposed ancestral arrangement of primate ancestor chromosome 6. A centromeric fission disrupted the short arm–long arm organization in EMA. In the latter species, a further rearrangements of PA6p sequences with sequences of HSA4 and HSA18 generated EMA8 (Cardone et al. 2002). (In *Lemur catta* [LCA], the long arm of LCA2 chromosome corresponds to EMA11, and 6p sequences of EMA8 are part of LCA4 chromosome [Cardone et al. 2002]). A single inversion generated the arrangement of chromosome 6 of Catarrhini ancestor (CA in fig. 1*a*), which descended unchanged to great apes. A further inversion in the CA form, involving markers K, L, and M, led to the OWM ancestor (OA). The two rearrangements found in PCR appear to be species specific.

Contrary to the relatively simple evolutionary history of marker arrangement, the centromere position was found in three distinct locations, delimited by markers F-G, L-M, and A-E. No hint of rearrangements that could explain centromere movement was detected. The only explanation emerging from our study is that two distinct centromere repositioning events occurred during the evolutionary history of this chromosome. The centromere in PA had, very likely, the same location as in the cat, CJA, and LLA. From this region it moved, in Hominoidea ancestor, to the present day location in great apes and man. An independent repositioning occurred, in Cercopitecoidea ancestor (CA in fig. 1*a*), from the ancestral location to the present day location in OWM, between markers L and M. The timing of the movement could not be established with certainty. Both repositionings occurred after the divergence of Catarrhini from Platyrrhini, which took place about 33 MYA (Glazko and Nei 2003).

Our data add further support to the idea that centromere repositioning is a relatively frequent phenomenon. Examples of centromere repositioning in primates, indeed, have been reported for most of the chromosomes whose marker order arrangement was investigated in detail: chromosome 9 (Montefalcone et al. 1999), chromosome X (Ventura, Archidiacono, and Rocchi 2001), chromosome 10 (Carbone et al. 2002), chromosomes 14 and 15 (Ventura et al. 2003), and chromosome 6 (present paper). Karyotype evolution of nonprimate mammals have been studied only by painting probes that, as stated, are not appropriate in this respect. Radiation hybrids data, however, have started to pinpoint a relative frequent occurrence of this phenomenon also in nonprimate mammals (Band et al. 2000).

The finding of a cluster of intrachromosomal segmental duplications encompassing the region 25.5 to 33.2Mb (UCSC) adds strong support to the hypothesis that, in the great apes ancestor, a functional centromere was silenced after the centromere moved to the present day location. This cluster has been already reported by Bailey et al. (2002). Clustering of segmental duplications around the centromere is a common feature of primate pericentromeric regions (Eichler, Archidiacono, and Rocchi 1999; Jackson et al. 1999; Bailey et al. 2002). It can be hypothesized that the strong constraint against recombination, typical of active centromeric/pericentromeric regions (see Kong et al. 2002), progressively weakens after inactivation, allowing the occurrence of ectopic nonhomologous exchanges that, very likely, trigger duplicon dispersal and an accelerated elimination of centromeric satellites. We have recently described a similar duplicon dispersal in the 15q25 region, where an ancestral centromere was inactivated after the chromosome fission that gave rise to the present day human chromosomes 14 and 15 (Ventura et al. 2003). An additional well-known example is present at 2q21, where the centromere of the phylogenetic chromosome IIq was inactivated after the telomere-telomere fusion that generated human chromosome 2 (Avarello et al. 1992; Baldini et al. 1993; Fan et al. 2002). The size of the region harboring the dispersed pericentromeric duplicons appears to be correlated with the time elapsed after inactivation. Indeed, the ancestral centromere region at 2q21 is relatively small (less than 4 Mb according to the data of Bailey et al. 2002), the inactivation dating back to 5 to 6 MYA, whereas it is much larger at 15q25 (about 13 Mb [Ventura et al. 2003]) and 6p22.1 (approximately 8 Mb [present work]), whose inactivation occurred before Hominoidea divergence.

Reshuffling of duplicons among pericentromeric regions is a well-documented phenomenon (Jackson et al. 1999; Horvath et al. 2000). In this respect, it is interesting to note that some large duplicons detected by GenAlyzer software are located in pericentromeric regions (see chromosomes 6 and 7 in fig. 3). Furthermore, the finding that the large clusters of duplicons reported at 6p22.1 and at 15q25 are the remains of silenced ancestral centromeres strongly reinforce our opinion that centromere repositioning is a relatively common occurrence in primates. We would not be surprised to find out that some other duplicon clusters are remains of ancestral pericentromeric regions. Sequence comparison reported in table 2 (last column) suggest that exchange events occurred during a long period of time and that they continued also after the centromere silencing, as documented, for example, by the duplicon on chromosome 12, which is almost 100% homologous and, therefore, very recent.

The BAC RP11-474A9 yielded clear signals on both sides of the MFA6 centromere, suggesting that the neocentromere was seeded inside this sequence. The clear-cut split looks like a breakpoint in a chromosomal rearrangement such as translocation. The results obtained by using BAC probes mapping very close to RP11-474A9 strongly reinforce this conclusion. The present data, therefore, provide a surprising scenario for the neocentromerization process. Apparently, the centromere recruited the huge amount of centromeric/pericentromeric sequences characteristic of a functional primate centromere without affecting the displaced flanking sequences.

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