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Many Yeast Chromosomes Lack the Telomere-Specific Y′ Sequence

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Chromosomal DNAs of 26 different strains representing *Saccharomyces* species were analyzed by pulsed-field gel electrophoresis and subsequent hybridization to Y′ telomere DNA. Hybridization to Y′ was found exclusively in *Saccharomyces cerevisiae* strains, and among these strains, Y′ sequences were found to be lacking in small, middle-sized, and large chromosomes.

Telomeres are at the ends of eucaryotic chromosomes. They help prevent degradation at the ends, and they ensure the replication of the ends without loss of information. A function in segregation of chromosomes is also very likely (4). The DNA composition of several telomeres is known. The very ends consist of tandemly repeated short sequences, e.g., C_3TA_2 in human chromosomes (17), C_4A_2 or C_4A_4 in ciliated protozoans (3, 14, 15, 19, 29), and less-regular repeats of $C_{1-3}A$ in *Saccharomyces cerevisiae* (24). This cytosine-rich strand is oriented 5′ to 3′ from the end toward the centromere (2, 10). The complementary guanosine-rich strand is slightly longer, creating 3′ overhanging termini (11, 15, 20).

In *S. cerevisiae*, the total lengths of the short tandem repeats are 200 to 600 base pairs (24, 28). The DNA immediately adjacent to the $C_{1-3}A$ repeats was found to consist of one to four copies of a conserved 6.7-kilobase (kb) sequence called Y′ and a much less conserved sequence called X (7, 8). Both sequences carry information for autonomous replication (16). Some *S. cerevisiae* chromosomes may lack Y′ sequences but still carry X sequences (Fig. 1).

In a recent study, four laboratory strains were examined and the absence of Y′ was found to be restricted to small chromosomes (30). Does this observation imply an important function of Y′ for maintenance of large chromosomes? In the absence of a functional assay for Y′, we tried to find an answer by examining the Y′ distribution in *S. cerevisiae* strains of widely different origins. In addition, we assayed strains representing other *Saccharomyces* species (1). A brief description of the 26 strains analyzed is listed in Table 1.

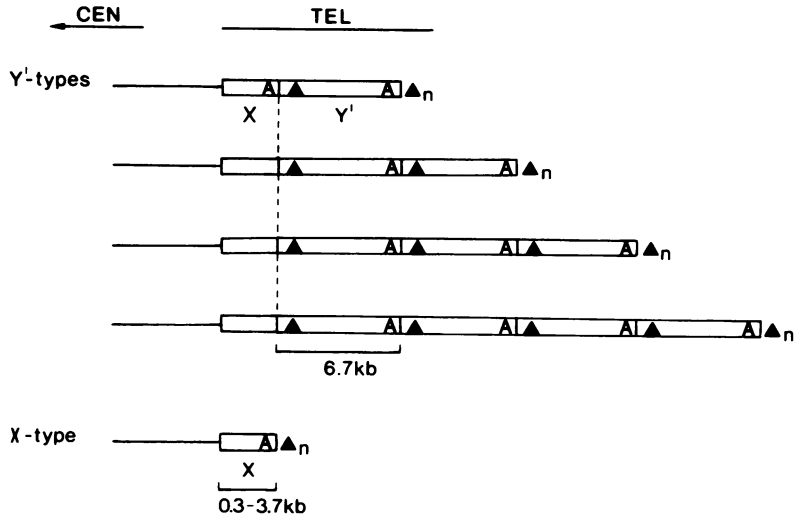

FIG. 1. Structures of *S. cerevisiae* telomeres. The figure is based on the findings of Chan and Tye (7) and Walmsley et al. (27). The telomeres consist either of X and Y′ sequences or of X sequences only, as also described by Button and Astell (5) and Horowitz et al. (12). The end of the chromosome is toward the right, and the centromere is toward the left. Abbreviations: X and Y′, X and Y′ telomere-associated sequences in *S. cerevisiae*; $\blacktriangle_n$, $C_{1-3}A$ repeats at the very ends of the telomeres and at the Y′-X junctions; A, autonomous replicating sequences found in X and Y′.

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TABLE 1. Y′ hybridization pattern of 26 *Saccharomyces* strains

Strain	Description	Source[a]	Our strain no.	No. of bands		No. of Y′-free chromosomes[b]		
				Total[c]	Lacking Y′	Large	Medium	Small
Laboratory								
S. cerevisiae X2180-1B[d]	Haploid	1	23	12	2	0	0	2
S. cerevisiae 702 a	Haploid	2	83	14	3	1	1	1
S. cerevisiae VB2-20A	Haploid	3	187	12	4	0	2	2
S. cerevisiae D7	Diploid; tester strain for mutagens	4	214	13	0	0	0	0
Domesticated								
S. cerevisiae 52/134	Diploid; port wine yeast	5	103	12	3	0	2	1
S. cerevisiae 63/228	Diploid; isolate from grapes	5	104	13	4	1	2	1
S. cerevisiae 81/835	Diploid; wine yeast	5	106	11	0	0	0	0
S. cerevisiae 81/170	Diploid; champagne yeast	5	108	12	1	0	0	1
S. cerevisiae 93/170	Diploid; wine yeast	5	110	11	4	1	3	0
S. cerevisiae CBS 1480	Diploid; wine yeast from Japan	6	138	12	3	1	1	1
S. cerevisiae CBS 4507	Brewery yeast hybrid	6	144	18	2	0	0	2
S. cerevisiae	Brewery yeast hybrid	7	279	20	8	2	5	1
Natural isolates								
S. cerevisiae CBS 1591	Diploid; from cocoa plants, Java	6	140	13	2	0	0	2
S. cerevisiae CBS 3081	Diploid; from soil, Spain	6	143	12	3	1	0	2
S. cerevisiae CBS 6333	Diploid; from banana, Equador	6	145	13	3	1	1	1
S. cerevisiae CBS 459	Diploid; from grape must, Italy	6	150	11	3	1	1	1
S. cerevisiae CBS 2908	Diploid; from soil, South Africa	6	153	14	12	4	3	5
S. cerevisiae	Diploid; from *Drosophila* spp., California	8	155	13	10	1	4	5
S. cerevisiae	Diploid; from *Drosophila* spp., California	8	156	15	9	3	3	3
S. uvarum[e] CBS 395	Diploid; from currant juice, Holland	6	131	15	15	n	n	n
S. uvarum[e]	Diploid; from *Drosophila* spp., California	8	157	14	14	n	n	n
Other								
S. exiguus CBS 397	Described by Hansen, 1888, as new species	6	230	11	11	n	n	n
S. unisporus CBS 398	Described by Jörgensen, 1909, as new species	6	231	10	10	n	n	n
S. dairensis CBS 421	Described by Naganishi, 1917, as new species	6	232	8	8	n	n	n
S. kluyveri CBS 3082	Described by Phaff, 1956, as new species	6	235	5	5	n	n	n
S. servazzii CBS 4911	Described by Capriotti, 1967, as new species	6	236	10	10	n	n	n

[a] 1, Yeast Genetic Stock Center, University of California, Berkeley, Berkeley, Calif.; 2, Collection of H. C. Douglas, University of Seattle, Seattle, Wash., via L. Magni, Milano, Italy; 3, L. Panzeri, University of Milano, Milano, Italy; 4, F. Zimmermann, University of Darmstadt, Darmstadt, Federal Republic of Germany; 5, Australian Wine Research Institute Yeast Culture Collection, Urrbrae, Australia; 6, Centraalbureau voor Schimmelcultures, Delft, Holland; 7, Versuchs- und Lehranstalt für Brauerei, Berlin, Federal Republic of Germany; 8, collection of H. F. Phaff, University of California, Davis, Davis, Calif.

[b] Large, 900 to 2,000 kb; medium, 500 to 900 kb; small, 250 to 500 kb. n, Chromosomes of all sizes lack Y′.

[c] Number of chromosomal bands seen on pulsed-field electrophoresis gels.

[d] X2180-1B most likely isogenic with S288C.

[e] *S. uvarum* is no longer accepted as independent species by Barnett et al. (1). Our data give indications that it may still represent a separate species (see text).

Chromosomal DNAs from all strains were separated by pulsed-field gel electrophoresis (6, 23), blotted to nitrocellulose membranes, and hybridized with a ^{32}P-labeled Y′ probe (25). The probe was derived from pSZ219-5 (26) and consisted of a 1.25-kb *Pvu*I-*Sca*I internal Y′ DNA fragment (18) cloned in pBR322 and labeled with ^{32}P by nick translation (21). The numbers and sizes of chromosomes lacking Y′ sequences are summarized in Table 1. Examples of electrophoretic karyotypes and Y′ hybridizations for five *S. cerevisiae* and two *Saccharomyces uvarum* strains are shown in Fig. 2.

The autoradiograms obtained with the laboratory strains 83 and 187 revealed that Y′ sequences can be absent from

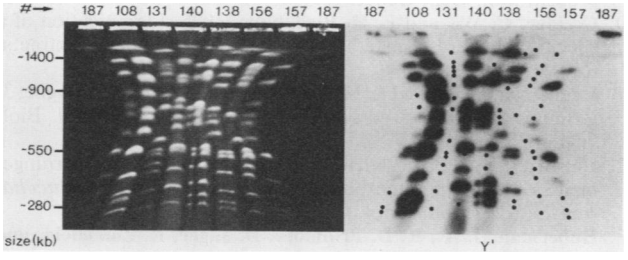

FIG. 2. Distribution of Y′ sequences among different *S. cerevisiae* and *S. uvarum* strains. DNAs were prepared from *S. cerevisiae* 108, 140, 138, 156, and 187 and from *S. uvarum* 131 and 157 essentially as described by Schwartz and Cantor (23). The gel was 1% in agarose and run at 300 V and 9°C for 17 h with a pulse time of 50 s in an apparatus constructed by the method of Carle and Olson (6). DNA was alkali denatured and transferred to a nitrocellulose membrane, and the blot was probed with plasmid pCE-T27 containing a 1.25-kb *Pvu*I-*Sca*I Y′ internal fragment. For reference, the sizes of the following chromosomes of strain 187 are indicated: 1,400 kb, chromosomes VII and XV; 900 kb, chromosome II; 550 kb, chromosomes V and VIII; 250 kb, chromosome I. Chromosomes that lack Y′ are labeled with a dot. #, Strain number.

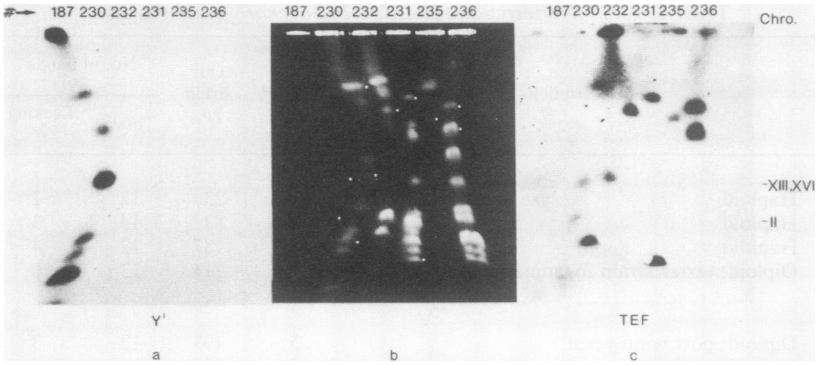

FIG. 3. Pulsed-field gel electrophoresis and hybridization analysis of the following *Saccharomyces* species; *S. exiguus* 230, *S. unisporus* 231, *S. dairensis* 232, *S. kluyveri* 235, *S. servazzi* 236, and *S. cerevisiae* 187. The conditions for DNA preparation and pulsed-field gel electrophoresis run were as described in the text. (a) Autoradiogram of hybridization with Y′ probe pCE-T27; (b) ethidium bromide-stained gel corresponding to the autoradiograms shown in panels a and c. White dots identify the chromosomes carrying *TEF* genes. For both hybridizations, the same blot was used. Instead of stripping the blot from the first probe, we waited until the ^{32}P label of the first probe (Y′) caused no more signals on X-ray film. After this, hybridization with the second probe (*TEF*) was performed. (c) Autoradiogram of hybridization with a 0.86-kb *Eco*RI-*Hin*dIII *TEF* probe isolated from plasmid pFS6 (22). The two *S. cerevisiae* chromosomes (XVI and II) known to carry the *TEF* genes are indicated on the right. The results obtained are in agreement with those of *TEF* hybridizations to whole-genome *Eco*RI digests of these *Saccharomyces* strains when signal intensity and copy numbers per genome are considered (22; F. Schirmaier, Ph.D. thesis, Biocenter, University of Basel, Basel, Switzerland, 1986). #, Strain number.

small (250- to 500-kb), middle-sized (500- to 900-kb), and large (900- to 2,000-kb) *S. cerevisiae* chromosomes. The same conclusions can be drawn from examinations of domesticated *S. cerevisiae* or *S. cerevisiae* isolated from different natural habitats. Within these two groups, there are several strains in which only a minority of chromosomes carry Y′ sequences, e.g., strains 153, 155, and 156. The genomes of these strains were independently analyzed with 10 different DNA probes from the widely used *S. cerevisiae* laboratory strain S288C. For all probes, hybridization signals were obtained with high conservation of restriction sites in strain 156 and low conservation in strains 153 and 155 (A. Stotz and P. Philippsen, unpublished data). We conclude from these data that the Y′ sequence is most likely not necessary in *cis* for the genetic stability of any of the *S. cerevisiae* chromosomes.

The complete lack of Y′ sequences in the genomes of strains 131 and 157 (Fig. 2) seems to confirm this conclusion because 131, originally the type strain of *S. uvarum*, was classified as *S. cerevisiae* in a recent revision of yeast taxonomy (1). However, strain 131 has barely detectable sequence homology to several *S. cerevisiae* genes (9, 13; Stotz and Philippsen, unpublished observations) and yields infertile hybrids when crossed with *S. cerevisiae* (D. Hawthorne, personal communication). Therefore, its new classification as *S. cerevisiae* is questionable, and it may still represent an independent *Saccharomyces* species.

Strains representing the accepted species of the genus *Saccharomyces* were also analyzed. They all lack Y′ sequences, as shown in the autoradiogram of Fig. 3a. Control hybridizations with the conserved *TEF* gene of *S. cerevisiae* (22) revealed substantial homology to all *Saccharomyces* genomes (Fig. 3c). Y′ is also a conserved sequence (7, 8, 29), and if it is present in *Saccharomyces* species other than *S. cerevisiae*, it should have been detected in our experiments.

The origin and function of Y′ sequences in *S. cerevisiae* strains remain obscure. Because of the variations in copy number and genomic location, they look almost like mobile elements with high sequence specificity for integrations at the chromosome ends.

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