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Specific cloning of human DNA as yeast artificial chromosomes by transformation-associated recombination

VLADIMIR LARIONOV*†‡, NATALYA KOUPRINA*‡, JOAN GRAVES*, X.-N. CHEN§, JULIE R. KORENBERG§, AND MICHAEL A. RESNICK*

*Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Box 12233, Research Triangle Park, NC 27709; and §Ahmanson Department of Pediatrics, Division of Genetics, Cedars–Sinai Research Institute, University of California, Los Angeles, CA 90048-1869

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ABSTRACT **DNA molecules undergoing transformation into yeast are highly recombinogenic, even when diverged. We reasoned that transformation-associated recombination (TAR) could be employed to clone large DNAs containing repeat sequences, thereby eliminating the need for *in vitro* enzymatic reactions such as restriction and ligation and reducing the amount of DNA handling. Gently isolated human DNA was transformed directly into yeast spheroplasts along with two genetically marked (*M1* and *M2*) linearized vectors that contained a human *Alu* sequence at one end and a telomere sequence at the other end (*Alu–CEN–M1–TEL* and *Alu–M2–TEL*). Nearly all the *M1*-selected transformants had yeast artificial chromosomes (YACs) containing human DNA inserts that varied in size from 70 kb to >600 kb. Approximately half of these had also acquired the unselected *M2* marker. The mitotic segregational stability of YACs generated from one (*M1*) or two (*M1* and *M2*) vector(s) was comparable, suggesting *de novo* generation of telomeric ends. Since no YACs were isolated when rodent DNAs or a vector lacking an *Alu* sequence was used, the YACs were most likely the consequence of TAR between the repeat elements on the vector(s) and the human DNA. Using the BLUR13 *Alu*-containing vector, we demonstrated that human DNA could be efficiently cloned from mouse cells that contained a single human chromosome 16. The distribution of cloned DNAs on chromosome 16 was determined by fluorescence *in situ* hybridization. We propose that TAR cloning can provide an efficient means for generating YACs from specific chromosomes and subchromosome fragments and that TAR cloning may be useful for isolating families of genes and specific genes from total genome DNA.**

The first step in the molecular characterization of eukaryote genomes generally involves cloning of large chromosomal fragments. This is usually accomplished by gentle DNA isolation, restriction enzyme cutting, ligation to cloning vectors, and transformation or packaging into the appropriate systems. For the case of mammalian DNA, these approaches have yielded up to megabase fragments within yeast artificial chromosomes (YACs) (1, 2) or 100- to 200-kb fragments as bacterial artificial chromosomes (BACs or PACs) (3, 4).

Because of their large size, YACs have proven essential in genome mapping of many organisms. However, artifacts such as chimeras and deletions can limit their use (5–8). These artifacts may result from *in vitro* DNA manipulation resulting in the DNA becoming broken or nicked. Also, there could be incomplete ligation of telomeric arms or ligation between different chromosomal fragments resulting in chimeric YACs. Damaged molecules undergoing transformation in yeast are recombinationally active and are a potential source of aberrant YAC clones (7, 9).

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We have taken an alternative approach that minimizes manipulation of cellular DNAs prior to transformation. The method is based on transformation-associated recombination (TAR). High levels of recombination between small homologous and diverged DNAs during transformation have been well documented in yeast (10, 11). Recently, we reported that during cotransformation an *Alu*-containing plasmid that had been linearized next to the *Alu* sequence could undergo recombination with a YAC that contained human DNA (9). Also, Ketner *et al.* (12) demonstrated that homologous recombination could be used during transformation to clone the 30-kb linear adenovirus DNA when present in a large excess of mouse DNA. In their experiments a DNA mixture was cotransformed into yeast along with two telomeric plasmids containing sequences homologous to adenovirus DNA.

Based on these observations, we have developed a method for cloning large human DNAs that uses recombinational interactions during transformation between telomeric plasmids containing *Alu* sequences or long interspersed repetitive elements (*LINE*s) and genomic DNA. This TAR cloning method is rapid and simple, greatly reduces *in vitro* handling of DNA, and eliminates the need for DNA enzymatic treatments such as restriction and ligation. Using this method, we demonstrate that human DNA can be efficiently cloned from mouse cells containing a single human chromosome. The approach can be extended to the isolation of any DNA in which there are frequent repeats.

MATERIALS AND METHODS

TAR Cloning Vectors. The vectors pVC1 (*Alu–CEN6–HIS3–TEL*), pVC3 (*LINE–CEN6–HIS3–TEL*), pVL13 (*Alu–ARSH4–TRP1–TEL*), and pVL27 (*Alu–URA3–TEL*) were used. To construct the pVC1 vector, a 0.4-kb *Nru* I fragment containing a *CEN6* sequence from the plasmid pMACS4 (provided by P. Philippsen, Department of Biotechnology, University of Basel) was cloned into the *Not* I site of plasmid pBP109 (13). When cut with *Sal* I, the plasmid pVC1 has an *Alu* sequence (BLUR13) at one end and a telomeric sequence at the other end. Digestion of pVC1 with *Xho* I yields a linear molecule with a 300-bp tail of nonhomologous DNA following the *Alu* sequence; digestion with *Bam*HI leaves no *Alu* sequence. To develop pVC3, an *Alu*-containing *Bam*HI fragment was replaced with a 2.9-kb *Bam*HI *LINE1.1* fragment from pBP111 (13) containing most of the *LINE* open reading frame 2 (ORF2). Cutting with *Sal* I yields a linear molecule bounded by a *LINE* and a telomere sequence. An acentric *ARS*-containing vector pVL13 was constructed by cloning a 0.8-kb *Not* I fragment containing two copies of the *Alu*

Abbreviations: TAFE, transverse alternating-field electrophoresis; TAR, transformation-associated recombination; YAC, yeast artificial chromosome.

†To whom reprint requests should be addressed.
‡On leave from: Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia.

sequence (BLUR8) from the plasmid pBP63A (14) into the unique *Not* I site of pJS98 (15). When cut with *Nru* I, the plasmid has an *Alu* sequence at one end and a telomeric sequence at the other end. The vector pVL27 was constructed by ligating the 2.2-kb *Pvu* I fragment from pBP109 with a 2.5-kb *Pvu* I fragment from pRS306 (16). The plasmid was cut with *Hin*dIII to yield a molecule bounded by an *Alu* and a telomere sequence.

Yeast Strains and Mammalian Cell Lines. *Saccharomyces cerevisiae* strain YPH857 with the *HIS3* gene deleted (*MATα, his3-Δ200, trp1-Δ1, ura3-52, leu2-Δ1, lys2-801, ade2-101*) was used (5). Human (HL-60), hamster (CHO-K1), and mouse (NIH 3T3) cells were obtained from the University of North Carolina Tissue Culture Facility. Mouse–human monochromosomal somatic cell hybrid CY18 with human chromosome 16 was provided by L. Deaven, Los Alamos National Laboratory. Yeast cells were grown on complete medium (YPD) or synthetic standard selective media (5).

Yeast Transformation. A protocol for spheroplasting cells was used that results in efficient transformation (9). The spheroplasts were used directly or dimethyl sulfoxide was added to a final concentration of 7% and the spheroplasts were stored at −70°C for later use. Agarose plugs (100 μl) containing ≈1 μg of gently prepared human or rodent DNAs were used for transformation (2). Linearized vector(s) (1 μg) was added to DNA-containing plugs after treatment with agarase and presented to spheroplasts.

Identification of YAC Clones. Colony hybridization was carried out to identify clones containing human YACs among yeast transformants generated from monochromosomal somatic cell hybrid DNA as described (17). Human and mouse probes were labeled by random priming. When a genomic human DNA probe was used, unlabeled BLUR13 *Alu* fragment (10 μg per reaction) and unlabeled mouse CotI DNA (100 μg per reaction) were added to the hybridization solution to suppress the hybridization of repetitive sequences. When a mouse DNA probe (B_2 or CotI mouse DNA) was used, unlabeled human CotI DNA (100 μg per reaction) was added to the hybridization solution.

Preparation of Chromosome-Size DNAs from Yeast. Low-melting-point agarose plugs were prepared from either primary transformants or subclones (9). Large DNAs were separated by transverse alternating-field electrophoresis (TAFE) (18). YACs were identified with randomly primed human or mouse genomic DNAs by using conditions described above for colony hybridization.

Analysis of YAC Propagation During Mitotic Growth. Loss of YACs was determined by measuring loss of the centromere-linked *HIS3* marker, as described (5), in primary transformants and in derived subclones. In brief, colonies were streaked to nonselective medium and the frequency of His^- colonies was determined by replica plating.

Inter-*Alu* PCR and *Alu* Profiles of YACs. Inter-*Alu* PCR was carried out in a 50-μl final volume containing 1 μg of total yeast DNA isolated from His^+ transformants as described (19). The *Alu* PCR primer sequence was either 5′-GGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGA-3′ or 5′-GGAGGCTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCGG-3′. To identify fragments containing *Alu* sequences (*Alu* profiles), 1 μg of total yeast DNA was digested to completion with *Taq* I. Samples were electrophoresed in 1.2% agarose gels, transferred to a nylon membrane, and hybridized to a BLUR13 *Alu* probe. Total yeast DNA for PCR analysis and *Alu* profiles was extracted from 5-ml cultures by standard methods.

Fluorescence *in Situ* Hybridization (FISH) Analysis. Clones containing human DNA derived from the chromosome 16 hybrid cell line were biotinylated by nick-translation in the presence of biotin-14-dATP with the BioNick Kit (GIBCO/BRL). FISH was performed as described (20). To distinguish precisely the chromosome sub-bands, a reverse banding technique was employed that used chromomycin A3/distamycin A double staining (20).

RESULTS

TAR Cloning of Human DNAs with Two Vectors. The TAR method for cloning human DNAs as YACs is based on the idea that YACs can be generated *in vivo* by recombination between human DNA and plasmids that contain human DNA repeats such as an *Alu* or *LINE* (Fig. 1). [While repeats in the human genome are diverged, recombination in yeast can occur between homologous as well as diverged sequences during transformation (10, 21).] The TAR cloning was investigated by using a mixture of human DNA and two plasmids with terminal *Alu* sequences; one contained an origin of replication (autonomously replicating sequence, *ARS*) and the other had a yeast centromere sequence to assure proper segregation. Human DNA and linearized plasmids pVC1 (*Alu–HIS3–CEN6–TEL*) and pVL13 (*Alu–TRP1–ARSH4–TEL*) were mixed and presented to spheroplasts. Between 800 and 2000 His^+ transformants were typically obtained by use of a mixture containing 1 μg of each plasmid and 1 μg of cellular DNA. Approximately 60% of the transformants also contained the unselected *TRP1* marker, which in all cases (110 examined) was linked to *HIS3* (i.e., they were lost simultaneously). Of the His^+Trp^+ transformants, about one-third (33/110) contained new chromosome-size (from 70 kb to >600 kb) molecules that hybridized with human DNA (data not shown). However, most of the His^+Trp^+ transformants lacked human DNA but contained small circular or linear molecules (data not shown) which probably arose by direct interaction between the vectors.

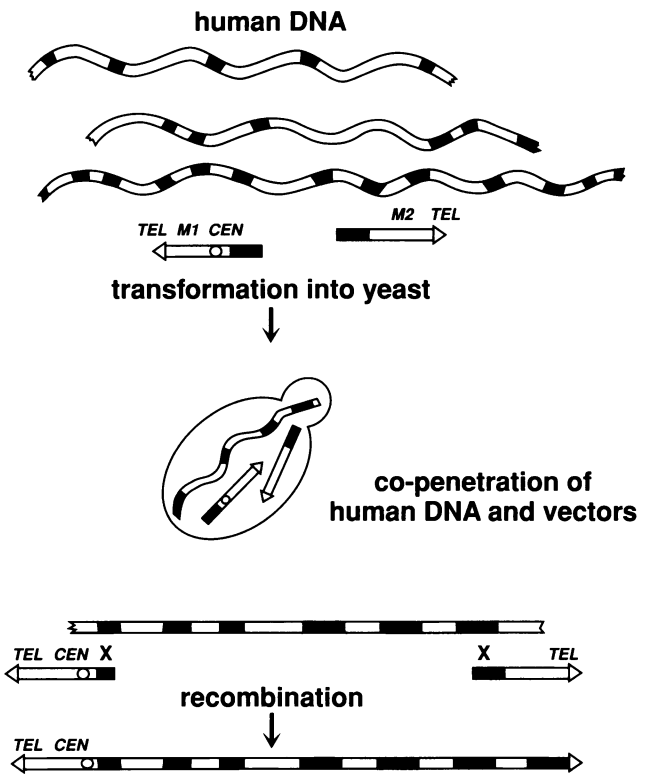

FIG. 1. Isolation of human DNAs as YACs by the TAR cloning method. Yeast spheroplasts are transformed with human DNA along with vectors containing *M1* and *M2* genetic markers. The filled-in blocks in human DNA and vectors identify repeated *Alu* (or *LINE*) sequences. After copenetration, YACs are generated by recombination between repeat sequences in human DNA and vectors.

To avoid the high background of clones containing only plasmid, TAR cloning with vectors lacking an *ARS* was investigated, since YAC replication might be initiated at the frequent *ARS*-related sequences in human DNAs (22, 23). Human DNA and linearized forms of pVC1 (*Alu–HIS3–CEN6–TEL*) and pVL27 (*Alu–URA3–TEL*) were mixed and presented to spheroplasts. Between 300 and 1000 His^+ transformants were typically obtained by use of a mixture containing 1 μg of each plasmid and 1 μg of cellular DNA (Table 1). Approximately 50% of His^+ transformants contained the unselected *URA3* plasmid marker. In 260 His^+Ura^+ transformants the *HIS3* and *URA3* markers were linked. All His^+Ura^+ transformants (55/55) contained DNA which hybridized with human DNA. New chromosome-size molecules were identified that varied from 70 kb to >600 kb, similar to YACs obtained when one vector had an *ARS* (data not shown). About 65% of His^+Ura^+ transformants (26/40) produced multiple inter-*Alu* PCR products (data not shown). The *Alu* profiles of the TAR-generated YACs (Fig. 2) are typical of YACs containing human DNA inserts. Thus, the use of vectors lacking an *ARS* eliminated the plasmid background and increased the yield of doubly marked YACs as compared with TAR cloning with vectors containing an *ARS*.

Approximately 50% of the His^+ transformants did not contain the unselected plasmid pVL27 marker *URA3*; however, almost all the transformants examined (28/30) also contained human YACs (data not shown). These singly marked YACs may have acquired telomeres utilizing internal yeast telomere-like sequences present in the human genome (see below).

TAR Cloning with One Vector. Because of the high yield of YACs containing only one telomere marker, TAR cloning of human DNA using only one vector lacking an *ARS* was examined. Transformation of yeast spheroplasts by a mixture of a linearized vector containing *Alu* (pVC1) or *LINE* (pVC3) plus an equal amount of human DNA yielded a high level of transformants (Table 1). Few transformants were obtained with plasmid only, and they arose by rare integration of the plasmid into yeast chromosomes (see below). A high frequency of transformation does not require that the *Alu* sequence be at the end of the vector. Comparable levels of transformation were observed when the *Alu* sequence was 300 bp from the end (the pVC1 plasmid was linearized by *Sal* I and *Xho* I). Transformation was low and comparable to that when human DNA was not included or the plasmid lacked an *Alu* sequence (digestion with *Bam*HI). The TAR cloning was specific, since transformation was lower by a factor of ≈100 when mouse or hamster DNA was used instead of human DNA (Table 1).

Table 1. Efficiency of transformation by *Alu*- and *LINE*-containing plasmids when various DNAs were included

Plasmid DNA*	Cellular DNA	No. of His^+ transformants†
pVC1	None	1–3
pVC1 + pVL27	None	0–7
pVC1 + pVL27	Human	300–1000
pVC1	Human	300–1000
pVC3	Human	50–600
pVC1	Hamster	1–10
pVC1	Mouse	1–20
pVC1	Hybrid cell line CY18	20–70

*YPH857 yeast spheroplasts were transformed with 1 μg of a *Sal* I-linearized pVC1 plasmid or with a mixture of linearized *Alu*-containing plasmids along with an equal amount of human or rodent DNA. pVC1, *Alu–CEN6–HIS3–TEL*; pVC3, *LINE–CEN6–HIS3–TEL*; pVL27, *Alu–URA3–TEL*.

†Five to 15 independent transformations were carried out for each condition.

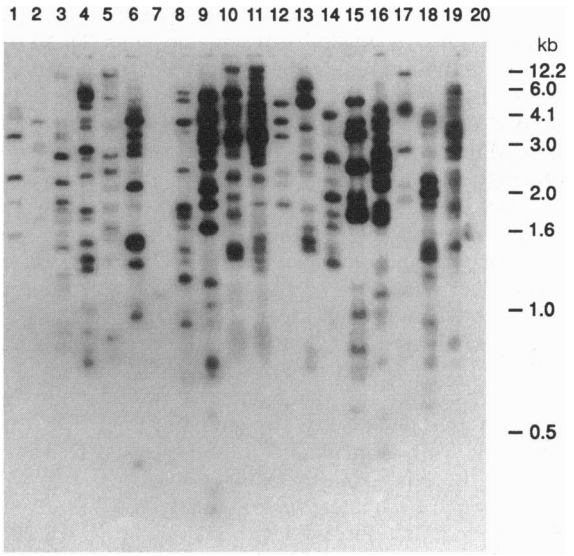

FIG. 2. *Alu* profiles of YACs generated by TAR cloning. The profiles were produced by hybridization of a BLUR13 probe with *Taq* I-digested DNA from 18 randomly selected clones. Lane 7 contained DNA of a host yeast strain; lane 20 contained the λ DNA size markers.

The high level of transformation observed with human DNA plus either pVC1 or pVC3 was primarily due to the production of YACs. Among 100 transformants analyzed, all contained new chromosome-size DNA molecules hybridizing with human DNA probe. Nearly 80% (82/100) of the transformants exhibited strong *Alu*-containing PCR bands (data not shown). The size distribution of YACs was comparable to that for YACs isolated with two vectors (Fig. 3). Four of the YACs were circular and were retained in the wells (data not shown).

Approximately 35% of the primary transformant colonies contained more than one YAC. Such clones are frequently observed in YAC libraries developed by standard methods (24) and could result from cotransformation. When six of these colonies were subcloned, most of the subclones contained only one stable band. While this result is consistent with cotransformation, it is also possible that, initially, cloned YACs are unstable.

In most primary transformant colonies obtained with a single vector the YACs were unstable (>70% of cells in the colony lacked the YAC marker), whereas the YACs were stable in most transformants obtained with two vectors (<10%

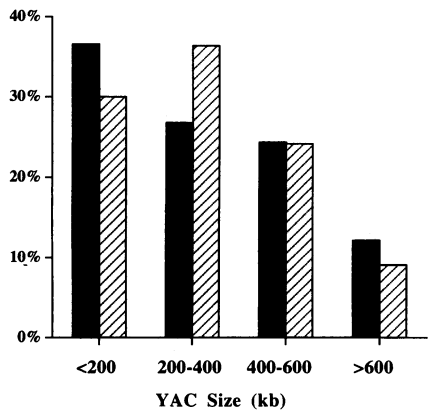

FIG. 3. Size distribution of YACs obtained by TAR cloning. The YACs that were isolated by cotransformation of human DNA along with two vectors (33 YACs; hatched bars) have the *HIS3* and *TRP1* markers. Also presented are results for 82 YACs (solid bars) obtained by cotransformation with a single *HIS3* vector lacking an *ARS*.

of cells in a colony lacked the YAC markers). Both types of YACs were stable in subclones of transformants. These results are consistent with a requirement for *de novo* generation of a telomere at the distal end (i.e., lacking the vector).

Specific TAR Cloning of Human DNA from a Mouse–Human Monochromosomal Cell Line. Since high levels of transformation were accomplished with the *Alu*-containing vector only when human DNA was present (Table 1), we investigated the cloning of human DNAs from a mouse–human monochromosomal cell line CY18 that carries a single human chromosome 16. Total genomic DNA was prepared as described above and transformed along with linearized pVC1 plasmid. The efficiency of transformation was always greater than that observed with mouse DNA only (Table 1). The transformants (from five independent transformations) were initially analyzed in two ways, by inter-*Alu* PCR or by colony hybridization with human and mouse probes. Nearly 20% (15/79) of the His^+ transformants contained human DNA inserts as judged from inter-*Alu* PCR analysis. This percentage was higher than that observed with hybridization, where 12% (42/350) hybridized to the human probe, presumably due to differences in sensitivity of the methods. Only 4% of colonies hybridized to the mouse probe and there were no transformants that could hybridize to both probes, suggesting that there were few, if any, chimeras. About three-quarters of the His^+ transformants lacked mammalian DNA and were most likely due to illegitimate recombination with yeast chromosomes (see *Discussion*). All the clones containing human DNAs (as judged from hybridization with human DNA probe, *Alu* profiles, and inter-*Alu* PCR) were examined by TAFE analysis and were found to contain additional chromosome-size bands, from 70 kb to >600 kb (Fig. 4).

The cytogenetic positions of 15 among 17 YAC DNAs tested were located on chromosome 16 by FISH analysis and are presented in Fig. 5. Four of the 15 YACs exhibited additional signals on chromosome 16. Some of these may be explained by the presence of chromosome-specific repeats, as has been described for cloned DNAs derived from this chromosome (25). For 9 of the YACs, signals were also detected on the short arm of the acrocentric chromosomes 13, 14, 15, 21, and 22, the most common of which was chromosome 21p. These are the well-described locations of simple repeats. It is of interest that many cloned DNAs are distributed to multiple sites in the short arm and, in addition, others are clustered at the junction of the heterochromatin and euchromatin in the pericentromeric region of the long arm.

Thus, human DNA can be efficiently cloned from hybrid cells. Since human DNA was only ≈3% of the total and the final ratio of human to mouse YACs was at least 3:1 (based on colony hybridization results), there was a >75-fold enrichment of human DNA during TAR cloning.

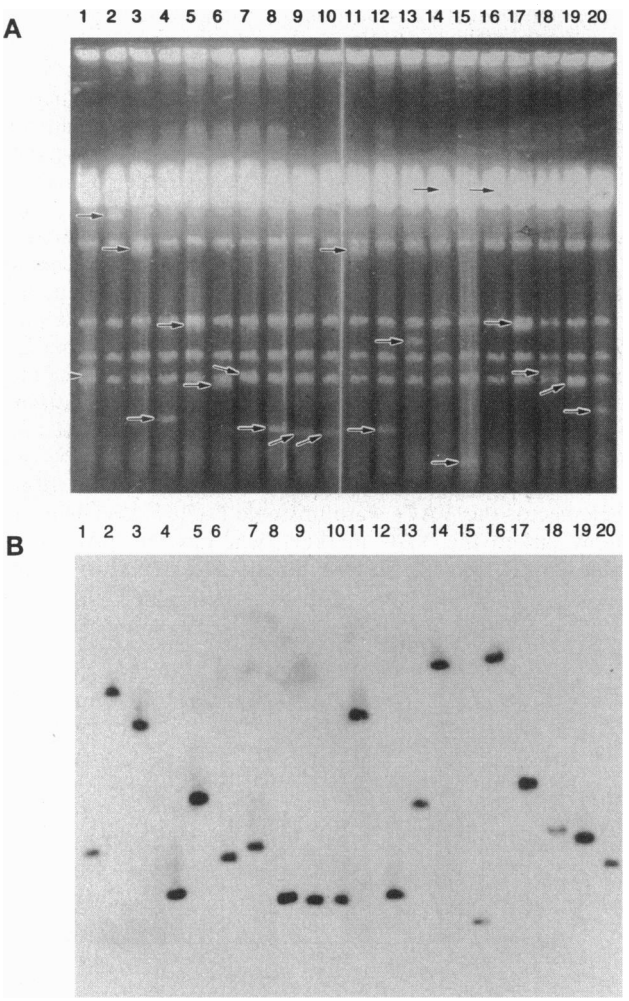

FIG. 4. Physical characterization of 20 randomly selected human YACs developed by TAR cloning from monochromosomal cell line CY18. (*A*) Ethidium bromide staining of the chromosome-size DNAs isolated from clones containing the YACs. Positions of YACs are shown by arrows. (*B*) The YACs identified by hybridization with a labeled human DNA probe.

DISCUSSION

We have described a method for cloning human DNAs which does not require *in vitro* enzymatic treatment of the DNAs. The development of YACs can be accomplished by using the intracellular enzymatic machinery that mediates efficient intermolecular recombination between homologous and diverged DNAs in yeast during transformation. That homology-driven recombination is the mechanism for YAC generation was demonstrated by the specificity of the bimolecular interaction. Large numbers of YAC clones were isolated when *Alu*-based plasmid was cotransformed with human DNA, whereas there were no YAC clones when the plasmid lacked

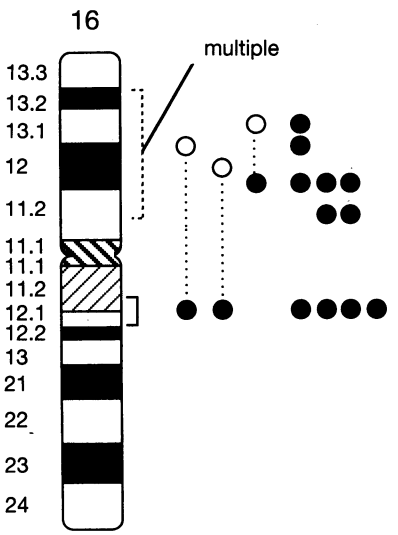

FIG. 5. Ideogram of chromosome 16 representing the distribution of signals generated by FISH mapping using whole YAC DNAs as hybridization probes. Primary signals are defined as those on chromosome 16 or, where secondary sites are also seen on 16, the site that is positive in the highest proportion of cells. Filled and open circles correspond to primary and secondary sites, respectively. One YAC hybridized as multiple positions.

a human repeat or when rodent instead of human DNA was used (Table 1).

The greatest yield of YACs (nearly 100%) was observed when a pair of TAR vectors was used that lacked an *ARS*. The efficient generation of YACs using a vector lacking an *ARS* indicates that there are frequent endogenous *ARS*-like elements (22, 23) that enable replication of YACs in yeast cells. Surprisingly, human DNA was also efficiently cloned with the use of only one TAR vector, suggesting that yeast telomere-like elements, as well as *ARS*-like sequences, are frequent in the human genome. Possibly, there is *de novo* generation of telomeres at the frequently repeated $(CA)_n$ sequences ($\approx 10^5$ copies) in the human genome (26).

While recombination during transformation can provide the means for cloning large molecules, we propose that the recombination is nonrandom. Given that the average distance between *Alu* sequences is 2–3 kb, it is surprising that nearly half of the YACs were >200 kb. Possibly there are homology limitations such that not all *Alu* and *LINE* sequences can recombine with the same efficiency. It is also possible that recombination interactions with molecules having double-strand breaks occur preferentially at homologous sequences near broken ends. This possibility is supported by the observation that the frequency of TAR between a human YAC and an *Alu*-containing telomeric vector is greatly increased when a YAC is broken (9).

The TAR method for cloning genomic fragments has many potential uses. The approach can be extended to the isolation of any DNA in which there are frequent repeats. For example, we have found that mouse DNA can be cloned by the TAR method using a 150-bp mouse-specific B_2 repeat (unpublished data). The use of the TAR method for generating genomic libraries remains to be established, since the randomness of cloned fragments and the level of chimeras need to be evaluated. We note, however, that this approach eliminates *in vitro* ligation as a potential source of chimeras. Furthermore, since chimeras can arise by recombination between copenetrating molecules (7, 9), it may be possible to decrease chimeras by dilution of human DNA with nonhomologus DNA during TAR cloning.

Except for flow-sorted chromosomes (27), the opportunities to isolate specific human chromosomal DNAs up to now have been limited. An important application of TAR cloning is the specific and direct cloning of human chromosomes (and possibly chromosomal fragments) from hybrid cell lines. We demonstrated that the TAR method led to a >75-fold enrichment in the isolation of human DNA from a mouse–human monochromosomal 16 hybrid cell line among the YACs containing mammalian DNA. There were no mouse–human chimeras. Human–human chimeras are unlikely on the basis of previous reports with hybrid cell lines (6, 8) and the low probability of copenetration of human DNAs.

To determine the distribution of DNAs isolated by TAR cloning with a single vector containing the BLUR13 *Alu* sequence, the YACs were examined by FISH analysis. Many of the YACs contained DNA from the pericentric region, although several identified other regions on chromosome 16. In the long arm, the cloned DNAs were clustered at the junction of the pericentromeric block of tandemly repeated DNA (16q11.2) and the euchromatic, gene-containing region. Several of the YACs identified other chromosomes, mainly the short arms of chromosomes 13, 14, 15, 21, and 22. This is not surprising, since the pericentromeric region of chromosome 16 has simple repeat DNAs related to the repetitive regions of the other chromosomes. While several regions of chromosome 16 were isolated by TAR cloning, the lack of randomness is most likely explained by the distribution of BLUR13 *Alu* or *ARS* sequences in the chromosome (28). Further investigation is necessary to understand the clustering of YAC signals at the junction of the pericentric heterochromatin and euchromatin of the long arm. Use of either less-diverged *Alu* repeats or a set of vectors containing different repeat sequences might result in a more random distribution of isolated DNAs.

During the cloning of human DNA from hybrid cells, approximately three-quarters of the transformants lacked mammalian DNA. These transformants presumably arose by illegitimate recombination between the vector and yeast chromosome(s), which resulted in the generation of yeast chromosome fragments that probably carried natural telomeres (unpublished data). When compared with YACs, these chromosome fragments were much more stable in the initial transformant colonies (<5% of cells in a colony lacked the *HIS3* marker). The false-positive clones can potentially be distinguished readily by including a color marker in the TAR cloning vector that can facilitate detection of chromosome malsegregation (29).

TAR cloning may also be employed to generate circular YACs. We have found that a linear plasmid containing human repeat at each end (i.e., *Alu*–Marker–*CEN*–*LINE*), when cotransformed along with human DNA, efficiently generates circular YACs containing large human DNA inserts (unpublished work). The circular molecules would facilitate handling as well as transfer to cells of other organisms.

Furthermore, we propose that the method could also be applied to the isolation of specific chromosomal regions, families of genes, and, perhaps, single-copy genes. Ketner *et al.* (12) demonstrated that a fragment of adenovirus DNA could be isolated from a mixture of virus DNA and mouse DNA when present at 10 copies per mammalian genome equivalent. The vectors used in their work contained an *ARS* which we have found would reduce the efficiency of cloning.

Although our results demonstrate that the TAR method is efficient for cloning large human DNA fragments, systematic studies will greatly enhance its usefulness. While YACs of >600 kb were obtained, we anticipate that conditions could be optimized to yield more and larger YACs. There may be limitations on TAR cloning that depend on the distribution of the TAR cloning sequences and ARS elements (when vectors lacking an *ARS* are used). Use of *Alu* and *LINE* sequences that are more representative (instead of BLUR13 *Alu* and *LINE1.1*) and less diverged from those used in this study may result in more efficient cloning.

There are several features of YACs, as compared with bacterial artificial chromosomes (BACs or PACs), that are desirable in the characterization and manipulation of genomes. Large fragments of DNA can be easily cloned or generated by *in vivo* recombination in yeast. YACs can be genetically manipulated in yeast, and they can be utilized in the generation of transgenic organisms. As discussed above, the TAR cloning method expands the usefulness of YACs in that it provides the possibility for direct cloning of DNA fragments by the use of homologous recombination and, therefore, could simplify isolation of chromosome-specific sequences and isolation of gene families.

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