Cytogenet Cell Genet 78:12–19 (1997)

Cytogenetics and Cell Genetics

Complex FISH probes for the subtelomeric regions of all human chromosomes: comparative hybridization of CEPH YACs to chromosomes of the Old World monkey *Presbytis cristata* and great apes

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Abstract. We have generated a human subtelomere probe panel, utilizing well characterized CEPH YACs, for the investigation of human chromosome pathology and evolution through fluorescent in situ hybridization (FISH). Region-specific FISH probes will be extremely valuable for detecting cytogenetically cryptic telomere abnormalities. Here, we present the first comparative mapping study (with 29 subtelomere probes and 6 chromosome paints) to the Old World monkey *Presbytis cristata*, followed by hybridizations to the great apes, gorilla and orangutan, when rearrangements were detected. We observed that the position of telomere-associated genomic sequences has been only moderately conserved during primate evolution. YAC 364f9, specific for the subtelomeric long arm of human chromosome 3, contains an evolutionary inversion breakpoint that was involved in independent chromosome rearrangements in *P. cristata* and gorilla.

The subtelomeric chromosome regions are proving to be a critical region of the human genome. Very high concentrations of genes are located in many human chromosome ends subtelomeric to simple and low copy repeat sequences (Saccone et al., 1992; Craig and Bickmore, 1994). A human subtelomere probe panel is applicable for a wide range of clinical as well as research purposes. It has recently been shown through DNA polymorphism analysis and confirmed with FISH that several percent of unexplained mental retardation may be accounted for by subtelomeric rearrangements (Flint et al., 1995). Because most of the chromosome ends stain Giemsa negatively, it is difficult to detect translocations and other subtelomere abnormalities by classical banding analysis. In many cases FISH will be the best method to detect cytogenetically cryptic subtelomere abnormalities. We have, therefore, generated a panel of non-chimeric subtelomeric FISH probes for all human chromosome ends (excluding the acrocentric short arms).

Received 7 March 1997; manuscript accepted 5 May 1997.

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In addition, subtelomere probes are ideal for comparative genome mapping. Although chromosome paints can be used to demonstrate gross chromosome homology, they fail to recognize intrachromosomal rearrangements. Due to the significance of the chromosome ends a subtelomere probe panel can provide valuable information for chromosome evolution studies. We report here on the hybridizations of human subtelomere YACs to chromosomes of the Old World monkey *P. cristata* (*P.cr.*). Our experiments begin to elucidate the overall chromosome homology between *P. cristata* and human as well as interesting intrachromosomal rearrangements involving the chromosome ends. We show that the positions of telomere-associated DNA sequences have been only moderately conserved during primate evolution.

Materials and methods

Chromosome preparation

Metaphase chromosomes were prepared from human (*Homo sapiens*, HSA) peripheral blood lymphocytes and from Epstein-Barr virus-transformed lymphoblastoid cell lines of gorilla (*Gorilla gorilla*, GGO), orangutan (*Pongo pygmaeus*, PPY), and Old World monkey (*P. cristata*). 0.1 µg/ml of colcemid (Gibco) was added to the culture medium 1 h before cell harvest to arrest cells in metaphase. Cell pellets were resuspended in a hypotonic solution consisting of 50 mM KCl. After 20 min of hypotonic treatment, cells

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were fixed overnight with 3:1 methanol:acetic acid. Slides were prepared using the conventional drop-splash technique.

DNA probes

Clones were selected from the CEPH mega-YAC library on the basis of genetic location and previous mapping data (Chumakov et al., 1995; Bray-Ward et al., 1996). YACs were obtained via the Reference Library Data Base, Berlin (Germany). The human inserts were isolated by pulsed-field gel electrophoresis (PFGE) and amplified by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) procedures as previously described (Telenius et al., 1992). DNA libraries derived from flow sorter-enriched human chromosomes were used as chromosome-specific painting probes (Vooijs et al., 1993). Cloned regions (pA and pB) of the transcribed part of the human rRNA gene were used as rDNA probe (Sylvester et al., 1986).

Fluorescence in situ hybridization

Standard FISH protocols were followed (Ward et al., 1995; Haaf and Bray-Ward, 1996). Briefly, the slides were treated with 100 µg/ml RNase A in 2 × SSC at 37 °C for 60 min and with 0.01 % pepsin in 10 mM HCl at 37 °C for 10 min, and then dehydrated in an ethanol series (70 %, 80 %, 90 %, 100 %). Slides were denatured at 80 °C in 70 % formamide, 2 × SSC, pH 7.0 and again dehydrated in an alcohol series. Probes were labeled by standard nick translation procedures with either biotin-16-dUTP or digoxigenin-11-dUTP (Boehringer Mannheim). 10 ng/µl of labeled probe DNA was coprecipitated with 100 ng/µl human cot-1 competitor DNA (Gibco) and 500 ng/µl salmon sperm carrier DNA, and redissolved in 50 % formamide, 20 % dextran sulfate, 2 × SSC. After 10 min denaturation at 70 °C, 30 µl of hybridization mixture was applied to each slide and sealed under a coverslip. Slides were left to hybridize in a moist chamber at 37 °C for 1–3 d. Slides were washed 3 times for 5 min in 50 % formamide, 2 × SSC at 42 °C followed by a 5 min wash in 0.1 × SSC at 65 °C. Biotinylated probes were detected by fluorescein isothiocyanate (FITC)-conjugated avidin (Vector) and digoxigenated probes by Cy3-conjugated anti-digoxigenin antibody (Dianova). Chromosomes and cell nuclei were counterstained with 1 µg/ml 4′,6-diamidino-2-phenylindole (DAPI) in 2 × SSC for 5 min. The slides were mounted in 90 % glycerol, 100 mM Tris-HCl, pH 8.0 and 2.3 % 1,4-diazobicyclo-2,2,2-octane (DABCO).

Digital imaging microscopy

Images were taken with a Zeiss epifluorescence microscope equipped with a thermoelectronically cooled charge-coupled device camera (Photometrics CH250), which was controlled by an Apple Macintosh computer. Oncor Imaging Software was used to capture gray scale images and to superimpose the images into a color image. Oncor Imaging Software was also used to invert the DAPI image into a G-banded metaphase for identification of the chromosomes.

Table 1. Subtelomere YAC probes; mapping and molecular data

Telomere[a]	YAC number	STS marker	cMpter[b]	Size (kb)
1p	273 d11	HKR3	17	1350
1q[a]	848 h10	D1S423	315	750
2p[a]	935 f4	D2S323	4	n.d.[c]
2q[a]	963 g7	D2S336	275	1300
3p[a]	852 b3	D3S1270	0	1100
3q[a]	364 f9	D3S1272	240	350
4p	32 g11	D4S412	0	1160
4q[a]	908 f5	D4S2930	218	n.d.
5p[a]	767 e1	D5S405	6	1430
5q[a]	856 e9	D5S469	201	500
6p[a]	954 h10	D6S344	0	1380
6q[a]	933 f7	D6S281	214	1650
7p[a]	855 a6	D7S481	10	640
7q[a]	965 c12	D7S550	201	160
8p[a]	931 b2	D8S264	1	1230
8q[a]	933 a5	D8S1837	167	1050
9p[a]	953 a7	D9S1813	8	1590
9q	415 c11	ABO,TSCI	148	100
10p[a]	889 c10	D10S558	1	1300
10q[a]	932 f11	D10S217	165	870
11p[a]	896 b12	D11S1323	13	1630
11q[a]	893 d11	D11S968	165	870
12p[a]	922 c8	D12S91	2	1390
12q[a]	751 b8	D12S1599	188	680
13q[a]	908 c3	D13S261	127	1420
14q[a]	960 d6	D14S267	136	1370
15q[a]	895 h10	D15S207	125	1670
16p	927 a8	D16S423	8	120
16q[a]	756 h3	D16S422	120	370
17p	762 b10	D17S786	17	270
17q[a]	946 e12	D17S840	102	n.d.
18p[a]	854 g8	D18S476	2	780
18q	932 b10	D18S554	125	1400
19p	661 f6	D19S209	10	1690
19q	965 c8	D19S218	107	n.d.
20p	777 a5	D20S103	3	1430
20q[a]	761 c3	D20S171	107	850
21q	265 h12	D21S266	52	1170
22q	712 g9	n.d.	n.d.	n.d.
Xp	210 b5	DXS1137	3	1130
Xq[a]	742 h5	DXS1200	191	1710
Yp	220 h5	DXYS28	n.d.	650
Yq	933 a6	DYS221	n.d.	n.d.

[a] Hybridization to the Old World Monkey *Presbytis cristata*.
[b] cMpter, distance in centimorgans from the short-arm telomere.
[c] n.d., not determined.

Results

Details of the subtelomere YAC panel that we have generated are shown in Table 1 and a composite image of all subtelomere probe hybridizations on human chromosomes, as well as representative metaphases and interphase nuclei, can be viewed in Fig. 1. Because of the large insert size and high-intensity FISH signals obtained, we have utilized the CEPH mega-YAC library. These YACs contain on average 1000 kb of human insert DNA. The selected YACs are on average 8 cM away from the telomere which avoids human telomeric and subtelomeric repeat sequences that are shared by multiple chromosomes. For each chromosome arm, at least 5 telomere-associated YACs were selected from Chumakov et al. (1995) and then tested by FISH; the non-chimeric chromosome-specific YAC closest to the telomere was used for further analyses. As shown in Table 1, the YACs of our subtelomere probe panel are well characterized with sequence tagged site (STS) markers, which will aid in further molecular studies when abnormalities are found. As shown in Fig. 1A, the subtelomere YAC chosen for the long arm of the Y chromosome is located just proximal of the large heterochromatic region present at the physical telomere (Jones et al., 1994). Due to low coverage in the CEPH-YAC library, probes for chromosomes 1pter, 17pter and 17qter are further from the telomere than would be optimal and the probe for chromosome 19pter showed a chimeric signal on 2q31→q33. It is interesting to note that out of 8 YACs from chromosome 19pter tested none proved to be chromosome-specific. For further information on markers and contigs of these YACs please see the Whitehead Institute internet site at http://www.genome.wi.mit.edu.

The human chromosome ends are characterized by specific structural and functional properties, including a high gene density, increased genetic recombination and a GC-rich base composition (Holmquist, 1992; Saccone et al., 1992; Craig and

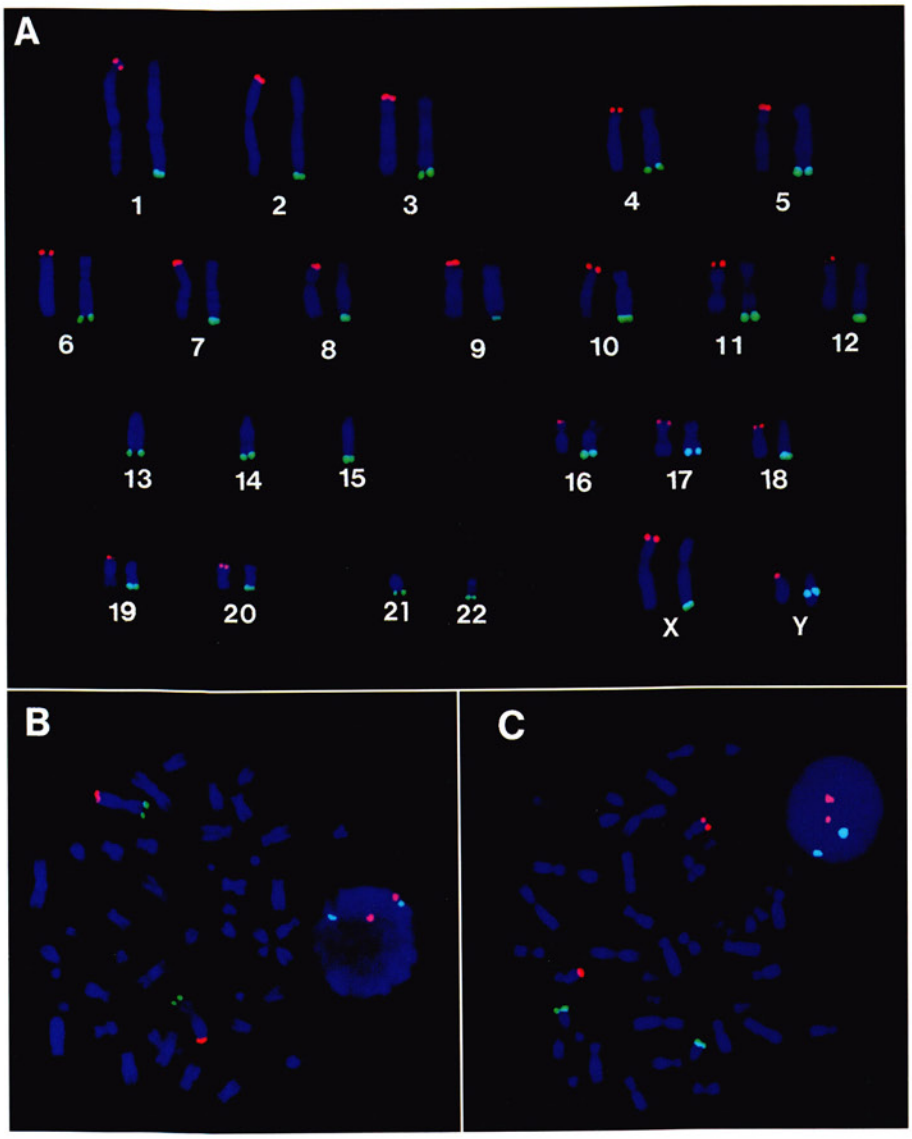

Fig. 1. FISH of subtelomere YACs on normal human chromosome preparations. YACs from the short-arm end are labeled with digoxigenin-Cy3 (red fluorescence) and from the long arm with biotin-FITC (green). Chromosomes are counterstained with DAPI. (**A**) Composite karyotype with chromosomes cut out from different metaphase spreads. All probes, except 19pter, are chromosome-specific. (**B**) Representative metaphase spread and interphase nucleus hybridized with probes specific for 2pter (green) and 2qter (red). (**C**) Metaphase and interphase nucleus hybridized with probes from 10qter (red) and 15qter (green).

Bickmore, 1994). We were interested to learn whether the position of telomere-associated DNA sequences has been conserved during human chromosome evolution. To this end, human subtelomere YACs were hybridized on chromosomes of the Old World monkey *P. cristata*. The first challenge of the comparative mapping was establishing the *P.cr.* karyotype. To our knowledge, only one RHG-banded karyotype of a male animal has been published (Dutrillaux et al., 1984). This karyotype shows four unpaired chromosomes including the sex chromosomes. The authors suggested this is probably due to a Y,autosome translocation which is characteristic of *P. cristata* and designated two of the unpaired chromosomes as Y_1 and Y_2 due to late-replicating regions noted on the short arms of these chromosomes. The largest of the unpaired chromosomes is placed as *P.cr.* 2 due to the size and the last unpaired chromosome is clearly the X. In the present study, our G-banded karyotype (Fig. 2A) confirms this rearrangement as typical of the species which suggests the need for a mechanism to ensure segregation of the resulting 2,X,Y_1,Y_2 tetravalent during male meiosis. Furthermore, chromosome painting demonstrated that all of *P.cr.* 2 as well as the long arms of Y_1 and Y_2 are homologous to human chromosome 5 (Fig. 2A, B). Hybridization with a human Y-chromosome DNA library revealed signals on both the short arm of Y_1 and the pericentromeric long arm of Y_2 (Fig. 2A, C). The short arm of Y_2 appears to consist mainly of constitutive heterochromatin. The human Y paint cross-hybridized with two distinct regions on the very distal short arm and the pericentromeric long arm of the *P.cr.* X. These regions are also labeled on the human X and are known to share homology to Y-chromosome sequences (Page et al., 1984; Cooke et al., 1985; Simmler et al., 1985). A human X-chromosome library exclusively painted the entire *P.cr.* X (data not shown). Hybridization with a human rDNA probe confirmed the presence of a large nucleolus organizer region (NOR) in the secondary constriction of *P.cr.* 19 (Fig. 2A), as reported previously (Stanyon et al., 1995).

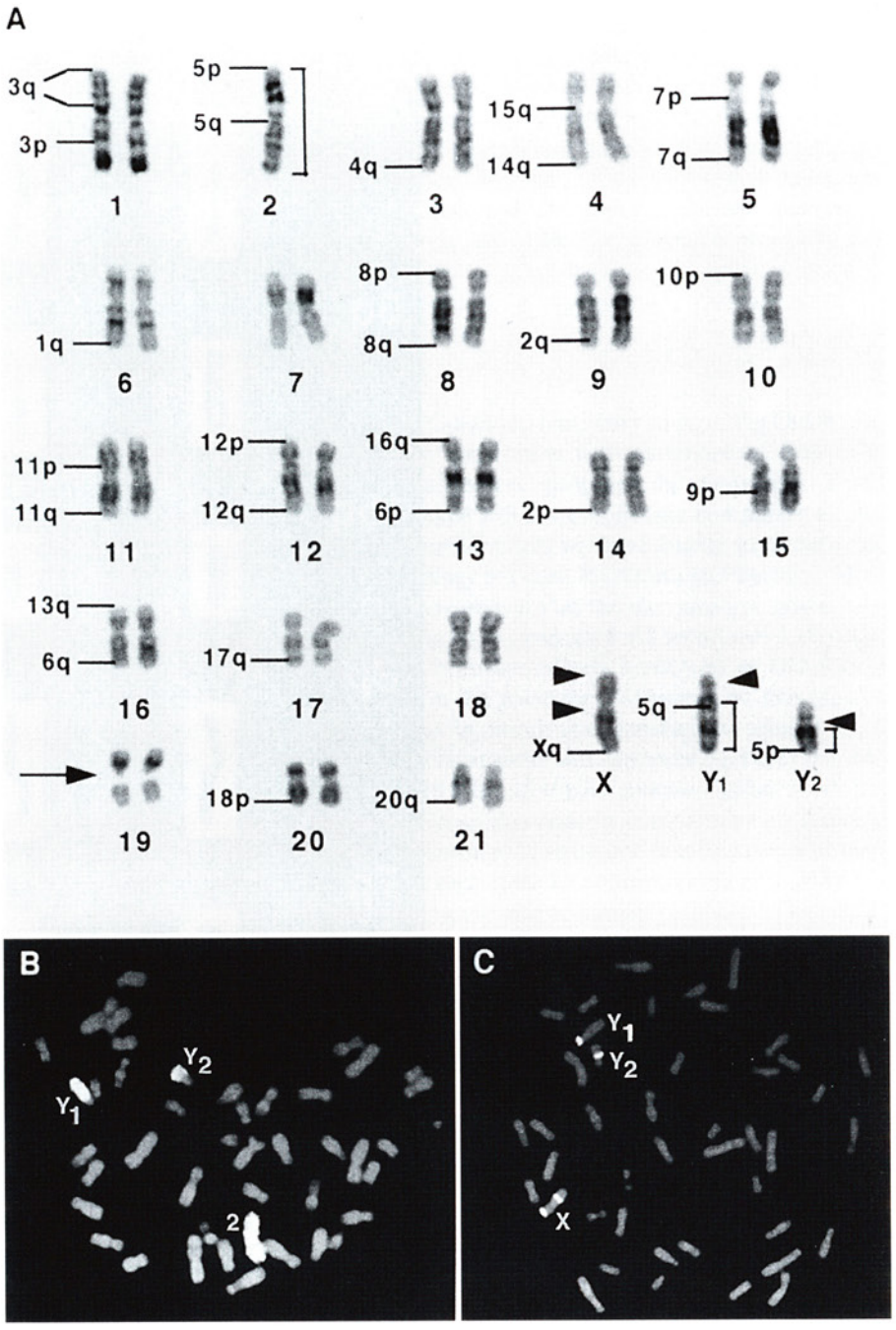

Fig. 2. (**A**) G-banded male karyotype of *P. cristata*. The dashes at the left-hand side of the chromosomes indicate the hybridization locations of human subtelomere YACs. Note the split hybridization signal of the human 3qter probe on *P.cr.* 1. Brackets indicate chromosome homologies identified with a painting probe for HSA5. Arrowheads mark the hybridization sites of a Y-specific DNA library. Location of the NORs is indicated by a long arrow. (**B**) Hybridization of a DNA library specific for HSA5 to *P.cr.* 2, Y_1 and Y_2 on a representative *P.cr.* metaphase spread. (**C**) Hybridization of a human Y paint on *P.cr.* X, Y_1 and Y_2. Chromosomes were pre-identified by inverted DAPI (G-like) bands.

Twenty-nine probes from the human subtelomere probe panel were hybridized to the Old World monkey *P. cristata* (see Table 1). The G-banded karyotype in Fig. 2A indicates the hybridization location of these YACs. Greater than twenty percent of human subtelomere YACs mapped to intrachromosomal locations in *P. cristata*, including human 3pter, 3qter, 5qter, 7pter, 9pter, 11pter, and 15qter. These probes were subsequently hybridized to gorilla and orangutan chromosomes to further determine the chromosome evolution of these regions (Fig. 3, Table 2). Previously published comparative banding studies (Dutrillaux, 1979; Yunis and Prakash, 1982) which have been recently confirmed with FISH (Jauch et al., 1992) were used to identify the great ape chromosomes. All human subtelomere probes hybridized to the respective homologous chromosomes in gorilla and orangutan.

The subtelomere YAC 364f9 from human 3qter showed a split hybridization signal on *P.cr.* 1 (Fig. 3, top panel). This narrows down the physical location of the evolutionary breakpoint to less than one megabase. One signal remained on the short-arm subtelomere and the second was close to the centromere also on the short arm, demonstrating a paracentric inversion. YAC 364f9 hybridized to a subtelomeric location on the

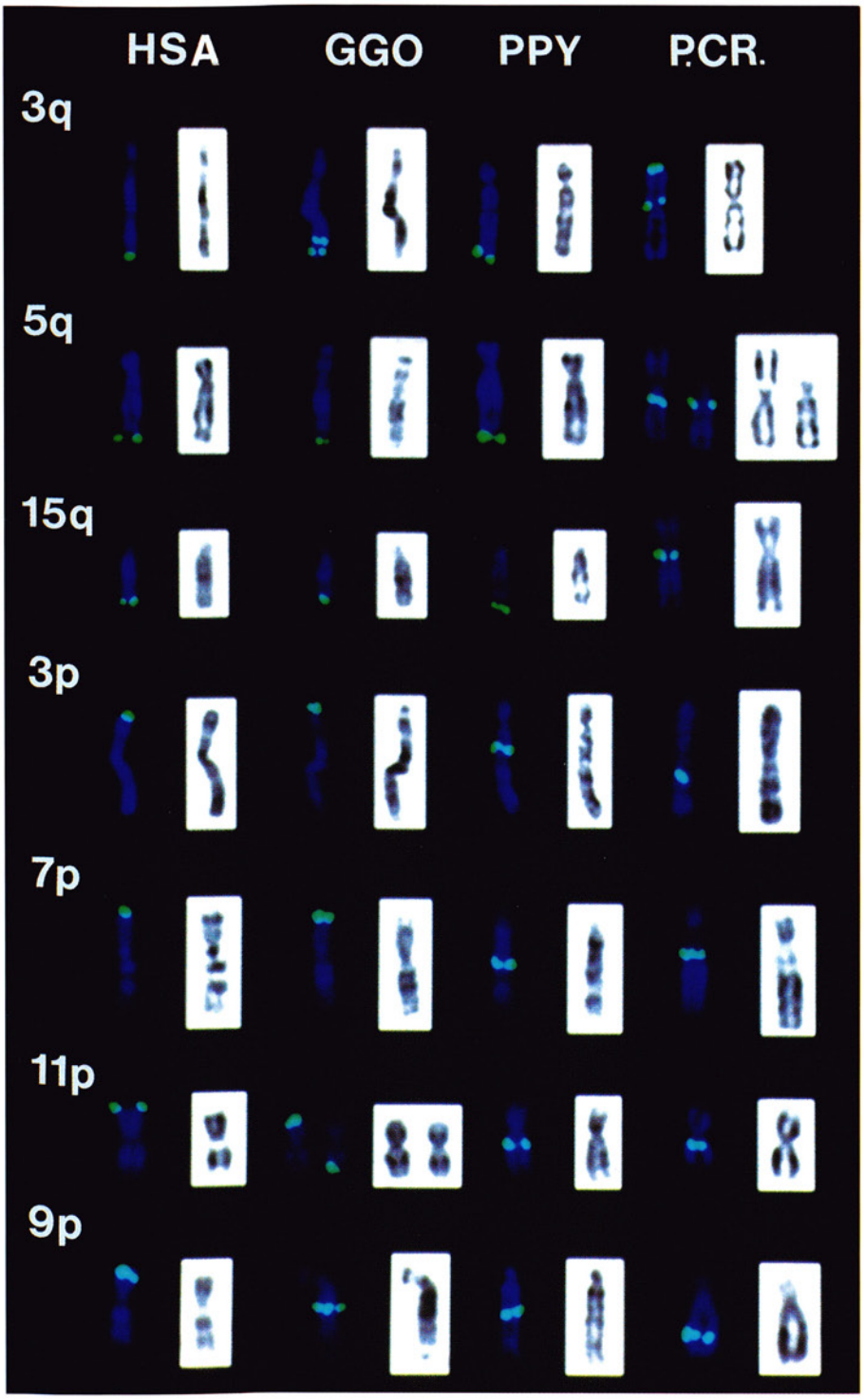

Fig. 3. Hybridization of human subtelomere YACs to homologous chromosomes of man (HSA), gorilla (GGO), orangutan (PPY) and *P. cristata* (P.CR.). White insets show the G-banding patterns of the hybridized chromosomes. Probes are labeled with biotin-FITC (green). The HSA column states the chromosome arm being analyzed in each panel. YAC 364f9 (HSA3qter) identifies paracentric inversions in GGO and *P.cr.* YACs 856e9 (HSA5qter) and 895h10 (HSA15qter) are located close to a centromere in *P.cr.* and close to a telomere in humans and great apes. YACs 852b3 (HSA3pter), 855a6 (HSA7pter), and 896b12 (HSA11pter) are telomeric in HSA and GGO, but intrachromosomal in PPY and *P.cr.* Note that 896b12 hybridizes to alternate arms of homologous gorilla chromosomes. YAC 953a7 (HSA9pter) hybridizes to intrachromosomal locations in great apes and *P.cr.*

orangutan chromosome known to be homologous to HSA3. Interestingly, it produced a split hybridization signal on the gorilla homolog of HSA3. However, the inverted chromosome segment between the two hybridization signals is much smaller than in *P. cristata*. This suggests that the paracentric inversions in *P. cristata* and gorilla represent independent events during primate chromosome evolution.

YAC 856e9 from human chromosome 5qter (Fig. 3, second panel) hybridized just distal of the centromeres on *P.cr.* 2 and Y_1. Similarly, YAC 895h10 from 15qter (Fig. 3, third panel) hybridized distal to the centromere of *P.cr.* 4. The same probes hybridized to subtelomeric sites in gorilla and orangutan (Table 2).

Hybridizations to *P. cristata* revealed that YAC 852b3 from human chromosome 3pter (Fig. 3, fourth panel) hybridized to

Table 2. Hybridization location of subtelomere probes in different primate species

Probe	Human	*Gorilla gorilla*	*Pongo pygmaeus*	*Presbytis cristata*
364 f9	3qter	Split signal	Subtelomeric	Split signal
856 e9	5qter	Subtelomeric	Subtelomeric	Intrachromosomal
895 h10	15qter	Subtelomeric	Subtelomeric	Intrachromosomal
852 b3	3pter	Subtelomeric	Intrachromosomal	Intrachromosomal
855 a6	7pter	Subtelomeric	Intrachromosomal	Intrachromosomal
896 b12	11pter	Subtelomeric	Intrachromosomal	Intrachromosomal
953 a7	9pter	Intrachromosomal	Intrachromosomal	Intrachromosomal

the middle of the long arm of *P.cr.* 1. YAC 855a6 from human 7pter (Fig. 3, fifth panel) and YAC 896b12 from 11pter (Fig. 3, sixth panel) hybridized just distal of the centromeres on *P.cr.* 5 and *P.cr.* 11, respectively. These results are consistent with the idea that whole chromosome arms are inverted during primate chromosome evolution and that breaks may preferentially occur near centromeric and telomeric regions. In addition, further hybridizations with all three of these probes displayed intrachromosomal locations in the orangutan (Fig. 3). This data further defines the inversions that have been established from classical cytogenetic banding studies for the orangutan homologs of HSA7 and HSA11 (Dutrillaux, 1979; Yunis and Prakash, 1982). The intrachromosomal signal seen with the human 3pter probe on orangutan (Fig. 3, fourth panel) elucidates a rearrangement that was not apparent through banding. Although an inversion on the orangutan homolog of HSA3 has been reported (Yunis and Prakash, 1982), the subtelomeric regions were not shown to be involved. This demonstrates that the chromosome location of subtelomeric sequences, even under 10 cM from the chromosome end, has not been conserved through primate evolution. This is especially evident with the human 3pter probe (852b3) which has markers located at 0 cM in humans, yet is still inverted in the orangutan. All three probes for human 3pter, 7pter and 11pter were located at subtelomeric sites on the respective homologous chromosomes in gorilla (Fig. 3). Interestingly, upon closer examination YAC 896b12 was found to label alternate ends on the gorilla chromosome pair homologous to HSA11 (Fig. 3, sixth panel). Additional individual gorillas should be tested to confirm this as a chromosome rearrangement that arose in tissue culture or as a chromosomal polymorphism within a gorilla population(s).

Only one probe, YAC 953a7 from human 9pter (Fig. 3, bottom panel), hybridized to intrachromosomal locations in all three primates tested, presenting an evolutionary very young inversion. Until humans YAC 953a7 had been conserved as an intrachromosomal sequence at least back to Old World monkeys. In *P. cristata* this probe hybridized to the long arm of chromosome 15. In gorilla and orangutan it was mapped to an intrachromosomal location on the HSA9 homologs, further elucidating these well known chromosome inversions (Tanabe et al., 1996).

Two chromosomes of *P. cristata, P.cr.* 8 and *P.cr.* 12, appeared upon G-banding analysis to be homologous to HSA8 and HSA12, respectively. In further support of this observation, the human probes for the long- and short-arm subtelomeres of these two chromosomes hybridized to both ends of the *P.cr.* homologs (Fig. 2A). Human chromosome paints were utilized and confirmed that *P.cr.* 8 and *P.cr.* 12 are entirely homologous to HSA8 and HSA12. In addition, chromosome painting revealed that *P.cr.* 3 is entirely homologous to HSA4. Since the banding pattern and centromere index of *P.cr.* 3 are different from its human homolog, an intrachromosomal rearrangement(s) must have occurred. These human chromosome paints were not detected on any other *P.cr.* chromosomes (data not shown).

Discussion

We display the G-banded male karyotype of the Old World monkey *P. cristata*, confirming with chromosome paints the Y,autosome rearrangement proposed by Dutrillaux et al. (1984). This karyotype will assist in further comparative cytogenetic studies. Furthermore, we have begun to discern the chromosome homology between *P. cristata* and humans. Most importantly, we have shown that the chromosome position of subtelomeric sequences, on average 8 cM away from the human telomere, has not been completely conserved in Old World monkey and even in the great apes. At first view, this may be surprising. Bearing in mind that genetic recombination is increased at telomeric regions and suppressed around the centromere (Bray-Ward et al., 1996), inversions of genetic material from subtelomeric positions close to a centromere are likely to be associated with changes in structural and functional properties of large DNA segments. The chromosome ends may not only represent a major source of human pathology (Flint et al., 1995). Inversions and other rearrangements involving the subtelomeric regions may also play an important role during chromosome evolution that has not been previously appreciated. Human chromosome pathology and evolution appear to be very closely interrelated. In this light, pathological chromosome rearrangements may be the consequence of an ongoing chromosome evolution (Canki and Dutrillaux, 1979; Dutrillaux, 1979; Haaf and Schmid, 1987).

In this context, it is interesting to note that YAC 896b12 hybridized to both the short-arm and the long-arm subtelomeres of a gorilla chromosome pair homologous to HSA11. Although this is assumed to be the result of clonal evolution in tissue culture involving an exchange of the short and long-arm subtelomeres, it is most likely representative of the rearrangements that occur in human chromosome pathology and phylogeny. In a recent study, van Deutekom et al. (1996) proposed frequent subtelomeric exchanges of repeat DNA sequences between human chromosomes 4qter and 10qter. Translocations of chromosome ends may represent a new type of chromosomal polymorphism and not always be of pathological significance.

YAC 364f9 showed a subtelomeric location in human and orangutan, whereas split hybridization signals were observed in gorilla and *P. cristata*. The most likely explanation may be that the HSA3q homologs in gorilla and Old World monkey underwent "convergent" chromosome mutations (paracentric inversions) involving the same subtelomeric breakpoint but differ-

ent interstitial breakpoints. This means that the chromosome type found in human and orangutan is the ancestral one. Convergent chromosome mutations have been observed previously in the pericentric region of the HSA2p homologs of gorilla and orangutan (Wienberg et al., 1994; Haaf and Bray-Ward, 1996) and may be more common than usually assumed. Another possibility may be that *P.cr.* 1 represents the ancestral chromosome type and a paracentric inversion occurred in the common ancestor of man and great apes. However, this would imply that a "reverse" chromosome mutation involving 364f9 occurred in the gorilla after divergence of the phylogenetic line leading to humans. Both scenarios suggest that chromosome evolution may re-use the same breakpoints (at least at the cytogenetic level) to generate karyotype diversity in closely related species.

In conclusion, we present here a YAC probe panel for all human chromosome ends with numerous possibilities for comparative FISH mapping. In addition, this panel is currently being used in several cytogenetic laboratories to perform a systematic search for subtelomere abnormalities in patients with unexplained mental retardation and/or congenital malformations. Chromosome-specific subtelomere probes may also prove extremely valuable to analyze the segregation behavior of balanced translocation chromosomes in sperm-cell nuclei by simple interphase FISH.

Our subtelomere YAC panel has certain advantages compared to cosmid probes that have been published recently (NIH and IMM Collaboration, 1996). Because CEPH mega-YACs contain on average one megabase of genomic DNA, they generate high-intensity FISH signals on both metaphase chromosomes and interphase nuclei which are clearly visible by eye through the microscope. We believe the size of subtelomere YACs will be very beneficial in detecting chromosome rearrangements involving the chromosome ends. CEPH YACs are available to the public and can be easily obtained from a number of human genome resource centers (for addresses see Chumakov et al., 1995). In addition, DOP-PCR products of PFGE-isolated YAC inserts can be rapidly re-amplified to generate complex FISH probes at larger scale. On the other hand, the telomere-specific cosmids available may be somewhat closer to the actual chromosome end than our YAC probes. For some telomeric regions, half-YACs with one *tetrahymena* and one human telomere and P1 clones have been isolated and extensively characterized (Vocero-Akbani et al., 1996).

In addition to the subtelomere YAC panel described here, our laboratory is also creating a larger set of some 1000 YAC probes, approximately one every 5–10 cM evenly spaced over the entire human genome, that will cover all chromosome bands. These probes which have been FISH-mapped and details on obtaining probes can be viewed at the Molecular Cytogenetics and Positional Cloning Center internet site (http://www.mpimg-berlin-dahlem.mpg.de). Hybridization of YACs with polymorphic STS markers on metaphase chromosomes allows the integration of genetic and cytogenetic maps (Bray-Ward et al., 1996; Haaf and Bray-Ward, 1996). A standard set of cytogenetically and genetically anchored YAC probes can be adapted with unlimited flexibility to specific FISH applications such as the study of pathological and evolutionary chromosome rearrangements. This may prove extremely useful in both research and routine cytogenetic laboratories.

Acknowledgements

We thank Johannes Wienberg for providing lymphoblastoid cells of *P. cristata*, Joe W. Gray for chromosome-specific DNA libraries, and the Reference Library Data Base (Heubnerweg 6, 14059 Berlin, Germany) for CEPH YACs. We also thank Dietmar Vogt for excellent technical assistance.

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