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Distribution of Telomere-Associated Sequences on Natural Chromosomes in *Saccharomyces cerevisiae*

VIRGINIA A. ZAKIAN* AND HARRIET M. BLANTON

Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104

Received 22 October 1987/Accepted 22 February 1988

Pulsed-field gel electrophoresis was used to examine the distribution of telomere-associated sequences on individual chromosomes in four strains of *Saccharomyces cerevisiae*. The pattern of X and Y′ distribution was different for each strain. At least one chromosome in each strain lacked Y′, and in some strains, chromosome I, the smallest yeast chromosome, lacked detectable amounts of both X and Y′.

A common theme is emerging for the structure of telomeric regions. The ends of chromosomes bear multiple numbers of tandem repeats of simple, satellitelike DNA (2). In addition to the simple sequences found at chromosome ends, regions adjacent to telomeres often carry long stretches of middle repetitive DNA (1, 10, 13, 15, 18, 24).

In *Saccharomyces cerevisiae*, chromosomes end in 200 to 600 base pairs of the irregular sequence $C_{1-3}A$ (17, 23; Fig. 1). In addition, two middle repetitive elements, called X and Y′, are found near DNA termini (8, 9). Y′ is a highly conserved element of 6.7 kilobases (kb) (8, 9). X, a less-conserved element than Y′, ranges in size from 0.3 to 3.75 kb and is found centromere proximal to Y′ (8, 9). Internal tracts of $C_{1-3}A$ repeats, as well as putative origins of DNA replication (autonomously replicating sequences) are found in association with X and Y′ (7, 21). These properties are consistent with telomere-associated sequences having roles in replication, recombination, or healing of telomeric regions.

Gel systems have been developed that allow separation of intact yeast chromosomal DNA molecules (4, 16). The behavior of each chromosome in one system (orthogonal field alternation gel electrophoresis [OFAGE]) has been documented for strains YNN281, A364a, DCO4, and AB972 (5). DNA was prepared from each of these strains by modifications (5) of the gel insert method (16) and subjected to OFAGE. The DNA was transferred to nitrocellulose and hybridized (20) to X- and Y′-specific probes (Fig. 2). An X-specific probe was prepared from YRp120 (9) by agarose gel isolation of a 1.7-kb *Nco*I fragment. A Y′-specific probe was prepared from YRp131b (9) by isolation of a 1.7-kb *Bgl*II fragment that was subcloned into *Bam*HI-digested M13 mp18. A 125-base-pair *Hae*III-*Mnl*I fragment containing an 82-base-pair tract of $C_{1-3}A$ repeats was excised from pYt103 (17). Hybridization probes were derived from regions of X and Y′ reported to be free of $C_{1-3}A$ repeats. This point was verified by the fact that bonafide $C_{1-3}A$ DNA derived from pYt103 did not hybridize to either the X or Y′ probe. The region of X chosen for the probe is conserved among different X elements (8, 9).

The data presented in Table 1 were compiled from 17 different gels run at switching intervals ranging from 20 to 80 s. The pattern of X and Y′ distribution was different in each strain (Fig. 2 and 3). At least one of the three smallest chromosomes in each strain did not hybridize to the Y′ probe, and in three strains, two of the five smallest chromosomes did not hybridize to Y′ (Fig. 2). For each strain, all of the bands seen in the ethidium bromide-stained profile of the gel that are derived from larger chromosomes hybridized to Y′ (Fig. 3; also see reference 25).

Most of the chromosomal bands in the four strains hybridized to the X probe (Fig. 3). However, the extent of hybridization varied widely for different chromosomes. The X panel in Fig. 2A was printed at an exposure chosen to emphasize some of these differences. For example, chromosome VI (band 2) from AB972 hybridized much more strongly to X than chromosome VI from strains YNN281 or DCO4. In addition, chromosome I in two strains (YNN281 and AB972) typically showed no hybridization to X (Fig. 2 and 3, rightmost gels). After removal of the X probe, subsequent hybridization of these gels with a sequence found in single copy on chromosome I (λPM4237; 4) demonstrated that chromosome I DNA from all strains was still readily detectable on the filter.

These data demonstrate that at least one of the three smallest chromosomes in every strain lacked Y′ on both telomeres. Previously it was inferred from hybridization data that some telomeres lack Y′ (12, 22), and direct analysis showed that the left arm of chromosome III in strain AB20α XP8-10B lacks Y′ (3). No chromosome larger than ~580 kb (size of chromosome VIII; 4) was found to lack Y′ (Table 1). Of course, when two chromosomes comigrate as a single band, it is not possible to know if both chromosomes in the band contain Y′ (or X).

Although most chromosomes hybridized to X, the amount of homology to X per chromosome varied widely (Fig. 2). Moreover, chromosome I in two strains lacked detectable amounts of X. This result was somewhat unexpected, in that

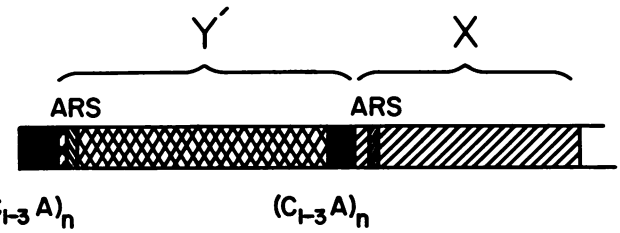

FIG. 1. Structure of DNAs. The structure of yeast telomeres is based on the findings of Chan and Tye (9) and Walmsley et al. (21). As drawn, the end of the chromosome is towards the left and the centromere is towards the right. The number of Y′ elements can vary from zero to four (9). As we show here, not all chromosomes carry X.

* Corresponding author.

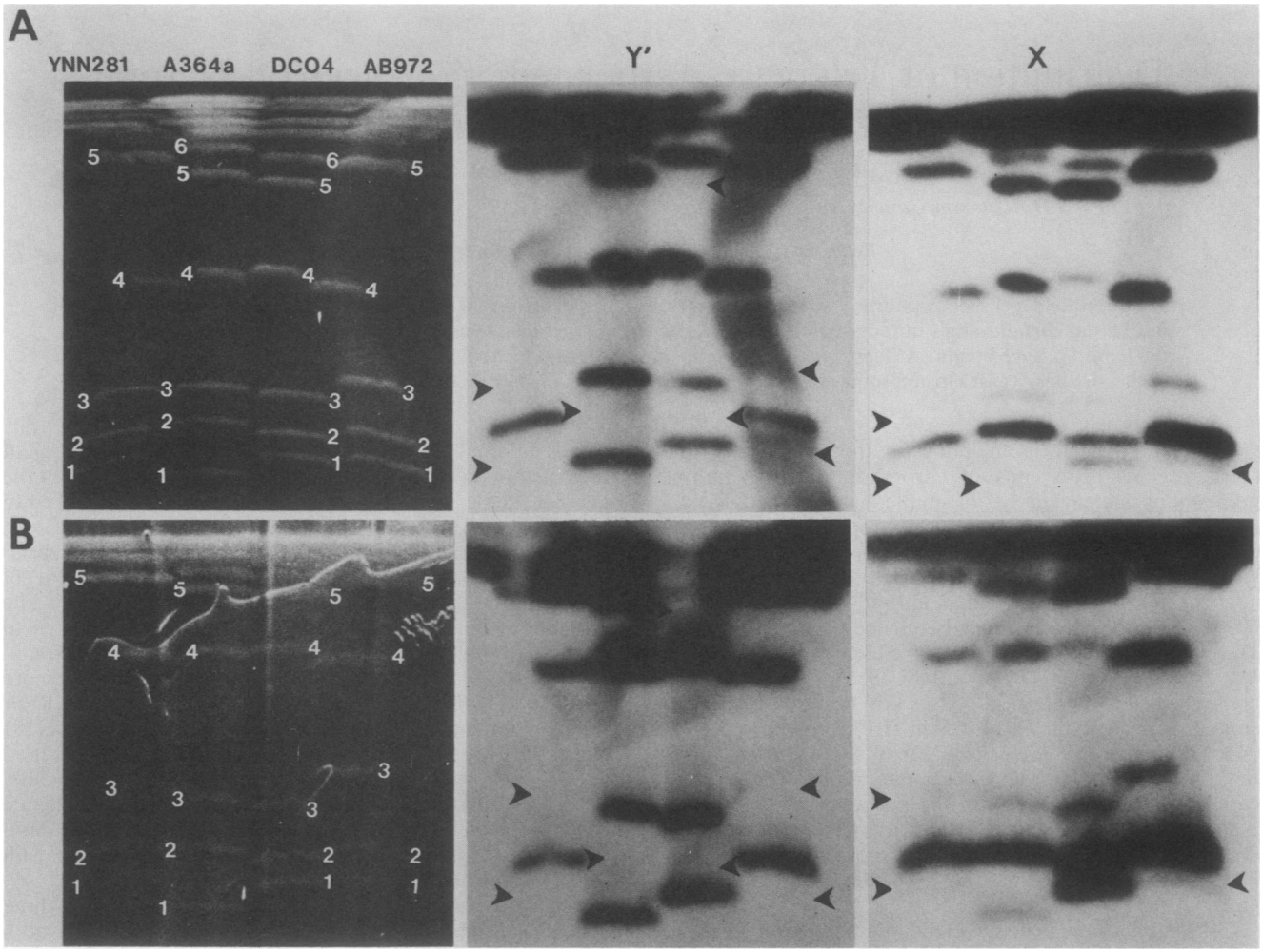

FIG. 2. Distribution of X and Y′ on the smaller yeast chromosomes. DNAs were prepared from yeast strains YNN281, A364a, DCO4, and AB972 and subjected to OFAGE in 1.5% agarose at 300 V for 20 to 22 h, with switching intervals of 30 (A) or 20 s (B). After electrophoresis, the gel was stained with ethidium bromide (left gels in panels A and B). The identities of chromosomes in each band (Table 1) are taken from Carle and Olson (5) and were confirmed for many of the bands in our laboratory. DNAs were transferred to nitrocellulose, and the blots were probed with Y′ (middle gels) or X (right gels). Ethidium bromide-staining bands that show little or no hybridization with X or Y′ are indicated by arrowheads.

TABLE 1. Pattern of X and Y′ distribution in four yeast strains

Band no.[a]	YNN281			A346A			DCO4			AB972		
	Chromosome[b]	Hybridization[c]		Chromosome	Hybridization		Chromosome	Hybridization		Chromosome	Hybridization	
		Y′	X		Y′	X		Y′	X		Y′	X
1	I	−	−	I	+	(+)	I	+	++	I	−	−
2	VI	+	++	VI	−	++	VI	−	++	VI	+	++
3	III	−	(+)	III	+	+	III	+	+	III	−	+
4	IX	+	+	IX	+	++	IX	+	+	IX	+	++
5	V, VIII	+	+	VIII	+	+	VIII	−	++	VIII, V	+	++
6	XI	(+)	+	V	+	+	V	+	+	XI	(+)	+
7	X	+	+	XI	+	(+)	XI	+	+	X	+	+
8	XIV	+	+	X	+	+	XIV, X	+	+	XIV	+	+
9	II	+	+	XIV, II	+	+	II	+	+	II	+	+
10	XIII	+	+	XIII, XVI	+	+	XIII, XVI	+	+	XIII, XVI	+	+
11	XVI	+	(+)	VII, XV	+	+	XV	+	+	VII, XV	+	+
12	VII, XV	+	+	IV	+	+	VII	+	+	IV	+	+
13	IV	+	+				IV	+	+			

[a] Bands refer to the ethidium bromide-stained profile and are numbered from the fastest (1) to the slowest (12 or 13) migrating bands.

[b] Assignments of bands to specific chromosomes are based on the findings of Carle and Olson (5) and were confirmed in our laboratory for the smallest chromosomes by hybridization to chromosome-specific probes (data not shown). The behavior of chromosome XII was not analyzed in this study.

[c] Hybridization to Y′- or X-specific probes. Symbols: −, no hybridization; (+), weak hybridization; +, strong hybridization; ++, very strong hybridization.

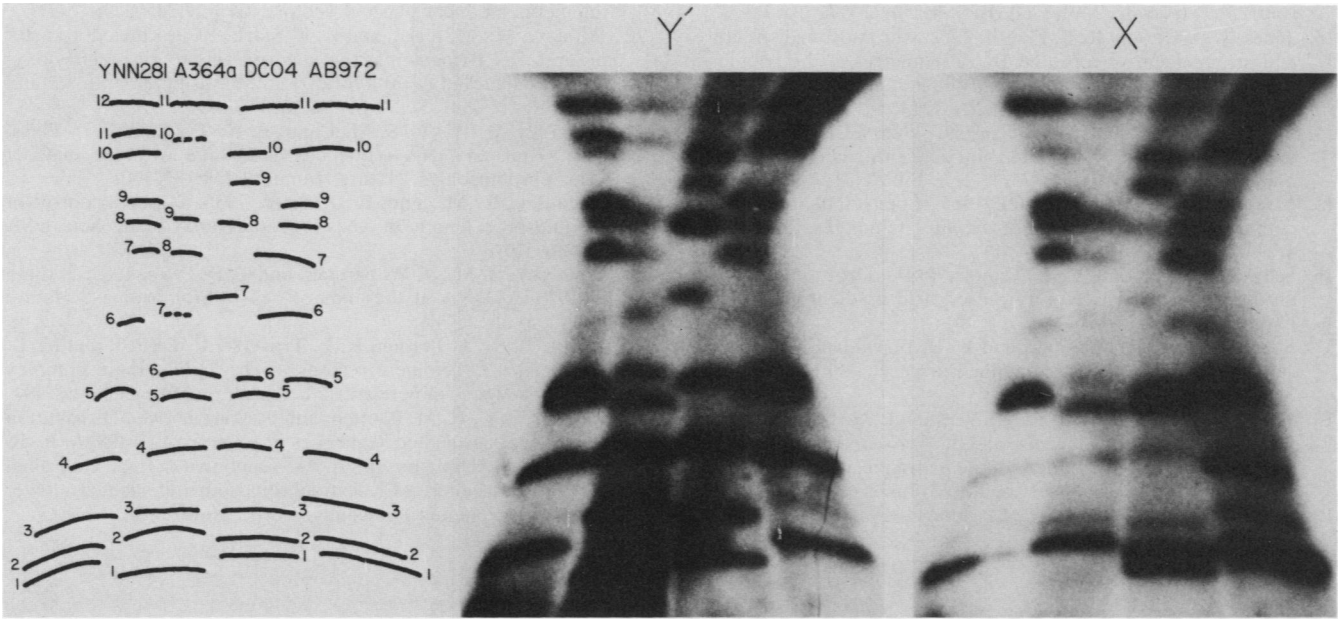

FIG. 3. Distribution of X and Y′ on yeast chromosomes. DNAs, agarose gels, and hybridizations are as described in the legend to Fig. 2, except that the switching interval was 40 s and electrophoresis was carried out for 25 h. The leftmost panel presents a tracing of band position, as revealed by the ethidium bromide-stained profile of the gel. Bands 7 and 10 in strains A364a (indicated by dashed lines) were underrepresented in the ethidium bromide-stained profile and hence in the hybridization profiles. Some bands (band 11, YNN281; bands 7 and 10, A364a) that display little, if any, hybridization to the X probe in this gel are clearly positive in other gels (see, for example, reference 25).

all telomeres examined previously were found to carry X (3, 6, 9, 19). A caveat to the conclusion that some yeast telomeres lack X is that X is a less well conserved element than Y′ (8, 9). Thus, chromosomes that do not hybridize to the probe may bear a highly diverged copy of X that has little homology to our probe. If so, a highly diverged copy of X must be present at both telomeres. In some gels, hybridization of X to chromosome I in strains YNN281 and AB972 can be detected after very long exposures of the autoradiogram. Although this fact is consistent with the presence of a highly diverged copy of X on these chromosomes, it is most likely due to nonspecific hybridization. Alternatively, it may reflect the acquisition of X on chromosome I by recombination (11) in a small subset of the cells in the culture.

Our data clearly demonstrate that Y′ is not universal on natural yeast chromosomes and suggest that in some strains chromosome I, the smallest yeast chromosome, lacks both X and Y′. These data are consistent with another study, in which a deletion derivative of chromosome III that lacks X and Y′ was shown to have a mitotic stability indistinguishable from that of an authentic chromosome III (14). However, the deletion derivative of chromosome III analyzed in that study carried terminal autonomously replicating sequences derived from *Tetrahymena* ribosomal DNA, which might have supplied a function normally carried out by X or Y′.

The absence of telomere-associated sequences on some natural chromosomes argues that they are unlikely to be important for any aspect of chromosome behavior, at least for the smallest yeast chromosomes. However, it is possible that the presence of X, Y′, or both is important, or even essential, for stable maintenance of large yeast chromosomes, all of which seem to carry both X and Y′.

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