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Characterization and Evolution of a Single-Copy Sequence from the Human Y Chromosome

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To study the evolution and organization of DNA from the human Y chromosome, we constructed a recombinant library of human Y DNA by using a somatic cell hybrid in which the only cytologically detectable human chromosome is the Y. One recombinant (4B2) contained a 3.3-kilobase *Eco*RI single-copy fragment which was localized to the proximal portion of the Y long arm. Sequences homologous to this human DNA are present in male gorilla, chimpanzee, and orangutan DNAs but not in female ape DNAs. Under stringent hybridization conditions, the homologous sequence is either a single-copy or a low-order repeat in humans and in the apes. With relaxed hybridization conditions, this human Y probe detected several homologous DNA fragments which are all derived from the Y in that they occur in male DNAs from humans and the apes but not in female DNAs. In contrast, this probe hybridized to highly repeated sequences in both male and female DNAs from old world monkeys. Thus, sequences homologous to this probe underwent a change in copy number and chromosomal distribution during primate evolution.

The Y chromosome is one of the smallest human chromosomes, comprising 0.5 to 1.0% of the diploid amount of genetic material (12). Its cytological staining properties indicate that the human Y chromosome is highly heterochromatic (19), and molecular studies indicate that at least 50% of the Y is composed of repeated DNA sequences (8, 15). Two groups of repeated DNAs on the Y, revealed as distinct bands of 3.4 and 2.1 kilobases (kb) in *Hae*III restriction digests of genomic male DNA, have been studied in some detail (9, 10, 15). It has been shown that these repeated sequences are not responsible for sex determination (16).

Comparison of the human Y chromosome with those of the great apes reveals marked cytological similarities (34). In contrast, molecular studies indicate that repeated DNAs from the long arm of the human Y chromosome are homologous, in part, with great ape autosomal sequences but not with their Y chromosome sequences (13). This finding suggests a relatively recent origin of a significant portion of the long arm of the human Y chromosome. Given that these human Y DNAs include several families of intermediate repeated DNAs with diverse evolutionary histories (13), that they are organized as complex clusters distributed throughout the length of the Y long arm (16), and that the region responsible for male determination is likely on the Y short arm (18), it is possible that the entire long arm is of recent origin. In addition, recent studies with a number of randomly selected single-copy sequences from the human Y chromosome demonstrate that many share homology with the X chromosome and some with autosomes (3, 21). At least one long stretch of DNA with X and Y chromosome homology in humans appears to be located on the X chromosome of apes but not on their Y (22). Such findings suggest that much of the Y chromosome may be the result of recent sequence rearrangements between chromosomes. Thus, those sequences which remain Y specific in related species may be of functional significance.

In an effort to further characterize the human Y chromosome with respect to its single-copy DNA content, function, and evolutionary history, we constructed a recombinant library of human Y chromosome sequences by using a rodent × human hybrid cell line in which the only cytologically identifiable human chromosome is the Y chromosome. We identified a series of clones containing single-copy sequences from the human Y chromosome and studied one in detail. We mapped it to the proximal region of the long arm of the human Y and showed it to be confined to the Y chromosome of great apes but to be highly repeated and present on one or more autosomes in old world monkeys.

MATERIALS AND METHODS

Cell lines and subjects. Cell line 7631 (Y hybrid) was derived by hybridization of Chinese hamster cells (CHW 1103) with fetal human male cells (GM-6317) (28) and was the kind gift of H. Wang and J. Hamerton. The Y hybrid cell line contains approximately a tetraploid number of Chinese hamster chromosomes and the Y chromosome as the only human chromosome detectable by cytological studies (28) (Giemsa, Giemsa at pH 11, and quinacrine banding). However, extensive isozyme analyses revealed the presence of the human isozyme nucleoside phosphorylase in all assays and occasionally phosphoglucomutase-2 and aromatic alpha-ketoacid reductase (H. Wang, personal communication). Thus, fragments of chromosome 14 (nucleoside phosphorylase) and possibly 4 (phosphoglucomutase-2) and 12 (aromatic alpha-ketoacid reductase) are present in this hybrid cell line even though they are cytologically undetectable.

Blood samples were obtained with informed consent from a normal 46,XY male, a normal 46,XX female, and six individuals with aberrant karyotypes. Subjects with aberrant karyotypes were as follows: individual 1, 47,XYY, a 32-year old male with hypogonadism and latent schizophrenia (16); individual 2, 46,XX,−14,+der(14),t(Y;14)(q12;p11), a 28-year old normal female [the presence of Y sequences in the

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t(Y;14) chromosome was confirmed by the presence of Y-specific repeated DNA in this individual (6)]; individual 3, 46,X,i(Yq), a 19-year old female, H-Y antigen negative, with gonadal dysgenesis and a gonadoblastoma (23); individual 4, 46,XY,del(Y)(q11), a 23-year old male with atrophic left testicle, gynecomastia, and schizophrenia (16); individual 5, 46,XX, a 27-year old male with gynecomastia and azoospermia (16); individual 6, 46,X,i(Yp), a newborn male (16). Primate samples were obtained as previously described (13).

Isolation and radiolabeling of DNA. Nuclear DNAs from cultured cells or from samples of primate and human peripheral blood were prepared as previously described (13, 16). DNA from *Alu*I repeated sequences was prepared by isolating the *Bam*HI insert from plasmid clones Blur 2 and 8 (the gift of T. Friedman) (11). DNA from a cloned representative of a long repeated DNA sequence, λHb2 (25), containing members of the Kpn LINES (29), was provided by B. J. Schmeckpeper. Two subclones of λHb2 (25), pBS70 and pBS18, containing the 3.3- and 4.5-kb *Eco*RI fragments respectively, were cloned in pBR322 (B. J. Schmeckpeper, unpublished data). Two repeated fragments from the Y chromosome, gel-purified genomic *Hae*III 3.4-kb (Y-3.4) and 2.1-kb (Y-2.1) probes, were prepared as previously described (6, 15). Phage were recovered from large-scale liquid lysate and purified by CsCl centrifugation. DNA was isolated as previously described (25).

DNA fragments from recombinant phage and plasmids were isolated from agarose gels by electroelution by published methods (17). DNA was radiolabeled with [^{32}P]dNTP by nick translation with *Escherichia coli* DNA polymerase I (25). Specific activities were 1×10^8 to 2×10^8 cpm/μg of DNA.

Enzyme digestion, gel electrophoresis, and DNA hybridization. Restriction endonuclease digestions of genomic or phage DNAs were performed as described previously (25). Briefly, DNA fragments were separated by electrophoresis in agarose slab gels, visualized by staining with ethidium bromide, and transferred to nitrocellulose filters. Molecular weights were estimated by comparison with fragments of known length generated by digestion of λ and φX174 phage DNA with *Hin*dIII and *Hae*III, respectively. Blot hybridization with radiolabeled DNA probes was performed by methods modified (25) from those described by Southern (31).

Filters were prehybridized at 37°C for 6 to 18 h in a solution containing 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 50% deionized formamide, 250 μg of salmon sperm DNA per ml, 1% glycine, 50 mM phosphate buffer (pH 6.8), 0.2% Ficoll, 0.2% polyvinylpyrrolidone (Pharmacia Fine Chemicals), and 0.2% bovine serum albumin. DNA probes were denatured by heating at 100°C for 5 min, quick-cooled on ice, and added directly to the prehybridization mixture. The filters were rotated at 37°C for 48 to 72 h. Gel-purified single-copy Y DNA probe was added to hybridization buffer containing 10% dextran sulfate and rotated with DNA filters for 14 h at 37°C. Hybridization solutions contained ca. 5×10^5 cpm/ml. After hybridization, filters were rinsed at room temperature with 3× SSC and washed by one of three procedures. Library screening filters were washed with four changes of 0.2× SSC containing 0.2% sodium dodecyl sulfate for 30 min each at 65°C. Filters of recombinant phage digests screened for single-copy sequences were washed at low stringency (65°C with a minimum of 0.5 M NaCl), as previously described (25). Restriction digest filters hybridized with single-copy probes were washed at low stringency and then with four changes of 0.1× SSC containing 0.1% sodium dodecyl sulfate for 30 min each at 65°C to ensure that only duplexes with considerable homology would be retained. In experiments to detect distant homology, the 0.1× SSC washes were omitted. Washed filters were dried and exposed to X-ray film (Kodak X-Omat) for various periods of time, depending on the amount of retained radioactivity.

Construction of a Y-hybrid library. Chimeric DNA molecules were formed between λ phage Charon 4A and fragments of genomic Y-hybrid DNA, generated by partial digestion with *Eco*RI. Charon 4A arms were generated by ligating the annealed ends with T4 ligase and digesting with *Eco*RI (17). The arms were separated from the internal fragments by centrifugation through a 5 to 20% (wt/vol) potassium acetate gradient in 10 mM Tris (pH 8.0)–5 mM EDTA at 27,000 rpm in a Beckman SW27 rotor for 16 h. Y-hybrid DNA was partially digested with restriction enzyme *Eco*RI according to the theoretical consideration of Seed et al. (26) to maximize sequence representation. DNA fragments of 12 to 20 kb were isolated from a 10 to 40% (wt/vol) sucrose density gradient in 1 M NaCl–20 mM Tris (pH 8.0)–5 mM EDTA and ligated with a 2.5 M excess of Charon 4A arms. In vitro encapsulation was carried out with Packogene (Bio-Tec), giving a total of 1.3×10^6 PFU. The library was plate amplified on *E. coli* DP50 *supF* with NZYDT agar (5), and the resulting lysates were pooled. Ninety-eight percent of plaques were judged to be recombinants by their failure to form blue plaques when grown in the presence of 5-bromo-4-chloro-3-indoyl-β-D-galactoside (Xgal) (5).

Given an estimated average insert length of 15 kb and a haploid length of ca. 6×10^6 kb for the tetraploid Y-hybrid genome and assuming random distribution of *Eco*RI sites, we calculate that the probability of finding a particular human Y chromosome sequence within the library is approximately 83% (7).

Y-HYBRID library screening, plaque purification and subcloning. Recombinant phage were plated at 10^4 PFU per 150-mm plate, and replicate nitrocellulose filters were prepared as described by Benton and Davis (2). The filters were hybridized with human male [^{32}P]DNA radiolabeled by nick translation (24). Phage hybridizing to human male DNA were replated at 10^2 PFU per 150-mm plate. Duplicate filters were prepared and hybridized with either human male [^{32}P]DNA or Chinese hamster [^{32}P]DNA. Plaques hybridizing with human but not hamster DNA were picked and subjected to another round of plaque purification.

Individual plaques were amplified, and the resulting phage were isolated from large-scale liquid lysates as described by Blattner et al. (5).

Gel-purified fragments resulting from *Eco*RI digestion of recombinant phage DNA were subcloned into the *Eco*RI site of pBR322 (17).

RESULTS

Identification and characterization of human DNA recombinants. Phage from the Y-hybrid library were screened for the presence of human repeated DNA by hybridization with radiolabeled human male or female DNA. Of 8×10^4 phage screened, 194 (0.24%) hybridized with each human DNA probe. No plaques hybridizing specifically with male DNA were detected.

To identify clones containing Y-sequences, we also screened the Y-hybrid library with human 47,XYY DNA and then examined it for coincident homology with three specific repeated sequences. A Y-3.4 probe hybridized with

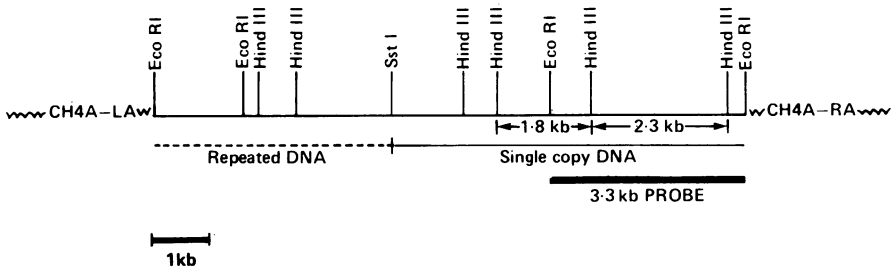

FIG. 1. Restriction map of the human DNA insert in recombinant phage 4B-2. Left (LA) and right (RA) arms of λ phage Charon 4A are indicated by (~). Repeated DNA fragments (----) were identified by their hybridization with radiolabeled 47,XYY DNA after restriction digestion and size fractionation on agarose gels. Presumptive single-copy fragments were identified by their lack of hybridization. The 3.3-kb *Eco*RI fragment (solid bar) was used as a probe either after isolation from the gel (see the text) or after subcloning in pBR322. This probe detected three fragments in *Hin*dIII digests of the recombinant phage: insert fragments of 1.8 and 2.3 kb and a 4.5 kb genomic fragment containing the 3′ end of the probe.

a majority of the clones detected with the human 47,XYY probe. *Alu*I family probes (Blur 2 and 8) hybridized with 90% of these clones, and the Kpn-LINE probes hybridized with 15%.

Nine independent recombinant phage containing human DNA inserts were purified for analysis. Insert sizes, determined by electrophoretic analysis of *Eco*RI restriction digests of the recombinant phage, ranged from 10.9 to 19 kb. Each of these clones contained sequences related to the *Alu*I family; two showed very weak homology, and a third had multiple copies. Four recombinants contained sequences homologous to subcloned fragments (pBS18 and pBS70) of λHb2, a cloned genomic example of a long repeated DNA element (Kpn-LINES) described by Schmeckpeper et al. (25); two others had weak homology for this element. Eight recombinants showed homology with the cloned Y-2.1 repeat although not in a 2.1-kb *Hae*III fragment, and six shared sequences with the genomic Y-3.4 probe but not in a 3.4-kb *Hae*III band. No consistent pattern of repeat organization was discernible among the recombinants. One recombinant phage appeared to contain human sequences derived from an autosome.

One recombinant, 4B-2, was studied further. The human insert DNA, as deduced from the sum of the human fragments released from the vector by digestion with *Eco*RI, was 10.0 kb. Total recombinant DNA and isolated insert DNA were digested with a variety of restriction enzymes to construct a partial restriction map of the insert DNA (Fig. 1). Digestion at the three *Eco*RI sites released insert fragments of 1.5, 5.2, and 3.3 kb. Digestion of the intact plasmid at the six *Hin*dIII sites produced insert fragments of 1.7, 0.7, 2.8, 0.6, 1.8, 2.3, and 0.5 kb.

To determine the organization of single-copy and repeated DNA within the human insert, we probed digests of clone 4B-2 with radiolabeled 47,XYY DNA. The repeated DNA appeared to occur in a single block with a maximum length of 4 kb at the 5′ end of the insert (Fig. 1). By subtraction, the minimum length of single-copy DNA is ca. 6 kb. Single-copy DNA is defined here by its failure to hybridize moderately repetitive DNA and can also contain sequences with low levels of repetition. The repeated DNA element shows little or no homology with the Y-3.4, Y-2.1, or *Alu*I (Blur 2 and 8) repeats but has extensive homology with the mixed subclones of the Kpn-LINE repeat (25).

The Y chromosome origin of clone 4B-2 was confirmed by probing genomic DNAs containing differing numbers of Y chromosomes per haploid genome with the 3.3-kb *Eco*RI fragment of single-copy DNA from the 3′ end of the insert (Fig. 1, solid bar). The results of this dosage analysis are shown in Fig. 2. The top panel shows the ethidium bromide-stained digests of nearly equal quantities of genomic DNA from 46,XX, 46,XY, and 47,XYY individuals. DNA in the left three lanes was digested with *Eco*RI and DNA in the right three lanes was digested with *Hin*dIII. The bottom panel shows the autoradiogram obtained after blot hybridization with the 3.3-kb *Eco*RI fragment. No hybridization was observed with female DNA, and the extent of hybridization with 47,XYY DNA was approximately twice that obtained with 46,XY DNA. The hybridization bands observed in each digest, 3.3 kb with *Eco*RI and 2.3 and 1.8 kb with *Hin*dIII, are the sizes predicted from the restriction map of clone 4B-2 (Fig. 1). The 0.5-kb *Hin*dIII 3′ fragment of the probe is included in a genomic 4.5-kb *Hin*dIII band and was seen after long exposure (data not shown). The detection of only those genomic restriction fragments predicted from the restriction map of the recombinant phage (Fig. 1) supports the single-copy designation of these fragments.

DNA from individuals with Y chromosome deletions or translocations was used to regionally map clone 4B-2 along the Y chromosome (Fig. 3). The *Eco*RI 3.3-kb probe hybridized with DNA from an i(Yq) individual (subject 3; Fig. 3, lane 2) but not an i(Yp) individual (subject 6; lane 5), indicating a Y long arm origin for this clone. No hybridization was obtained with DNA from an individual (subject 2; lane 1) carrying a translocation of the distal portion of the Y long arm (::q12→qter). Since DNA from an individual (subject 4; lane 3) with a deletion of the distal region of the Y long arm but retaining the short arm and the proximal region of the long arm (pter→q11) contained the probe sequence, the most likely location of clone 4B-2 is between Yq11 and the Y-chromosome subcentromeric region. DNA from a 46,XX male (Fig. 3, lane 4) did not hybridize with this probe; for comparison, hybridization with normal 46,XY male DNA is shown (lane 6).

Homology of the 3.3-kb *Eco*RI human Y fragment of clone 4B-2 with primate DNAs was determined by hybridization of various genomic restriction digests with a subcloned 3.3-kb *Eco*RI fragment probe. These studies included examination of humans (four males and three females), gorillas (three males and one female), chimpanzees (four males and two females), orangutans (one male and one female), and macaques (two males and three females). No variations were observed among individuals of the same sex and species. Results obtained with *Eco*RI, *Hin*dIII, or *Rsa*I digestion of male ape DNA are shown in Fig. 4. The patterns and intensities of hybridization obtained at high stringency (Fig.

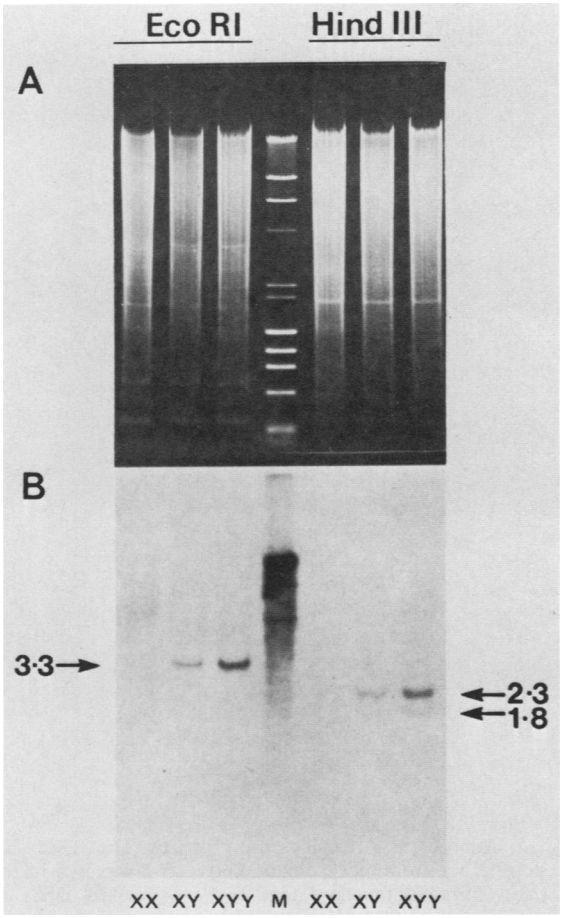

FIG. 2. Localization of clone 4B-2 to the Y chromosome. Human genomic DNAs from female 46,XX, male 46,XY, and a male with an extra Y, 47,XYY (individual 1, see the text) were digested with either *Eco*RI (left three lanes) or *Hin*dIII (right three lanes). (A) Ethidium bromide-stained gel of digested DNAs. The middle lane (lane M) contains size markers derived from *Hin*dIII digests of λ DNA and *Hae*III digests of ϕX174 DNA. Designated in kb from top to bottom, these markers are: 23.7, 9.5, 6.7, 4.3, 2.2, 2.0, 1.3, 1.1, 0.9, 0.6, and 0.3. (B) Autoradiogram of the samples from (B) after hybridization with the radiolabeled 3.3-kb *Eco*RI probe described in the legend to Fig. 1. Arrows at the side of the figure indicate bands of hybridization that dose in a Y-dependent fashion. Estimated band sizes are given in kb next to the arrows.

4A) indicate that, as in humans, this fragment is single copy. Since this fragment is not detected in female ape DNA at this stringency (Fig. 5A), it is also confined to the Y chromosome in apes. In all three digests, the pattern of hybridization bands observed in chimpanzee DNA (Fig. 4, lanes C) is identical to that observed in human DNA (lanes H), although there may be intensity differences between some bands. The restriction pattern obtained with gorilla DNA (Fig. 4, lanes G) suggests a shorter fragment, whereas the result with orangutan DNA (lanes O) suggests a longer fragment with homology for this human Y fragment. It is unclear whether these changes represent altered restriction sites or insertion-deletion events. Although differences in restriction patterns among the species may be the result of sequence divergence, leading to altered restriction sites, the stringent wash conditions ensuring extensive sequence homology between reacting fragments and the similarity of hybridization intensity among fragments of different lengths in different species suggest that the changes may be due to insertion-deletion events. Figure 4B displays the results of a similar experiment performed under conditions of low stringency. In all species, including humans, additional high-molecular-weight fragments are seen. Thus, there are multiple copies of related sequences in each genome. No homology was seen with male mouse or Chinese hamster DNA at high or low stringency (data not shown).

The Y specificity of the additional fragments detected at low stringency was examined by comparison of hybridization of restriction digests of male and female primate DNAs at both high and low stringency. Equivalent results were obtained in all digests examined. The results achieved after digestion with *Rsa*I are shown in Fig. 5. The male-specific hybridization expected at high stringency (Fig. 5A) was observed in all species except the old world monkey *Macaca mulatta*. In *M. mulatta*, equivalent hybridization was seen with both male and female DNAs (Fig. 5A, M lanes). Thus, this fragment must exist on chromosomes other than the Y, and the heterogeneous nature of its hybridization suggests that the related sequences in this species are highly repeated.

Figure 5B shows the results obtained at low stringency. Although some hybridization is seen with female DNAs in all species, the principal hybridization bands are confined to males in all species of apes; thus, these related fragments are also confined to the Y chromosome. No detectable hybridization was obtained with DNA from a somatic cell hybrid in which the only human chromosome is the X (25), indicating that the weak hybridization seen with female DNA is due to autosomal homology (data not shown). The intensity of hybridization to *Macaca* DNA is increased in both male and female DNAs at low stringency.

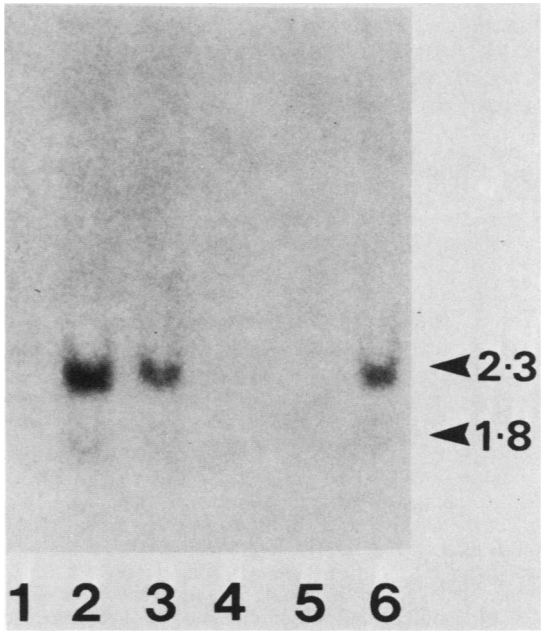

FIG. 3. Southern blot analysis of variant Y DNAs with the Y-specific probe. DNAs were digested with *Hin*dIII, and a blot experiment was performed with the radiolabeled 3.3-kb *Eco*RI 4B-2 probe (see the legend to Fig. 1). DNAs were 46,XX,−14,+der(14), t(Y;14)(q12;p11) (lane 1); 46,X,i(Yq) (lane 2); 46,XY,del(Y)(q11) (lane 3); 46,XX (lane 4); 46,X,i(Yp) (lane 5); and 46,XY (lane 6).

DISCUSSION

Although repeated DNA sequences from the human Y chromosome have been studied in some detail (9, 10, 14), much less is known about single-copy DNA sequences from the Y chromosome. Further questions relating to the origin, function, and evolution of the Y chromosome require a representative source of Y sequences. The construction of several recombinant libraries of Y sequences should provide the necessary material. One such library has been reported, and several clones containing repeated and single-copy sequences have been described (4). The library of Y sequences we describe here, which in theory should be highly representative of the Y, is, in fact, deficient in some known tandemly repeated Y sequences, such as the Y-3.4 and Y-2.1 *Hae*III repeated fragments. Deletion of tandem sequences are known to occur when recombinants constructed in λ vectors are amplified in *E. coli* (30). Nevertheless, this library does contain a large number of clones with both repeated and single-copy DNA from the Y.

We have used one such clone to extend our analysis of the evolution of Y chromosome DNA organization. This recombinant, 4B-2, contains single-copy DNA which is confined to the Y chromosome in humans and maps to the proximal portion of the long arm (subcentromeric → q11). In addition, similar sequences appear to be confined to the Y chromosome of gorillas, chimpanzees, and orangutans. This is in contrast to the majority of single-copy Y sequences which have homologs on other chromosomes (20, 33) and to at least one long region of single-copy sequence on the human Y which is not present on the Y chromosome of apes (22). Unlike the predominant repeated DNA fragments of the human Y long arm, which are of recent origin on the human Y (13), the single-copy DNA sequence of the Y long arm described here has a more ancient association with the Y chromosome of primates. Even in this case, the specific association with the Y is not constant. In old world monkeys, this human single-copy sequence is part of a highly repeated DNA family with homologous sequences on one or more chromosomes other than the Y. Two explanations are possible for this observation. (i) An ancestral primate species may have contained a family of repeated DNA sequences which were retained in old world monkeys but became reduced in number during the radiation of the apes. (ii) The sequences may originally have been single copy, as in present-day apes, and been amplified in old world monkeys. Although there is no indication of Y specificity for homologs of this probe in the old world monkey digests, the presence of multiple Y-specific fragments in the apes suggests that the association with the Y chromosome may be relatively ancient and that the sequences have been eliminated from other chromosomes in the ape genomes.

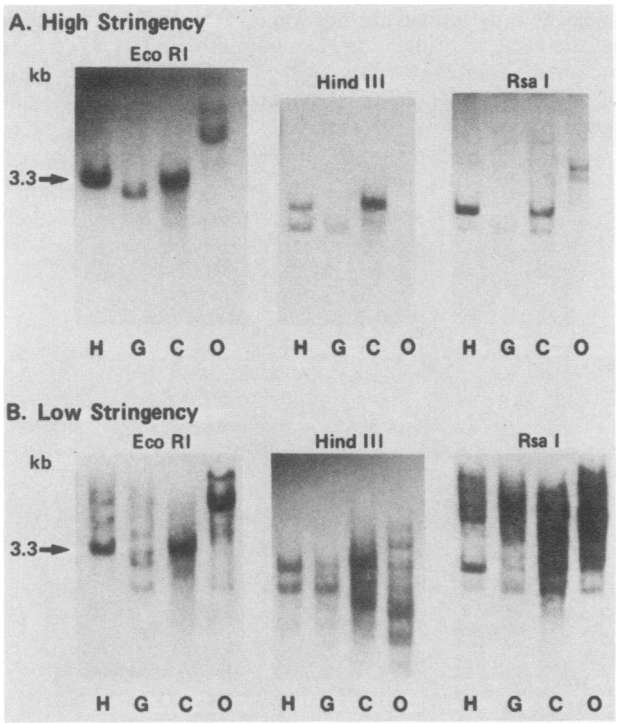

FIG. 4. Analysis of human Y probes in male ape DNA. The 3.3-kb *Eco*RI single-copy Y probe from recombinant phage 4B-2 (see the legend to Fig. 1) was hybridized to restriction digests of humans (lanes H), gorillas (lanes G), chimpanzees (lanes C), and orangutans (lanes O) as indicated. The stringency was established by the wash conditions after hybridization. For low stringency (B), all filters were washed at 65°C in buffers with at least 0.5 M NaCl. Under these conditions, all hybrids formed during hybridization (25 to 30% base mismatch) would be retained. High stringency (A) was established by several washes at 65°C in 0.1× SSC. Under these conditions, greater than 90% homology is required for retention of stable hybrids.

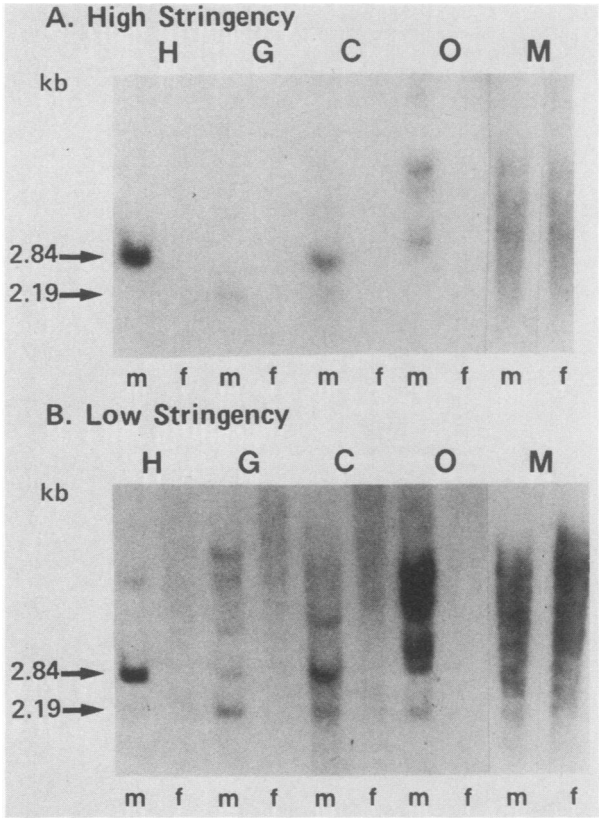

FIG. 5. Analysis of Y specificity in primates. The 3.3-kb *Eco*RI single-copy probe from recombinant phage 4B-2 was hybridized to male (lanes m) and female (lanes f) *Rsa*I-digested DNA from humans (H lanes), gorillas (G lanes), chimpanzees (C lanes), orangutans (O lanes), and *M. mulatta* (M lanes). Filters were washed at high (A) and low (B) stringency as described in the legend to Fig. 4. The *M. mulatta* lanes were exposed two to three times longer than those for the other species.

The association of this single-copy sequence with the Kpn-LINE sequence is intriguing. Certain features of the organization pattern of this LINE element appear to be identical throughout the genome of humans, apes, and old world and new world monkeys (26). Furthermore, these elements are closely associated with single-copy sequences on the human autosomes (1, 25) and X chromosome (B. J. Schmeckpeper, A. F. Scott, and K. D. Smith, unpublished data). Thus, the physical linkage of Kpn-LINE elements and single-copy sequences of 4B-2 DNA is similar to that found elsewhere in the genome.

The presence of this single-copy sequence on the proximal portion of the Y long arm may coincide with a region known to control spermatogenesis, which when deleted, results in azoospermia (32). Whether the single-copy sequence we have identified is functional or is linked to such functional sequences remains to be determined. Its failure to hybridize with DNA from a 46,XX male indicates that its presence is not required for male determination.

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