MOLECULAR AND CELLULAR BIOLOGY, Apr. 1986, p. 1352–1356 Vol. 6, No. 4
0270-7306/86/041352-05$02.00/0
Copyright © 1986, American Society for Microbiology

The *Saccharomyces cerevisiae* Chromosome III Left Telomere Has a Type X, but Not a Type Y', *ARS* Region

LINDA L. BUTTON AND CAROLINE R. ASTELL*

Department of Biochemistry, Faculty of Medicine, The University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada

Received 26 August 1985/Accepted 17 December 1985

A yeast *Saccharomyces cerevisiae* telomeric region was isolated by chromosome walking from *HMLα*, the most distal known gene on the chromosome III left (IIIL) end. The terminal heterodisperse 3.3-kilobase (kb) *Sal*I fragment on chromosome IIIL, 8.6 kb distal to *HMLα*, was cloned in a circular vector to generate a telomeric probe. Southern hybridization and DNA sequencing analyses indicated that 0.6 kb (±200 base pairs) of 5'-C_{1-3}A-3' simple tandem repeat sequence, adjacent to a 1.2-kb type X *ARS* region, constitutes the telomere on the chromosome IIIL end, and no type Y' *ARS* region homologies exist between *HMLα* and the IIIL terminus.

Salient properties of telomeric DNA have been deduced primarily from studies conducted on amplified macronuclear DNA molecules in lower eucaryotic organisms (3, 4, 6) and a yeast *Saccharomyces cerevisiae* telomere isolated by selection for telomeric function with a linear plasmid vector (27). Southern hybridization with a probe prepared from the cloned yeast telomere indicates that the ends of most yeast chromosomes are homologous for at least the terminal 3 to 4 kilobases (kb) (16, 27). Autonomously replicating sequences (*ARS*), identified as types X or Y' according to relative homologies, have been mapped to the telomere-associated region of yeast chromosomes (9, 10), and yeast telomeres are categorized into two classes, according to the presence or absence of a type Y' *ARS* region (16, 29, 30) (Fig. 1a). The telomere previously isolated and characterized (9, 15, 16, 27) is representative of the Y'-containing telomere class, although the existence of the Y'-lacking class has been spec-

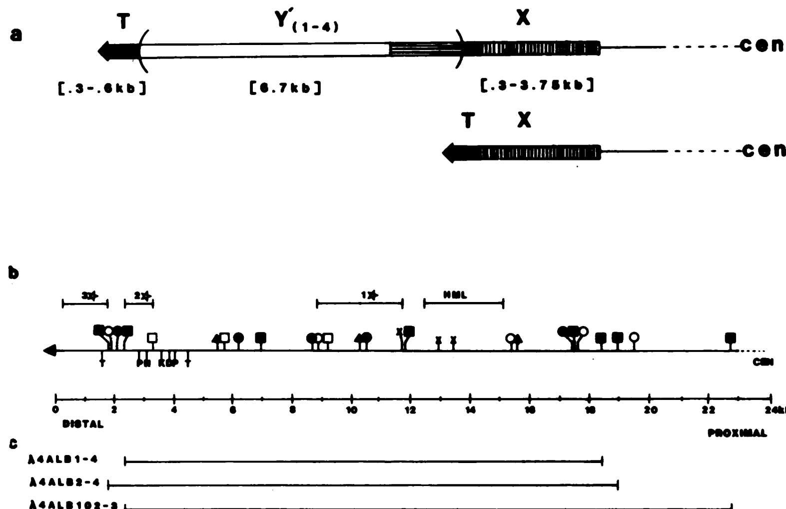

FIG. 1. Organization of telomeric repeat sequences in yeast and map of the chromosome IIIL telomeric region. (a) The telomeric arrangement described initially by Chan and Tye (9) and modified by Walmsley et al. (30) contains yeast *ARS* elements of the Y' and X classes and the T region, 5'-C_{1-3}A-3' simple repeat sequences, at the terminus and the X-Y' junction region. The T-Y'-X-class telomere has one to four conserved 6.7-kb Y' elements, consisting of Y (▭) and 131 (▭) regions, adjacent to a single, heterogeneous X element (▭). The second proposed T-X-class telomere has a single heterogeneous X element adjacent to the T region at the chromosome end. (b) Restriction maps of the chromosome IIIL end with sites mapped with multiple enzyme digestions and Southern analyses. Symbols above the chromosome designate those enzymes mapped for the entire 23-kb region, and the letters below are sites mapped for only the distal 5-kb portion. ●, *Bam*HI; ■, *Eco*RI; ○, *Hin*dIII; □, *Sal*I; ▲, *Xho*I; ×, *Xba*I; T, *Bst*EII; K, *Kpn*I; N, *Nco*I; D, *Nde*I; P, *Pvu*II. Probes 1*, 2*, and 3* (1☆, 2☆, and 3☆, respectively) represent progressive chromosome walking steps toward the chromosome IIIL terminus. (c) Extent of yeast partial *Eco*RI insert fragments in representative phage clones, isolated with probes 1* or 2* from a λCh4A yeast genomic library.

ulated

* Corresponding author.

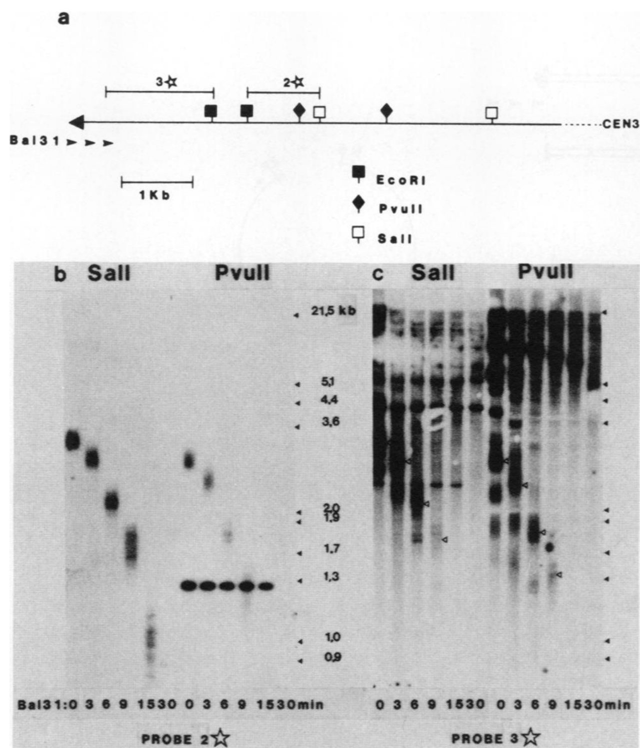

FIG. 2. Chromosome IIIL distal probes hybridize with BAL 31-sensitive regions in yeast genomic DNA. (a) Chromosome IIIL end region map indicating positions of probe 2* and 3* (2☆ and 3☆, respectively) hybridization, salient restriction sites, and region of BAL 31 nuclease digestion. (b and c) Yeast AB20α XP8-10B, treated with BAL 31 nuclease (0.1 U/μg, 30°C) for up to 30 min, such that between 125 and 150 bp are removed per minute from the chromosome end. DNA was digested with *Sal*I or *Pvu*II and fractionated on a 0.65% agarose gel (3.3 μg of DNA per lane). After bidirectional Southern transfer to GeneScreen plus, duplicate filters were screened with the 2* (2☆, panel b) or 3* (3☆, panel c) M13 probes. Size markers are λ-*Hin*dIII-*Eco*RI fragment positions. (c) The positions of the chromosome IIIL terminal fragments, (Δ) are indicated.

ulated (16, 29, 30). We report here the isolation of a T-X class telomere by chromosome walking from *HML*α to the chromosome III left (IIIL) end (11, 18, 19, 23). The telomeric region, localized to a heterodisperse 1.8-kb (±200 base pairs [bp]) *Eco*RI fragment on IIIL, consists of a 1.2-kb X region adjacent to a 0.6-kb (±200 bp) T region of 5′-C_{1-3}A-3′ simple repeat sequence.

Cloning of chromosome IIIL telomeric region. A genomic DNA library of yeast strain AB20α XP8-10B (24) was prepared by cloning partial *Eco*RI digest fragments (15 to 20 kb) in the λ Charon 4A (λCH4a) vector (20, 21) and screened by plaque hybridization (1, 20) with a nick-translated *HML*α distal probe (a 2.2-kb *Hin*dIII-*Xba*I fragment) (24). Probe 1* (Fig. 1b) resulted in a set of similar clones (Fig. 1c), but attempts to isolate further distal telomeric clones with probe 2* (Fig. 1b, a 1-kb *Eco*RI-*Sal*I fragment 8.6 kb distal to *HML*α) were unsuccessful. Either identical inserts to those obtained with probe 1* or inserts extending in the centromeric direction (Fig. 1c, λ4ALB 102-3) were present in all probe 2*-selected clones. Besides plaque hybridization, ΠVX recombinant screening (20, 25) with a ΠAN7 vector (H. Huang, Washington University, St. Louis, Mo.) was used, but only clones identical to those obtained with probe 1* were isolated. These results suggested probe 2* was very close to the telomeric region, and the terminal *Eco*RI fragment on chromosome IIIL was not included in the *Eco*RI partial digest genomic library.

Two properties observed for terminal restriction fragments on chromosomes are length heterogeneity, due to variable amounts of simple repeat sequences (9, 26, 29), and susceptibility to digestion with the double-stranded exonuclease BAL31, as shown in *S. cerevisiae* (26, 29) and trypansosomes (2, 5, 12, 28, 31). Southern analysis indicated that probe 2* hybridized to a single heterodisperse 3.3-kb (±200 bp) *Sal*I fragment and to two *Pvu*II fragments, a heterodisperse 3.0-kb (±200 bp) fragment and a discrete-length 1.2-kb fragment. The *Pvu*II site within probe 2* (Fig. 2a) explains the dual *Pvu*II fragment hybridization, with the heterodisperse 3.0-kb fragment mapped distal and the 1.2-kb fragment mapped proximal. Only the heterodisperse *Sal*I 3.3-kb fragment and the 3.0-kb *Pvu*II fragment were readily digested with BAL 31 nuclease, and both fragments extended about 2.3 kb (±200 bp) distal to probe 2*, indicating that the IIIL terminus was included in these probe 2* homologous fragments.

To clone the IIIL end, high-molecular-weight yeast DNA was treated briefly (2 and 4 min) with BAL 31 nuclease, and ends were repaired with DNA *Pol*I Klenow fragment (20). After *Sal*I complete digestion and low-melting-point agarose gel fractionation, fragments in the 3- to 4-kb-size range were isolated by electroelution (Fig. 3a). *Sal*I-*Sma*I-digested M13mp9RF (22) was ligated to the yeast fragments (5× molar excess of insert to vector) in a 20-μl ligation mix containing 1 mM spermidine and 2 U of T4 DNA ligase at 16°C for 24 h of incubation. After transformation of JM101 cells (20, 22), plaques were screened for the terminus of chromosome IIIL with a nick-translated probe 2* fragment. Clone MTLB6411 had a 3.0-kb *Sal*I-BAL 31 insert and contained the 0.5-kb *Eco*RI fragment distal to probe 2* as well as a 1.5-kb *Eco*RI fragment, presumably the terminal fragment with 300 bp (±200 bp) removed from the terminus by the BAL 31 nuclease treatment. The terminal 1.5-kb *Eco*RI-BAL 31 fragment (probe 3*, Fig. 3a) was subcloned to remove upstream probe 2* sequences that would confuse the characterization of the IIIL terminus. The probe 3* region was released from MTLB6411 by *Eco*RI digestion, made blunt ended with DNA *Pol*I Klenow fragment, and cloned into the *Sma*I site of M13mp18RF (32). Probe 3* was thereby flanked by several restriction sites in the polycloning region of M13mp18 to facilitate DNA sequencing.

Chromosome IIIL end contains a class X *ARS* region but lacks a class Y′ *ARS* region. As expected for a telomeric fragment which contains repetitive Y′, X, or T regions, probe 3* hybridized with multiple yeast genomic fragments (Fig. 4). The two classes of yeast telomeres are distinguished either by homology with the Y′ *ARS* region or by the distance between the X *ARS* region and the chromosome end. Clones with X and Y′ regions have been isolated and characterized; YRp131A and YRp131B contain X and Y′ *ARS* region *Sal*I fragments, respectively (9, 10). The telomere on chromosome IIIL belongs to the T-X class, as shown by hybridization of probe 3* with the X-region clone YRp131A (Fig. 4, lanes 5 and 6) and the lack of homology with the Y′-region clones Yrp131B or pSZ220 (Fig. 4, lanes 4 and 7). The relatively short telomeric repeat length on the chromosome IIIL end (1.8 kb ±200 bp) confirms the T-X classification of the telomere.

Chromosome IIIL terminal fragment is homologous exclusively with chromosome ends. To estimate the distances

a

Yeast High MW DNA

Bal31

SalI

S S S S

Isolate 3-4 Kb fragments

H S

2☆ R

M13mp9RF

Ligate & Transform

Probe 2☆

2☆ R R 3✪

MTLB6411

Subclone 3✪

R 2☆R 3✪

MTLB6S12

b

```
                    20                  40                  60                  80                  100                 120
5' CCCACACACCACACCCACACCACACCCACACACCACACACACCACACCCACACACCCACACCACACCACACCCACACCACACCCACACACCCACACCCACACACCACACCCACACACACC
3' GGGTGTGTGGTGTGGGTGTGGTGTGGGTGTGTGGTGTGTGTGGTGTGGGTGTGTGGGTGTGGTGTGGTGTGGGTGTGGTGTGGGTG.GTGGGTGTGGGTGTGTGGTGTGGGTGTGTGTGG

                    140                 160                 180                 200                 220                 240
ACACCCACACACACCACACCCACACACCACACCCACACACACACCACACCCACACACACCACACCACACCCACACCACACCCACACCCACACACCACACCACACCACACCCACACCACAC
TGTGGGTGTGTGTGGTGTGGGTGTGTGGTGTGGGTGTGTGTGTGGTGTGGGTGTGTGTGGTGTGGTGTGGGTGTGGTGTGGGTGTGGGTGTGTGGTGTGGTGTGGTGTGGGTGTGGTGTG

                    260                 280                 300                 320                 340                 360
ACCACACCCACACACACCACACCACACACCACACCACACCCACACACCACACCCACACACCCACACCCACACACACCACACCCACACCACACCCACACCCACACACCCACACCCTAAC
TGGTGTGGGTGTGTGTGGTGTGGTGTGTGGTGTGGTGTGGGTGTGTGGTGTGGGTGTGTGGGTGTGGGTGTGTGTGGTGTGGGTGTGGTGTGGGTGTGGGTGTGTGGGTGTGGGATTG

                    380                 400                 420                 440                 460                 480
ACTACCCTAACACTACCCTATTCTAACCCTGATTTTACCTGTCTCCCAACTTACTCTCCATTACCCTACCTCTCCACTCGTTACCCTGTCTCATTCAACCGTACCACTCCCAACCACCAT
TGATGGGATTGTGATGGGATAAGATTGGGACTAAAATGGACAGAGGGTTGAATGAGAGGTAATGGGATGGAGAGGTGAGCAATGGGACAGAGTAAGTTGGCATGGTGAGGGTTGGTGGTA

                    500                 520                 540                 560                 580                 600
CCATCTCTCTACTTACTACCACCAACCCACCGTCCACCATAACCGTTACCCTCCAACTACCCGTATCCAACTCCACTACCGCTTACCCTACCATCGACCATGTCCTACTCACTGTACTGT
GGTAGAGAGATGAATGATGGTGGTTGGGTGGCAGGTGGTATTGGCAATGGGAGGTTGATGGGCATAGGTTGAGGTGATGGCGAATGGGATGGTAGCTGGTACAGGATGAGTGACATGACA

                    620                 640                 660                 680                 700                 720
TGTTCACCCCACCATATTGAAACGTCTACAAATGATCGTAAATAATACACATATACTTATCCTACCACTCTAATCCCACTACCACATGCCATACTCACCTTCACTTGTATTCTGATCGGTC
ACAAGTGGGGTGGTATAACTTTGCAGATGTTTACTAGCATTTATTATGTGTATATGAATAGGATGGTGAGATTAGGGTGATGGTGTACGGTATGAGTGGAAGTGAACATAAGACTAGCCAG

                    740                 760                 780                 800                 820                 840
ATACGCACACGGATGCTACAGTATATACCATCTCAAACTTACCTACTTTCATATTCATCATCACCCATCTCTCCCATCATCAGTACCAATGCACTCGCATCATTATGCACGGCTTGCCTCAG
TATGCGTGTGCCTACGATGTCATATATGGTAGAGTTTGAATGGATGAAAGTATAAGTAGTAGTGGGTAGAGAGTGGTAGTCATGGTTACGTGAGCGTAGTAATACGTGCCGAACGGAGTC

                    860                 880                 900                 920                 940                 960
CGGTCTATACCCTGTGCCATTTACGCATAACGCCCATCATTATCCACATTTTAATATCTATATCTCATTCGGCGACACCAAATATTGTATAACTGCCCTTAATACATACGTTATACCACT
GCCAGATATGGGACACGGTAAATGCGTATTGCGGGTAGTAATAGGTGTAAAATTATAGATATAGAGTAAGCCGCTGTGGTTTATAACATATTGACGGGAATTATGTATGCAATATGGTGA

                    980                 1000                1020                1040                1060                1080
TTTACACCATATACTAACCACTCAATTTATACACACTTATGTCAATATAACCACAAAATCACCACTAAAATCACCTAAACATAAAAATATTCTACTCTTCAACAATAATACATAAACACA
AAATGTGGTATATGATTGGTGAGTTAAATATGTGTGAATACAGTTATATTGGTGTTTTAGTGGTGATTTTAGTGGATTTGTATTTTTATAAGATGAGAAGTTGTTATTATGTATTTGTGT

                    1100                1120                1140                1160                1180                1200
CTCAATTGCGTATCTATACCACCATGACGTCATTAACGTAAAAGTTCCTTAATATTGCAAATTGCTTGAACGGATGCCATTTCATGTTGAATGTGGTAACCCAATAGCATGATATGAGTA
GAGTTAACGCATAGATATGGTGGTACTGCAGTAATTGCATTTTCAAGGAATTATAACGTTTAACGAACTTGCCTACGGTAAAGTACAACTTACACCATTGGGTTATCGTACTATACTCAT

                    1220                1240                1260                1280                1300                1320
ATGCTTTAGTATTGTTTCAGAGTTGTTTCAGTAATGTTTTAGACAAGGAGAACATATAGTAGCAAACCTCTAATCCGGTAGTACTTAAGAAACTACAGTTTCTATGTACGAAAGCAGTAA
TACGAAATCATAACAAAGTCTCAACAAAGTCATTACAAAATCTGTTCCTCTTGTATATCATCGTTTGGAGATTAGGCCATCATGAATTCTTTGATGTCAAAGATACATGCTTTCGTCATT

                    1340                1360                1380                1400                1420                1440
CTATGTAATTATTACATTTACATGACATATAGGAAGGTCCAATAAACTTACTACATTATGACCTATAAGCTAGATCGTAATTCATTACGTCAACAGGTTATGAGCCCTAGAGCAATGCTT 3'
GATACATTAATAATGTAAATGTACTGTATATCCTTCCAGGTTATTTGAATGATGTAATACTGGATATTCGATCTAGCATTAAGTAATGCAGTTGTCCAATACTCGGGATCTCGTTACGAA 5'

               1455           1470           1485           1500           1515           1530
CTGAGAACATAATCAATCTATCTAGCCCCAACAATTATAAACAGTGGCTGTACGGTATCGAGACCGCTGCTGAATATGCTAACGAATATATGAACGAAT
GACTCTTGTATTAGTTAGATAGATCGGGGTTGTTAATATTTGTCACCGACATGCCATAGCTCTGGCGACGACTTATACGATTGCTTATATACTTGCTTA
```

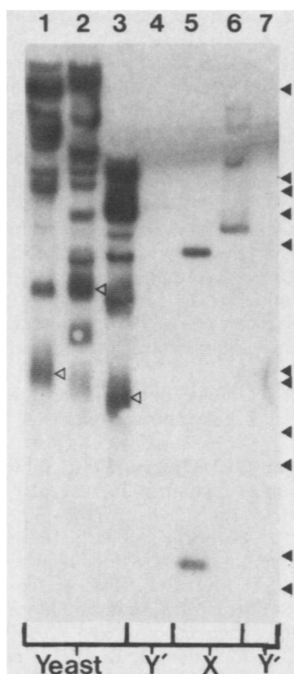

FIG. 4. Southern analysis of yeast genomic DNA and *ARS* clone restriction digests with the chromosome IIIL telomeric probe 3*. Lanes: 1, AB20α XP8-10B-*Bam*HI (4 μg); 2, AB20α XP8-10B-*Pvu*II (4 μg); 3, AB20α XP8-10B-*Hin*dIII (4 μg); 4, Yrp131B-*Sal*I-*Nco*I (0.12 μg); 5, Yrp131A-*Sal*I-*Nco*I (0.12 μg); 6, Yrp131A-*Sal*I-*Hin*dIII (0.12 μg); 7, pSZ220-*Alu*I (0.25 μg). Clones Yrp131B and pSZ220 contain a Y' region, and clone Yrp131A has an X-region insert (9, 10). The chromosome IIIL end fragment positions in lanes 1 to 3 (△) are indicated. The posiitions of λ-*Hin*dIII-*Eco*RI fragments (▲) are indicated: 21.5, 5.1, 5.0, 4.4, 3.6, 2.0, 1.9, 1.7, 1.3, 1.0, and 0.9 kb.

between the probe 3* homologous regions and the respective chromosome ends, we used progressive BAL 31 nuclease digestion and Southern analysis with probe 3* (Fig. 2c). Only a subset of *Sal*I fragments was shortened, whereas all *Pvu*II fragments were BAL 31 nuclease sensitive. Given that BAL 31 nuclease initiates digestion at the terminus of the chromosome in high-molecular-weight DNA (2, 5, 12, 26, 28, 29, 31), all *Pvu*II bands must include the chromosome end. The difference between *Sal*I and *Pvu*II sensitivity patterns with BAL 31 nuclease is a result of restriction map differences for these enzymes in the type X *ARS* distal region. In the Y'- and X-region representative clones (9, 10), there are no *Pvu*II sites in the Y' region or the *ARS* distal portion of the X region. Conversely, *Sal*I sites separate the Y' and X regions in all isolated clones. As a result, with probe 3* telomeres of the T-Y'-X class should display BAL 31 nuclease-sensitive bands with *Pvu*II-digested DNA, while *Sal*I-digested DNA bands homologous with probe 3* would be insensitive, at least until the T region and the 6.7-kb Y' repeat region are degraded with BAL 31 nuclease. Alternatively, chromosome ends belonging to the T-X class should show BAL 31 nuclease sensitivity with both *Pvu*II- and *Sal*I-digested DNA, assuming the restriction map in the X region, distal to *ARS*, agrees with those previously isolated (9, 10) (i.e., there are no *Pvu*II or *Sal*I sites in this region). Assuming all telomeres of the T-Y'-X class are at least 7-kb long (Fig. 1a), the *Pvu*II bands homologous with the chromosome IIIL X region (probe 3*) and shorter than 7 kb must represent telomeres of the T-X class. Based on this reasoning, along with the proportion of *Sal*I bands that display BAL 31 nuclease sensitivty, we estimate that at least 30% of the chromosome ends in the haploid yeast genome belong to the T-X class.

Nucleotide sequence of the chromosome IIIL telomere. To establish the T-region length and the position of the *ARS* consensus sequence within the X region, to search for repeat sequences within the X region, and to compare the telomeric sequence with that reported for a Y'-containing telomere, we determined the complete nucleotide sequence of the probe 3* fragment (1,539 bp). The terminal fragment contained 356 bp of 5'-C_{1-3}A-3' repeat units, or 53 repeats of the more defined 5'-$C_{2-3}A(CA)_{1-4}$-3' unit (Fig. 3b). A 80-nucleotide-repeat region from a Y'-containing telomere previously sequenced (26) has the same repeat unit, except a maximum of three tandem CA dinucleotides exists in these repeats. The four tandem dinucleotides observed at the chromosome IIIL end are present in only one repeat unit, nucleotides 152 to 163 (Fig. 3b). Telomeres are presumed to have the simple repeat region extending to the chromosomal teminus (3–6, 26, 28, 29). A section of 5'-C_{1-3}A-3' repeats adjacent to the telomere-associated *ARS* region is sufficient for telomere function in yeast (13, 26; L. L. Button and C. R. Astell, manuscript in preparation), which supports the assumption that the chromosome IIIL teminus removed with BAL 31 nuclease during the cloning process is additional 5'-C_{1-3}A-3' repeat sequences. Adjacent to this T region were X *ARS*-region sequences (Fig. 3b); the 260 bp adjacent to the T region is 80 to 90% homologous to that portion of X regions sequenced (30). An *ARS* consensus sequence (7, 8, 17), 5'-TTTTATGTTTTA-3', is located at nucleotides 1036 to 1048 in the cloned end, or about 1.2 kb from the intact chromosome IIIL end. The *ARS* consensus in the sequenced Y' region (26) is 700 bp from the chromosome end and in the opposite orientation with respect to the 5'-C_{1-3}A-3' repeat DNA strand from that on the chromosome IIIL end; however, the orientation effect on *ARS* activity has not been reported. The *ARS*-flanking regions do participate in *ARS* function but vary to a large extent between different *ARS* regions (7, 8, 17). Consequently, the strengths of X and Y' *ARS* regions cannot be compared by DNA sequence analyses; functionality tests are essential. Nucleotide sequence

FIG. 3. Isolation and DNA sequence of the chromosome IIIL terminal fragment. (a) High-molecular-weight (MW) yeast DNA was BAL 31 nuclease and DNA *Pol*I Klenow fragment treated to yield clonable, blunt chromosome ends. After *Sal*I digestion, fragments encompassing the 3.3-kb (±200 bp) *Sal*I-chromosome IIIL end fragment were cloned into the M13mp9RF-*Sal*I-*Sma*I vector (22). Probe 2* (2☆) served to identify the telomeric clone MTLB6411, which contained a 3-kb *Sal*I fragment corresponding to the chromosome IIIL end lacking the terminal 0.3 kb (±200 bp) due to the BAL 31 nuclease treatment. MTLB6411 was *Eco*RI digested, the ends were rendered blunt ended with DNA *Pol*I Klenow fragment, and the probe 3* (3☆), 1.5-kb fragment was cloned in the *Sma*I site of M13mp18RF (32). Abbreviations: H, *Hin*dIII; S, SalI; R, *Eco*RI; Z, *Sma*I; B, *Bam*HI; P, *Pst*I. (b) The entire DNA sequence for the forward and reverse strands of the cloned chromosome IIIL telomeric region determined from the sets of exonuclease III-S1 nuclease deletion clones prepared from the MTLB6S12 and MTLB6S21 probe 3* subclones (14). A perfect *ARS* consensus sequence (7, 8, 17), is underlined (▬) at positions 1036 to 1048. The T-region repeat units are described by the formula 5'-$C_{2-3}A(CA)_{1-4}$, and the single $C_3A(CA)_4$ block is at nucleotides 152 to 163.

studies with the Y'-containing clone pSZ220 (15, 27) revealed a region of tandem direct repeats which were proposed to play a role in telomeric replication, maintenance, and homogenization of telomeric sequences. No direct repeat region is apparent in the X region on chromosome IIIL, and the lack of G residues in the $5'$-$C_{1-3}A$-$3'$-containing strand is its only distinguishing feature.

In summary, we have isolated the telomeric region from a specific yeast chromosome and have shown that it conforms to the structure determined for a yeast DNA fragment that functions as a yeast telomere (9, 26, 27). We have shown that the telomeric region of IIIL contains an X *ARS* region but lacks a class Y' *ARS* region and hence is the first reported isolation of a yeast telomeric region of the type T-X. The T-X telomeric probe hybridized with multiple genomic fragments, located exclusively at chromosome ends. Other properties of our cloned chromosome IIIL telomere are that the length varies by ±200 bp within a given yeast strain, and the average chromosome IIIL end length varies among different yeast strains from that observed for AB20α XP8-10B by ±200 bp (data not shown). Presumably this results from variable average amounts of $5'$-$C_{1-3}A$-$3'$ repeat sequences, as previously described with a T-region probe (29). Finally, the telomeric fragment from chromosome IIIL has been shown to function as a T-X telomere on a linear vector in yeast (Button and Astell, in preparation).

We thank A. Klar for yeast strains, J. W. Szostak for telomeric clone pSZ220, and C. Chan for the *ARS* clones. We also thank T. Snutch and I. Kovesdi for assistance in construction of the λCh4A genomic library.

This work was supported by grants from the Medical Research Council of Canada and the British Columbia Health Care Research Foundation. C.R.A. L.L.B. is a recipient of a Medical Research Council of Canada studentship.

LITERATURE CITED

1. **Benton, W. D., and R. W. Davis.** 1977. Screening lambda gt recombinant clones by hybridization to single plaques in situ. Science **196**:180–182.
2. **Bernards, A., P. A. M. Michels, C. R. Lincke, and P. Borst.** 1983. Growth of chromosome ends in multiplying trypanosomes. Nature (London) **303**:592–597.
3. **Blackburn, E. H.** 1984. Telomeres: do the ends justify the means? Cell **37**:7–8.
4. **Blackburn, E. H., M. L. Budarf, P. B. Challoner, J. M. Cherry, E. A. Howard, A. L. Katzen, W. C. Pan, and T. Ryan.** 1983. DNA termini in ciliate macronuclei. Cold Spring Harbor Symp. Quant. Biol. **47**:1195–1207.
5. **Blackburn, E. H., and P. B. Challoner** 1984. Identification of a telomeric DNA sequence in *Trypanosoma brucei*. Cell **36**:447–457.
6. **Blackburn, E. H., and J. W. Szostak.** 1984. The molecular structure of centromeres and telomeres. Annu. Rev. Biochem. **53**:163–194.
7. **Broach, J. R., Y.-Y. Li, J. Feldman, M. Jayaram, J. Abraham, K. A. Nasmyth, and J. B. Hicks.** 1983. Localization and sequence analysis of yeast origins of DNA replication. Cold Spring Harbor Symp. Quant. Biol. **47**:1165–1173.
8. **Celniker, S. E., K. Sweder, F. Srienc, J. E. Bailey, and J. L. Campbell.** 1984. Deletion mutations affecting autonomously replicating sequence *ARS1* of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:2455–2466.
9. **Chan, C. S. M., and B.-K. Tye.** 1983. A family of *Saccharomyces cerevisiae* repetitive autonomously replicating sequences that have very similar genomic environments. J. Mol. Biol. **168**:505–523.
10. **Chan, C. S. M., and B.-K. Tye.** 1983. Organization of DNA sequences and replication origins at yeast telomeres. Cell **33**:565–573.
11. **Chinault, A. C., and J. Carbon.** 1979. Overlap hybridization screening: isolation and characterization of overlapping DNA fragments surrounding the *leu2* gene on yeast chromosome III. Gene **5**:111–126.
12. **DeLange, T., and P. Borst.** 1982. Genomic enviroment of the expression-linked extra copies of genes for surface antigens of *Trypanosoma brucei* resembles the end of a chromosome. Nature (London) **299**:451–453.
13. **Dunn, B., P. Szauter, M. L. Pardue, and J. W. Szostak.** 1984. Transfer of yeast telomeres to linear plasmids by recombination. Cell **390**:191–201.
14. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted break points for DNA sequencing. Gene **28**:351–359.
15. **Horowitz, H., and J. E. Haber.** 1984. Subtelomeric regions of yeast chromosomes contain a 36-base pair tandemly repeated sequence. Nucleic Acids Res. **12**:7105–7121.
16. **Horowitz, H., P. Thorburn, and J. E. Haber.** 1984. Rearrangements of highly polymorphic regions near telomeres of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:2509–2517.
17. **Kearsey, S.** 1984. Structural requirements for the function of a yeast chromosomal replicator. Cell **37**:299–307.
18. **Klar, A. J. S., J. McIndoo, J. B. Hicks, and J. N. Strathern.** 1980. Precise mapping of the homothallism genes *HML* and *HMR* in *Saccharomyces cerevisiae*. Genetics **96**:315–320.
19. **Klar, A. J. S., J. N. Strathern, J. B. Hicks, and D. Prudente.** 1983. Efficient production of a ring derivative of chromosome III by the mating-type switching mechanism in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **3**:803–810.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Maniatis, T., R. C. Hardison, E. Lacy, J. Lauer, C. O'Connell, and D. Quon.** 1978. The isolation of structural genes from libraries of eucaryotic DNA. Cell **15**:687–701.
22. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–79.
23. **Mortimer, R. K., and D. Schild.** 1980. Genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44**:519–571.
24. **Nasmyth, K. A., and K. Tatchell.** 1980. The structure of transposable yeast mating type loci. Cell **19**:753–764.
25. **Seed, B.** 1983. Purification of genomic sequences from bacteriophage libraries by recombination and selection *in vivo*. Nucleic Acids Res. **11**:2427–2445.
26. **Shampay, J., J. W. Szostak, and E. H. Blackburn.** 1984. DNA sequences of telomeres maintained in yeast. Nature (London) **310**:154–157.
27. **Szostak, J. W., and E. H. Blackburn.** 1982. Cloning of yeast telomeres on linear plasmid vectors. Cell **29**:245–255.
28. **VanderPloeg, L. H. T., A. Y. C. Liu, and P. Borst.** 1984. Structure of the growing telomeres of trypanosomes. Cell **36**:459–468.
29. **Walmsley, R. M., and T. D. Petes.** 1985. Genetic control of chromosome length in yeast. Proc. Natl. Acad. Sci. USA **82**:506–510.
30. **Walmsley, R. W., C. S. M. Chan, B.-K. Tye, and T. D. Petes.** 1984. Unusual DNA sequences associated with the ends of yeast chromosomes. Nature (London) **310**:157–160.
31. **Williams, R. O., J. R. Young, and P. A. O. Majiwa.** 1982. Genomic enviroment of *T. Brucei* VSG genes: presence of a minichromosome. Nature (London) **299**:417–421.
32. **Yanisch-Perron, C., J. Viera, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequence of the M13mp18 and pUC19 vectors. Gene **33**:103–119.