The Subtelomeric Y′ Repeat Family in *Saccharomyces cerevisiae*: An Experimental System for Repeated Sequence Evolution

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ABSTRACT

The subtelomeric Y′ repeated sequence families in two divergent strains of the yeast *Saccharomyces cerevisiae* have been characterized in terms of copy number, location and restriction site differences. The strain YP1 has 26 to 30 Y′s that fall into two previously described, long (6.7 kb) and short (5.2 kb), size classes. These Y′s reside at 19 of the 32 chromosome ends and are concentrated in the higher molecular weight chromosomes. Five ends contain tandem arrays, each of which has only one size class of Y′s. There is restriction site homogeneity among the Y′s of YP1 even between size classes. The Y′s of strain Y55 contrast sharply with the Y′s of YP1 in terms of copy number, location and sequence differences. There are 14 to 16 Y′s, both long and short, most of which are found at different chromosome ends than those of YP1. None of these are tandemly arrayed. Four to six of the Y′s appear degenerate in that they have homology with a telomere distal end Y′ probe but no homology with sequences at the telomere proximal end. The majority of the Y55 Y′s have the same restriction sites as in YP1. Despite the conservation of restriction sites among Y′s, a great deal of restriction fragment length heterogeneity between the strains is observed. The characterized Y′ repeated sequence families provide an experimental system in which repeated sequence interactions and subsequent evolution can be studied.

REPEATED homologous sequences are ubiquitous among eukaryotic genomes (ARNHEIM 1983). These multigene families can be tandemly arrayed (as in rDNA genes and chorion genes) or dispersed (as in heat shock protein genes and actin genes). They can exist in few copies (mammalian globins) or many thousands of copies (AluI sequences). They can be virtually homogeneous (rDNA genes) or highly polymorphic (class I genes of the mammalian histocompatibility complex). Although the functions of many repeated sequences are known, many have unknown functions (AluI and other satellite DNAs, for example). Postulated reasons for the existence of repeated sequences range from coordination of gene regulation (DAVIDSON and BRITTON 1979) to maintenance of population cohesiveness and species divergence (DOVER 1982; DOVER *et al.* 1983; FLAVELL 1983) to parasitism (DOOLITTLE and SAPIENZA 1980; ORGEL and CRICK 1980).

Repeated sequences are generally observed to share a level of homogeneity among themselves greater than expected for independent evolution (SLIGHTOM, BLECHL and SMITHIES 1980; BALTIMORE 1981; LEIGH BROWN and ISH-HOROWITZ 1981; SELKER *et al.* 1981; HAYASHIDA and MIYATA 1983; IATROU, TSITILOU and KAFATOS 1984). This within-population and -species homogeneity contrasts with the divergence observed in single copy sequences and in related repeat families in different species (DOVER 1982; ARNHEIM 1983; OHTA 1983). This apparent "concerted" evolution is generally attributed to reciprocal crossing over and gene conversion between homologous sequences at non-allelic (ectopic) locations and to unequal crossing over within tandem arrays of repeats [see ARNHEIM (1983) and OHTA (1983) for reviews]. Other explanations for the apparent "concerted" evolution include rapid turnover of sequences duplicated, via transposition, from a donor with subsequent loss of diverged copies by segregation (SELKER *et al.* 1981).

Recombinational interactions between repeated sequences have been observed in many organisms, mostly in microbes [see PETES and HILL (1988) for review]. Both naturally occurring repeats and artificially constructed repeats have been used to monitor and select recombinational interactions during mitosis and meiosis. Theoretical models of recombination among repeated elements, incorporating experimental observations, can predict long term evolutionary consequences of such recombination (OHTA 1983; NAGYLAKI 1984a, b; SLATKIN 1986; WALSH 1986). The feedback between these theoretical models and

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experimental observations can lead to an understanding of repeated sequence evolution. No experimental system has been developed yet in which both short term and long term recombinational interactions can be monitored and manipulated. Such a system is necessary in order to test theoretical expectations experimentally.

The Y′ repeated sequence family in *Saccharomyces cerevisiae* is a typical repeated sequence family. Y′s have so far been found only at chromosome ends in association with other telomeric sequences (SZOSTAK and BLACKBURN 1982; CHAN and TYE 1983; WALMSEY *et al.* 1984). Figure 1 displays the sequences associated with the telomere in yeast. Y′s are highly conserved sequences that exist in long (about 6.7 kb) and short (about 5.2 kb) forms apparently due to an insertion-deletion difference. They can exist in 0 to 4 tandem copies at any particular chromosome end adjacent to the $(C_{1-3}A)_n$ repeats of the functional telomere (SZOSTAK and BLACKBURN 1982; CHAN and TYE 1983; WALMSEY *et al.* 1984). Different yeast strains vary with respect to copy number and location of Y′s (CHAN and TYE 1983; HOROWITZ and HABER 1984; ZAKIAN and BLANTON 1988). A less highly conserved repeated sequence family, X, is found at every chromosome end between Y′ and the unique chromosomal sequences of the end (CHAN and TYE 1983; ZAKIAN and BLANTON 1988). X and Y′ and tandem Y′s are separated by variable stretches of $(C_{1-3}A)_n$ repeats (WALMSEY *et al.* 1984). Within Y′s there is a tandem array of imperfect 36 bp repeats that exist in 8–20 copies (HOROWITZ and HABER 1984). There is evidence for strain differences in sequence composition of Y′s as well based on hybridization intensities to different probes and restriction fragment size differences (HOROWITZ and HABER 1984).

Some forms of recombination among Y′s have been observed experimentally. Y′s can exist as autonomously replicating circles (HOROWITZ and HABER 1985) that can integrate into other Y′s at different chromosomal locations. Y′s are able to undergo high levels of meiotic recombination (HOROWITZ, THORBURN and HABER 1984). Linear plasmids have also been shown to be able to acquire Y′ sequences via recombination (DUNN *et al.* 1984). These and other recombinational interactions may generate strain differences in terms of copy number and location of Y′s.

The Y′ family represents a system in which it may be possible to observe recombinational interactions among repeats over a large number of generations as well as from generation to generation. An opportunity for measuring and observing these long term consequences is provided by the ability to culture yeast in chemostats for several thousand generations. A more thorough study of the structure of the Y′ family and recombination among the Y′s is necessary for an understanding of their evolution. As a prerequisite to such study, we map the genomic distribution of Y′s in two yeast strains, characterizing copy number, location and restriction site differences. In the accompanying paper (LOUIS and HABER 1990) we characterize mitotic recombination events among Y′s using a genetic marker system in which duplications and losses can be selected and monitored.

MATERIALS AND METHODS

Media and growth conditions: Strains were grown at 30°. Sporulation was carried out at 25°. Rich (YEPD) media, synthetic complete (SC) media, SC without specific amino acids added, sporulation media, and canavanine containing and cycloheximide containing media were prepared as described in SHERMAN, FINK and HICKS (1986).

Plasmids and construction: pHinfI1–6 (HOROWITZ and HABER 1984) consists of Y′ sequence from the *Pvu*I site through 6–7 copies of the 36 bp repeats (see Figure 1). pEL16 contains the *Asp*718 (an isoschizomer of *Kpn*I) to *Bam*HI fragment of Y′ from pHH4 inserted into pGEM3. pHH4 consists of an entire Y′ marked with *URA3* adjacent to the 36-bp repeats (HOROWITZ and HABER 1985), inserted into a *LEU2* containing pBR322 vector. YRp14 and YRp15 (HIETER *et al.* 1985) contain *SUP11* and *URA3*. The *SUP11* in YRp15 is a less efficient ochre suppressor than the one in YRp14 (P. HIETER, personal communication). The two plasmids differ in orientation of the *SUP11*. The plasmid of pEL2 was constructed by inserting the *Nco*I to *Pst*I (blunt ended with T4 DNA polymerase) fragment of YRp15 containing part of *URA3, SUP11* and approximately 1400 bp of pBR322, including the origin of replication, into pTU10 cut with *Nco*I and *Sma*I. pTU10 (HOROWITZ, THORBURN and HABER 1984) consists of *URA3* flanked by Y′ sequences adjacent to the 36 bp repeats. The relevant portions of these plasmids with respect to Y′ sequence are shown in Figure 1.

Plasmids containing *TRP1, MET14, MAT* distal sequences, *LYS2, LEU2* and *URA3* were used for chromosome identification and strain comparison. pYeMET14-27 contains *MET14* and *CEN11* sequence as well as *TRP1* and *ARS1* sequence in a pBR322 vector (FITZGERALD-HAYES *et al.* 1982). pSE271 (NICKOLOFF, CHEN and HEFFRON 1986) contains *TRP1, ARS1* and *CEN4* sequence in pUC19. pCW7, obtained from C. WHITE, contains unique sequences centromere distal to *MAT* from a *Hin*dIII site to an *Eco*RI site in pGEM3. pLEM2, obtained from R. H. BORTS, contains *URA3* sequence in pBR322. pEL12 contains *LEU2* sequence in pGEM3. p2L4 consists of about 10 kb of *LYS2* and surrounding sequence in YEp24 (2μ and *URA3* in pBR322) and was obtained from C. FALCO.

Plasmids with Ty1 and Ty917 specific sequences as well as rDNA sequences were also used in comparison of the strains. pB161 contains a 0.6-kb *Bgl*II internal fragment of a Ty1 inserted into the *Bam*HI site of pBR322 (SIMCHEN *et al.* 1984). pB205 contains a 1.8-kb *Cla*I fragment of Ty917 inserted into pBR325. These were obtained from F. WINSTON. Ty1 and Ty917 are members of divergent classes of Tys (ROEDER and FINK 1983). pSES5 contains about 0.6 kb of rDNA sequence comprised of the upstream transcriptional regulatory sites (STEWART and ROEDER 1989) inserted into pBR322.

Strain construction: Yeast strains used in this study are shown in Table 1. YP1 and YP3 (HIETER *et al.* 1985) are

TABLE 1

Strains

Name	Genotype
YP1	*MAT***a** *ho ura3-52 ade2-101 lys2-801*
YP3	*MAT***a** *ho ura3-52 ade2-101 lys2-801* / *MATα ho ura3-52 ade2-101 lys2-801*
EJL8-4B	*MATα ho ura3-52 ade2-101 lys2-801 can1^R cyh2^R*
Y55	*MAT***a** *thr4 HO* / *MATα thr4 HO*
EJL9	*MAT***a** *THR4 ho ura3-52 ade2-101 lys2-801* / *MATα thr4 HO URA3 ADE2 LYS2*
ELT2.1	YP1 with marked Y′ at end E3 of chromosome *XII*
ELT2.3	YP1 with marked Y′ at end E5 of chromosome *VII* or *XV*
ELT2.4	YP1 with marked Y′ at end E2 of chromosome *IV*
ELT2.5	YP1 with marked Y′ at end E9 of chromosome *XIII*
ELT2.7	YP1 with marked Y′ at end E1 of chromosome *IV*
ELT2.9	YP1 with marked Y′ at end E21 of chromosome *V* or *VIII*
ELT2.14	YP1 with marked Y′ at end E6 of chromosome *VII* or *XV*
ELT2.15	YP1 with marked Y′ at end E11 of chromosome *XVI*
ELT2.21	YP1 with marked Y′ at end E4 of chromosome *XII*
ELT2.32	YP1 with marked Y′ at end E10 of chromosome *XIII*
ELT2.33	YP1 with marked Y′ at end E2 of chromosome *IV*

related strains. Y55 (McCUSKER and HABER 1988) is a wild-type isolate, that has never been crossed to other strains, in which hundreds of auxotrophic mutations have been selected. The standard map order and map distances (MORTIMER and SCHILD 1980) are conserved in this strain (McCUSKER and HABER 1988). EJL9 resulted from a cross between YP1 with Y55. The 35 transformants ELT2.1–35 were obtained by gene transplacement (ROTHSTEIN 1983) of resident Y's with Y′ sequence containing *URA3* and *SUP11* and pBR322 sequences using the lithium acetate procedure (ITO *et al.* 1983) and the *Pvu*I to *Eco*RI (about 10 bp from the end of Y′ sequence) fragment of pEL2. The locations and contexts of the eleven different marked Y's of transformants ELT2.1, 3, 4, 5, 7, 9, 14, 15, 21, 32, and 33 (Table 1) were determined using chromosome and Southern blot (SOUTHERN 1975) analysis.

Gel electrophoresis and Southern analysis: DNA samples were prepared from overnight cultures grown in 5 ml of YEPD broth, using a scaled down version of the rapid yeast DNA preparation (SHERMAN, FINK and HICKS 1986). Restriction endonuclease digestion was carried out on 0.5–1 μg of yeast DNA and fragments were separated on a 0.5% (w/v) agarose gel run in 1× TBE (89 mM Tris, 89 mM boric acid and 2 mM EDTA) (MANIATIS, FRITSCH and SAMBROOK 1983). All enzymes were obtained from New England Biolabs or Boehringer Mannheim and used according to suppliers instructions. The separated DNA fragments were transferred to Biorad Zetaprobe membrane as per manufacturers instructions. Hybridization with radioactive probe was carried out according to manufacturers instructions. Exposure of Kodak XAR-5 film was carried out at −70° with intensifying screens.

Preparation of radioactive probe: ^{32}P-labeled probes were prepared by nick translation (RIGBY *et al.* 1977) or by random primer extension (FEINBERG and VOGELSTEIN 1983, 1984). Y′-specific probe was prepared using pHinfI1–6 or pEL16. Transformant-specific probe was prepared using pBR322. Chromosome-specific probes were obtained using plasmids described above.

Chromosome preparation and electrophoretic separation: Intact chromosomal DNA was prepared from 5 ml overnight cultures in YEPD using a scaled down version of the standard chromosome preparation procedure (CARLE and OLSON 1984). Chromosomes were separated on a 1% agarose gel using a contour-clamped homogeneous electric field (CHEF) apparatus (CHU, VOLLRATH and DAVIS 1986) or an internally clamped homogeneous field (ICHF) apparatus (H. E. CHIKARMANE and E. J. LOUIS, unpublished). Small sections of the agarose plug containing chromosomal DNA were placed directly in the wells of a 30 ml 10 cm × 10 cm gel. Separation was accomplished in 20–24 h with constant switching times (60, 80 or 93 sec) at 250 V. The running buffer (1/4 × TBE) was maintained at a temperature of 11° by rapid recirculation while running the gel in an ambient room temperature of 4°. Separated chromosomal DNA was transferred to Biorad Zetaprobe membrane. Hybridization was carried out as described above.

Individual chromosome restriction analysis: Individual chromosomes from ethidium bromide stained 1% low melt agarose ICHF gels were cut out and destained overnight in a large volume of TE at 4°. Restriction endonuclease digestion of the chromosome was carried out in the agarose plug by immersing the plug in the appropriate restriction enzyme buffer. A 20–50-fold excess of restriction endonuclease was added and digestion was carried out at 37° with slight agitation. After 4–6 hr of digestion, the plug was melted at 65°, mixed with loading dye and loaded onto a 0.5% (w/v) agarose gel. The separated chromosomal fragments were transferred to membranes and hybridized to Y′ specific probes as described above.

Cloning of Y's: DNA from strains with Y's marked with *URA3, SUP11* and pBR322's origin of replication was digested with *Xho*I. This digested DNA was ligated under dilute conditions. A *pyrF*$^-$ version of *Escherichia coli* strain HB101 (that can be complemented with the *URA3* gene from yeast) was transformed directly with this DNA using electroporation (CALVIN and HANAWALT 1988). *URA3* function was selected.

RESULTS

Y′ restriction fragment analysis and segregation: From the Y′ restriction map in Figure 1 it can be seen that certain restriction endonuclease digestions can uniquely define particular Y's as they will yield fragments with lengths dependent on the probe and the location of restriction sites in Y′-adjacent unique chromosomal sequence. For example, *Xho*I is diagnostic for nearly every individual chromosome end bearing a Y′. The size of each *Xho*I fragment with pHinfI1–6 homology depends on the location of a telomere-adjacent *Xho*I site in the unique sequences centromere-proximal to Y's. Similarly, *Asp*718 is diagnostic for chromosome ends bearing the short form of Y′ as Y′-shorts are missing the telomere distal *Asp*718 site (Figure 1) such that the fragment length depends on the next telomere adjacent *Asp*718 site in the unique chromosomal sequences. Y′-longs will all yield about

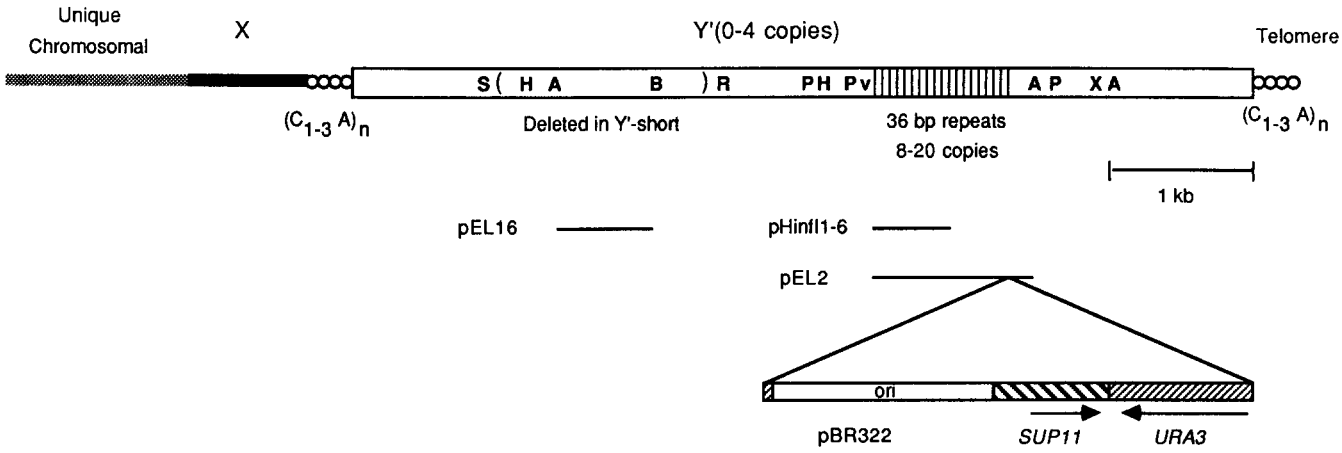


FIGURE 1.—Structure of the chromosome ends of *S. cerevisiae*. Adjacent to the unique sequences (shaded) at the end of a chromosome are *X* sequences (solid), which comprise a not very highly conserved repeated sequence family. Adjacent to *X*, on some chromosome ends are 1 to 4 tandem copies of Y′ sequences (open). These sequences are separated by variable numbers of $C_{1-3}A$ repeats (open circles) which are also at the end of the chromosome and are part of the functional telomere. There is a tandem array of variable numbers of a 36-bp repeat (vertical lines) between the *Pvu*I (Pv) and the middle *Asp*718 (A) sites of Y's. The sequences between the parentheses, including a *Hin*dIII (H), the telomere distal *Asp*718 (A) and *Bam*HI (B) sites are missing in Y′-shorts. The restriction sites shown are conserved among Y's and include *Pst*I (P), *Xho*I (X), *Sal*I (S), and *Eco*RI (R). The relevant portions of plasmids used in this study are shown below the Y′ map. pEL16 contains Y′ sequence from the telomere distal *Asp*718 site to the *Bam*HI site. *p*HinfI1-6 contains Y′ sequence from the *Pvu*I site through 6 to 7 copies of 36-bp repeats. pEL2 contains 1400 bp of pBR322 including the origin of replication, *SUP11* and *URA3* inserted at the telomere proximal end of the 36-bp repeats in Y′ sequence from the *Pvu*I site to near the middle *Asp*718 site.

the same 3.6-kb *Asp*718 fragment with pHinfI1-6 homology. Other restriction enzyme digestions are diagnostic for the Y′ structures at chromosome ends. For example, *Hin*dIII digested DNA probed with pHinfI1-6, will yield a dispersed band for the endmost Y's of about 3.0 kb in size (see Figure 1 and Figure 4 below). This is due to variable length fragments containing the chromosome terminus in the population of cells (CHAN and TYE 1983). If there are any tandem arrays of Y's there will also be larger sharp bands resulting from Y's internal to other Y's (see Figure 4 below).

Eight entire tetrads from EJL9, the diploid formed by crossing YP1 and Y55, were analyzed using *Xho*I, *Hin*dIII and *Asp*718. Figure 2A shows the *Asp*718 fragments with pHinfI1-6 homology in strains YP1 (lane 2) and Y55 (lane 3) as well as a single complete tetrad (spores A through D) from EJL9. The *Asp*718 fragments in YP1 and Y55 are labeled A1–A13 (YP1) and B1–B7 (Y55) by descending size. The segregation of these fragments as always 2:0, always 4:0, or mixed 4:0, 3:0 and 2:0 determines whether there is one, many or a few Y's that have the same restriction fragment length. For fragments that segregate 2:0, allelism can be determined by the segregation patterns of pairs of fragments. If a pair of Y's from YP1 and Y55 are at the same chromosome end, their restriction fragments will always segregate away from each other, whereas they will segregate randomly if nonallelic. Fragments A1–A8, A11, A13, B1–B3, and B7 segregate 2:0 in all eight tetrads. Fragments A10, B5 and B6 segregate in mixed patterns and therefore representative of a few but not many Y's. Fragment A12 segregated 4:0 in every case and represents many Y's with this fragment size. Fragments A4 and A8 always cosegregate and are at the same chromosome end. These fragments also cosegregate with a higher molecular weight *Hin*dIII fragment (data not shown) and are therefore in a tandem array. Fragments A2 and B2 always segregate away from each other and are alleles. Fragments A9 and B4 comigrate but segregate in a mixed fashion and therefore represent a few nonallelic Y's with this fragment size.

Chromosomal locations of Y's: Figure 2B shows YP1 and Y55 chromosomes separated and probed with pHinfI1-6 and pEL16. The difference in locations of Y's is evident. In YP1 there are only three chromosome bands that have no pHinfI1-6 homology: *I, III* and *XI*. In Y55 many of the chromosome bands have no pHinfI1-6 homology: *III, X, II, XIII, VII, XV, XII* and *IV*. These include the majority of the larger molecular weight chromosomes. Chromosomes *IV* and *VII* or *XV* in Y55 have homology to pEL16 but not to pHinfI1-6. These additional Y′ homologous sequences may be degenerate in that they do not consist of entire Y's (see below). No additional pEL16 homology is evident in YP1.

In order to match a particular Y′ restriction fragment to a particular chromosome, individual chromosomes were cut out and digested with restriction endonucleases. Analysis of the individual chromosomes was used to assign the location of particular fragments. The number of Y's per chromosome can also be determined. Figure 2C displays individual Y55

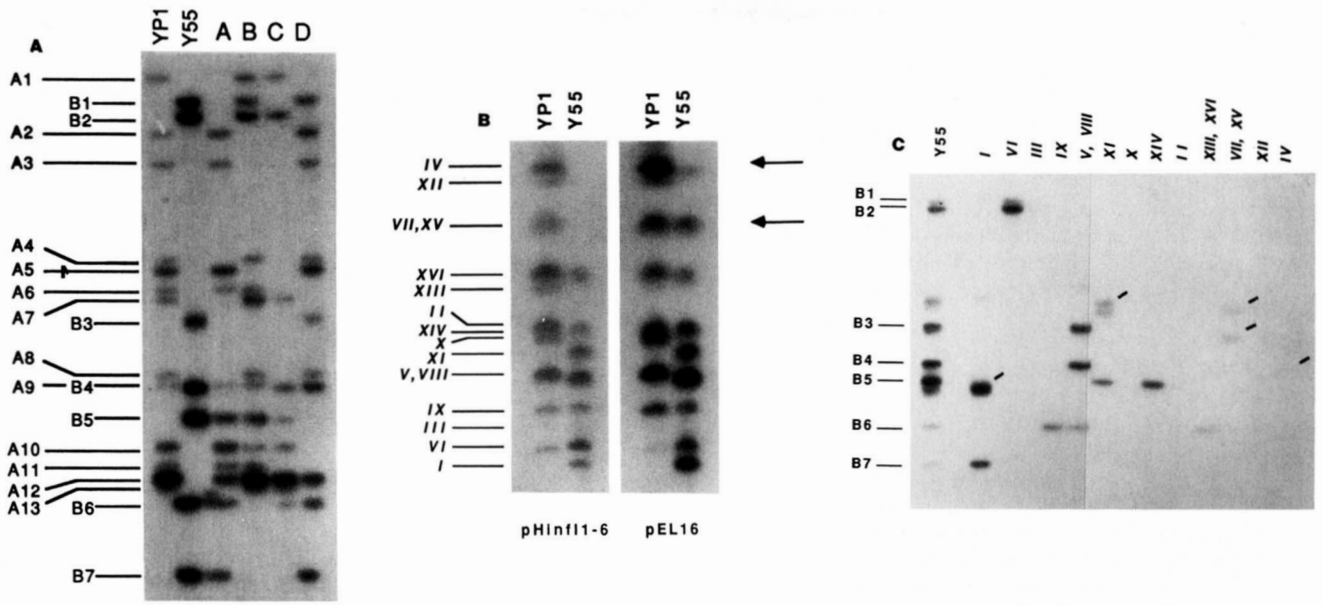


FIGURE 2.—Y′ segregation and location as determined by genetic crosses and chromosome separating gels. (A) The *Asp*718 fragments with pHinfI1–6 homology in YP1, Y55 and the four spores A, B, C and D of a tetrad from EJL9 (YP1 × Y55). The fragments are labeled by descending size, A1 to A13 (YP1) and B1 to B7 for Y55. Fragments A1–A8, A11, A13, B1–B3, and B7 all segregate 2:0. Fragments A10, B5 and B6 segregate 3:0 in this tetrad and fragment A12 segregates 4:0. Fragments A9 and B4 comigrate but segregate independently. (B) Separated chromosomes of YP1 and Y55 with pHinfI1–6 and pEL16 homology. Chromosome determination was made with chromosome specific probes and by inference from previously published chromosome identifications. In Y55 there are two chromosome bands, *IV* and *VII/XV* that have pEL16 homologous sequences but not pHinfI1–6 homologous sequences (marked by arrows). Three chromosomes in YP1 have no Y′ homology (*I, III* and *XI*) whereas chromosomes *III, X, II, XIII* and *XII* have no Y′ homology in Y55. (C) *Asp*718 digestions of individual Y55 chromosomes probed with pEL16. The pHinfI1–6 homologous fragments are labeled and the pEL16 specific fragments are marked with arrows. The faint band in chromosome *IV* is more clearly evident with longer exposure. Three chromosomes (*IX, V* or *VIII*, and *XIII* or *XVI*) have restriction fragment B6 and two (*XI* and *XIV*) have restriction fragment B5.

chromosomes digested with *Asp*718 and probed with pEL16. As can be derived from these and the above data (Figure 2A), there are ten Y′s in Y55 that have pHinfI1–6 homology. Three of them have *Asp*718 restriction fragment B6 (chromosomes *IX, V* or *VIII* and *XIII* or *XVI*) and two have restriction fragment B5 (chromosomes *XI* and *XIV*). The arrows (Figure 2C) indicate an additional 6 pEL16 homologous fragments that do not have pHinfI1–6 homology. These six additional pEL16 homologous fragments, at chromosomes *I, XI, VII* or *XV* and *IV*, which have no pHinfI1–6 homology, represent 4 to 6 Y′s that are degenerate. These *Asp*718 restriction fragments appear to have characteristic variable sized telomere-containing fragments. Similar analysis of YP1 chromosomes was performed using *Xho*I and *Pvu*II as well as *Asp*718 (data not shown).

These combined data lead to the structures displayed in Figure 3 for the Y′ families in YP1 and Y55. Chromosomal designations were determined with the probes described in MATERIALS AND METHODS or derived from previously published designations when probes were not available (CARLE and OLSON 1985). The left and right arm orientation of Y′ locations is not known relative to the standard yeast genetic map (MORTIMER and SCHILD 1980). The two ends of a chromosome could be distinguished by the unique *Xho*I and *Pvu*II Y′ homologous fragments upon Southern analysis. The chromosome pairs *V, VIII* and *VII, XV* could not be separated on chromosome separating gels and therefore are combined.

YP1 has 26 to 30 Y′s that reside mostly in the higher molecular weight chromosomes. Five ends have tandem arrays of Y′s, none of which are mixed for long and short versions. The copy number of the tandemly arrayed Y′s could not be determined completely. When three copies are displayed there are at least three copies but possibly four (ends E2 and E3). The tandem array at chromosome end E1 could have two to four copies of Y′s. The other tandem arrays have only two Y′s (ends E4 and E10). Thirteen chromosome ends have no Y′s. There are 9 Y′-shorts and 17 to 21 Y′-longs.

In contrast, Y55 has only ten Y′s with pHinfI1–6 homology, none of which are tandemly arrayed. The three Y′-shorts and seven Y′-longs are concentrated in the lower molecular weight chromosomes. This is not consistent with the idea that the function of Y′s is to stabilize the larger molecular weight chromosomes (ZAKIAN and BLANTON 1988). There are also four to six degenerate Y′s in Y55 that do not exist in YP1. There are apparently six allelic pairs of Y′s between

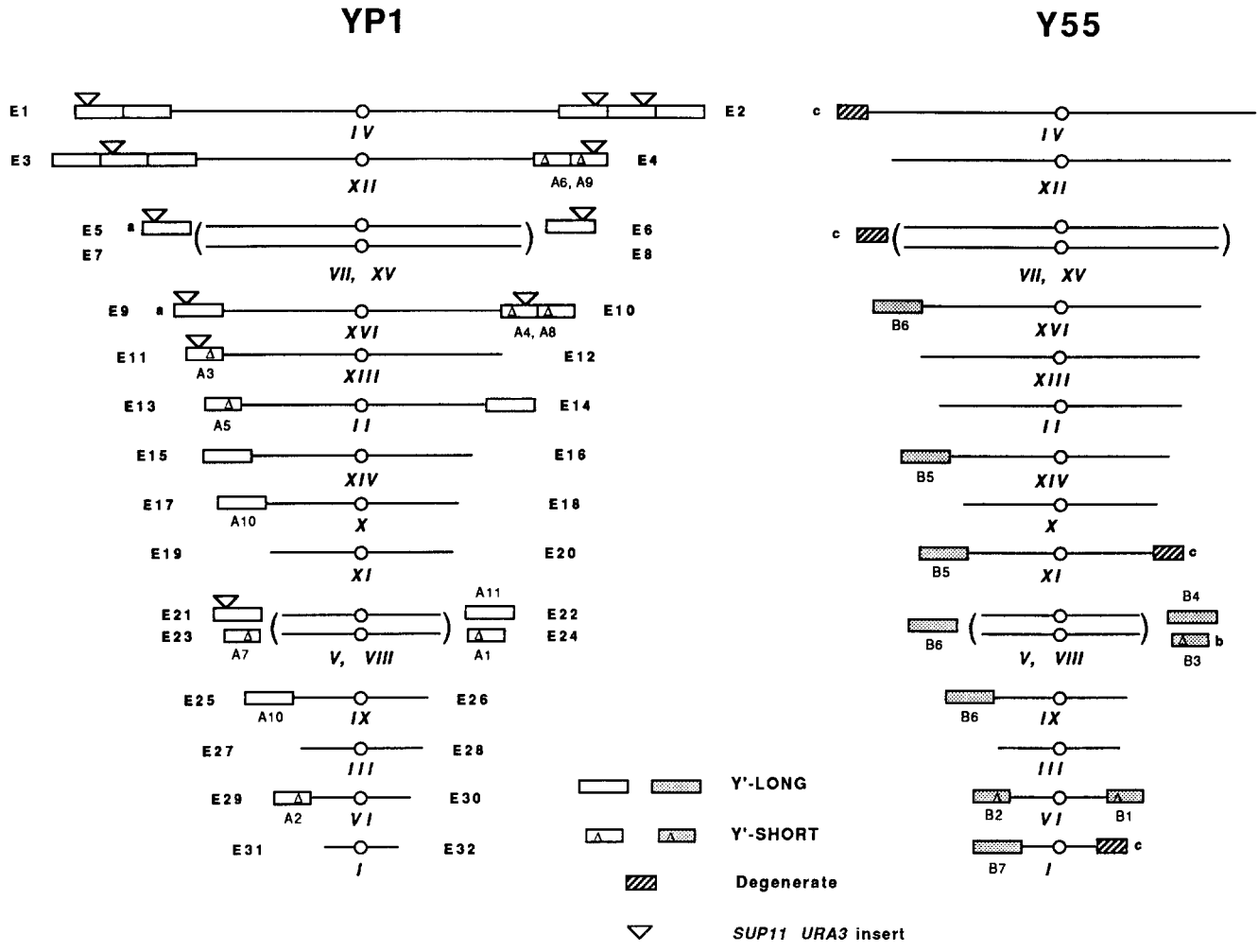


FIGURE 3.—Y′ families in YP1 and Y55. The location of Y′-shorts and Y′-longs (and degenerate Y's) in YP1 and Y55 are shown. Determination of left and right arms of chromosomes was not made, though they are distinguishable by Southern analysis. The chromosome pairs *VII, XV* and *V, VIII* could not be separated and are combined. Each chromosome end is labeled E1 through E32 for discussion purposes. The *Asp*718 fragments A1 to A13 and B1 to B7 are assigned to their particular Y's. All unlabeled Y′-longs in YP1 have restriction fragment A12 except for one of ends E1, E2, E3 or E6 which has restriction fragment A13, the location of which was not determined completely. The independently *SUP11–URA3* marked Y's in YP1 are indicated with inverted triangles. (a) The Y′-longs at ends E5 and E9 share restriction sites up to 5 kb internal to the Y′ sequence and may include sequence homology of internal chromosomal DNA as well as X sequences. (b) The Y′-short of Y55 with *Asp*718 fragment B3 is unique in that it is missing the telomere proximal *Asp*718 site and possibly other restriction sites (see text for details). (c) These Y's in Y55 are degenerate in that they do not have homology to the 36-bp repeat probe pHinfI1–6 but do have homology to pEL16 (see text for details).

the strains if the degenerate Y's are taken into account (Figure 3).

Y′ heterogeneity: Southern analysis of DNA from YP1 and Y55 digested with each of *Asp*718, *Bam*HI, *Bgl*II, *Eco*RI, *Eco*RV, *Hin*dIII, *Pst*I, *Sal*I, *Xba*I and *Xho*I restriction endonucleases. After electrophoresis, the separated fragments were probed with pEL16 and pHinfI1–6 to assess heterogeneity among Y's within and between the strains. In Figure 4, the pHinfI1–6 homologous fragments from YP1 (lanes 1A–10A) and Y55 (lanes 1B–10B) for each of the ten enzymes are shown. Restriction enzymes that cut on the telomere distal side of the 36-bp repeats but not on the telomere proximal side result in the characteristic dispersed bands indicative of variable $C_{1-3}A$ repeats, at each telomere, within the population of cells (CHAN and TYE 1983). The arrow (1) in lane 4B indicates the dispersed *Hin*dIII bands for Y55 Y's. Similar dispersed bands are found for *Eco*RI (lane 1), *Eco*RV (lane 2), *Bam*HI (lane 3), *Sal*I (lane 7) and *Xba*I (lane 8) for both strains. The dispersed bands in the two strains are of similar sizes indicating the presence of these restriction sites in approximately the same location in YP1 and Y55 Y's. Tandem arrays of Y's result in higher molecular weight fragments that do not yield dispersed bands in addition to dispersed bands for these enzymes. The arrow (2) in lane 4A shows the higher molecular weight *Hin*dIII fragments in YP1 indicative of tandem arrays of Y's. The absence of these higher molecular weight fragments in Y55 indicates its lack of tandem arrays. Similarly, the comigrating higher molecular weight fragments for *Eco*RI,

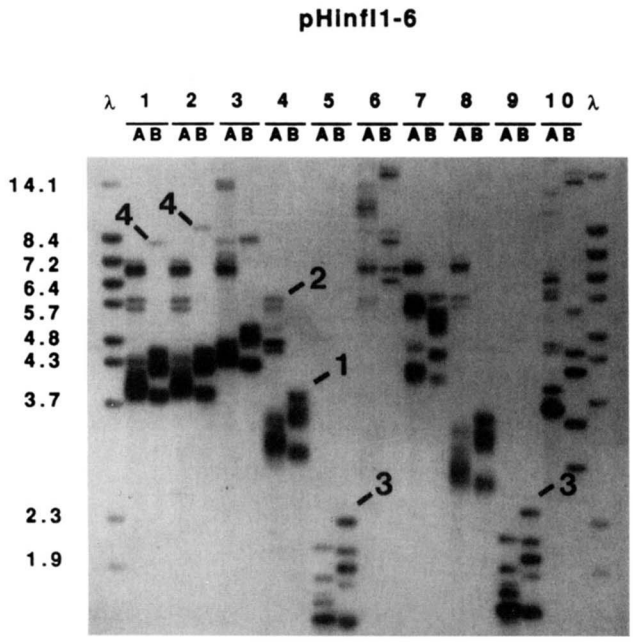


FIGURE 4.—Y′ heterogeneity. DNA fragments from strains YP1 (lanes A) and Y55 (lanes B) resulting from digestion with each of *Eco*RI (1), *Eco*RV (2), *Bam*HI (3), *Hin*dIII (4), *Pst*I (5), *Xho*I (6), *Sal*I (7), *Xba*I (8), *Bgl*II (9) and *Asp*718 (10) are probed with pHinfI1–6 to assess heterogeneity within and between strains. The lanes marked λ are molecular weight markers of sizes shown (in kb). Arrow 1 (lane 4B) indicates that variable lengths of *Hin*dIII fragments in which one end is the telomere. Arrow 2 (lane 4A) shows discrete higher molecular weight *Hin*dIII fragments indicative of tandem arrays of Y's. Arrows 3 (lanes 5 and 9) indicate the 36-bp repeat homologous *Pst*I (5) and *Bgl*II (9) fragments consistent with variation in copy number of the 36-bp repeats. Arrows 4 (lanes 1B and 2B) indicate restriction fragments consistent with a Y′ missing an *Eco*RI site (1B) and an *Eco*RV site (2B).

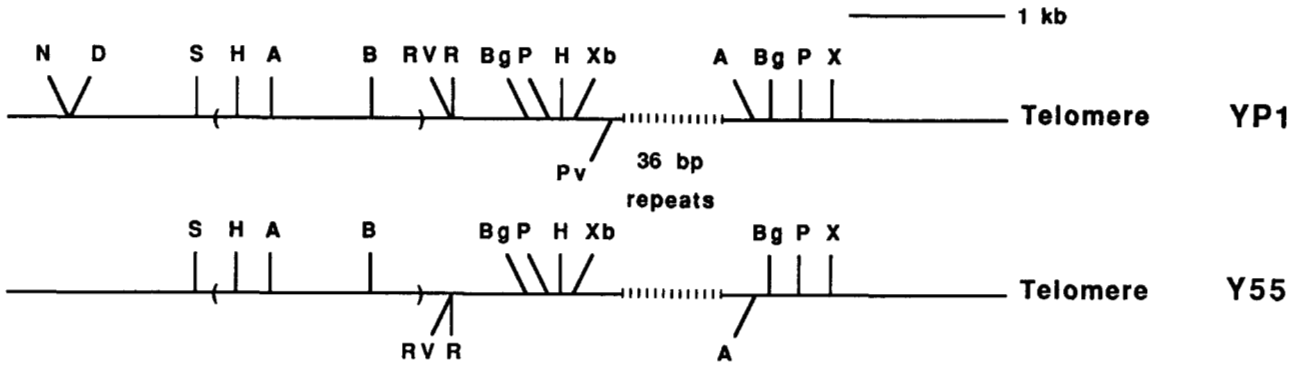
*Eco*RV, *Bam*HI, *Sal*I and *Xba*I digested YP1 DNA, all of which cut once within a Y′, are also indicative of tandem arrays and are of sizes consistent with unit Y′-longs (about 6.7 kb) and Y′-shorts (about 5.2 kb).

The restriction maps of Y's in YP1 and Y55 are summarized in Figure 5A. The Y's of YP1 and Y55 are virtually homogeneous with respect to restriction site presence. All of the Y's of both strains apparently have *Hin*dIII, *Sal*I, and *Xba*I sites as there are no fragments greater than unit Y′ size that would result from a Y′ missing any of these sites. The higher molecular weight *Bam*HI and *Asp*718 fragments are consistent with the number of Y′-shorts that are missing the *Bam*HI and *Asp*718 sites. The Y's of both strains all have *Pst*I and *Bgl*II sites flanking the 36-bp repeat region as indicated by a characteristic ladder of fragment sizes. In Y55 there is one large molecular weight fragment for *Eco*RI and *Eco*RV (arrows 4 in lanes 1 and 2). These are indicative of a Y′ missing each of these sites. These sites could be missing in a single Y′ or in two different Y's. The rest of the Y's in both strains have the *Eco*RI and *Eco*RV sites. All of the Y's apparently have a telomere-proximal *Xho*I and all but one have the telomere-proximal *Asp*718 site as none of these fragments yields a dispersed band. The Southern band B3 (segregants B and D in Figure 2A and lane 10B in Figure 4) has a dispersed nature indicative of a single Y55 Y′ missing this *Asp*718 site. It has not been determined whether this Y′ is the same one missing the *Eco*RI and/or *Eco*RV sites. Except for possibly one Y55 Y′, there is conservation of the restriction sites mapped in all pHinfI1–6 homologous Y's in both strains.

In contrast to the restriction site conservation there is a great deal of restriction fragment length polymorphism between the strains. Figure 5B summarizes the restriction fragment length variations found for Y′ internal sites flanking the probe sequences. For the 17 to 21 Y′-longs in YP1, nearly all of the *Asp*718 fragments are the same 3.6 kb in length. Four of the Y′-longs vary to some extent in *Asp*718 fragment size. One is slightly shorter (A11) and one is slightly longer (A13) (both about 100 bp different). The other two Y's share the same difference of being about 300 bp larger (A10). The *Asp*718 fragments of the 7 Y′-longs in Y55 fall into four size classes that vary from 2.9 kb (B7) to 4.4 kb (B4). The approximately 30 Y's of YP1 fall into six size classes of 36 bp repeat regions based on *Pst*I and *Bgl*II digests [arrows (3) in lanes 5 and 9 of Figure 4]. The 10 pHinfI1–6 homologous Y's of Y55 fall into five different size classes in the same size range as the YP1 Y's. The differences in size each can be explained by differences of a few copies of the 36-bp repeats. Most of the 17 to 21 Y′-longs of YP1 have nearly the same 2.1-kb *Hin*dIII fragment when probed with pEL16 (data not shown). There is a minor band less than 100 bp shorter. The pEL16 homologous Y55 *Hin*dIII fragments fall into four size classes that vary from 1.6 kb to 2.1 kb. There is apparently more restriction fragment length variation within Y55 Y's than in YP1 Y's.

Repeated sequences within Y's: In addition to the 36-bp repeats within Y's there are apparently other duplicated sequences within Y's. The probe pEL16 was constructed to distinguish between Y′-long and Y′-short versions in that the *Asp*718 to *Bam*HI fragment is entirely contained within the sequences missing from Y′-shorts (see Figure 1). When DNA fragments from various restriction endonuclease digestions are probed with pEL16 (which has no cross homology with pHinfI1–6), the Y′-short specific fragments show homology with the probe. Therefore, Y′-shorts have pEL16 homology outside of the deletion. The four size classes of *Hin*dIII fragments homologous to pEL16 in Y55 (Figure 5) are reminiscent of variable numbers of tandem repeats. This same type of ladder is seen for the tandemly arrayed 36-bp repeats as indicated by the five to six sizes of *Pst*I and *Bgl*II fragments with pHinfI1–6 homology. These

A



B

	YP1	Y55
*Asp*718 fragments (Y'-longs)	4 size classes: 3.9 kb (2) 3.7 kb (1) 3.6 kb (13-17) 3.5 kb (1)	4 size classes: 4.4 kb (1) 4.1 kb (2) 3.4 kb (3) 2.9 kb (1)
*Hin*dIII fragments (Y'-longs)	2 size classes: 2.1 kb (16-20) 2.0 kb (1)	4 size classes: 2.1 kb (2) 1.9 kb (3) 1.7 kb (1) 1.6 kb (1)
Pst I fragments (Y'-longs and Y'-shorts)	6 size classes: 2.1 kb 1.9 kb 1.8 kb 1.7 kb 1.6 kb 1.55 kb	5 size classes: 2.3 kb 2.1 kb 1.9 kb 1.8 kb 1.6 kb

FIGURE 5.—Restriction map of sites analyzed (A) and summary of variation in YP1 and Y55 (B). (A) The restriction sites mapped for the Y's of YP1 and Y55 include *Nco*I (N), *Dra*I (D), *Sal*I (S), *Hin*dIII (H), *Asp*718 (A), *Bam*HI (B), *Eco*RV (RV), *Eco*RI (R), *Bgl*II (Bg), *Pst*I (P), *Xba*I (Xb), *Pvu*I (Pv), and *Xho*I (X). The parentheses indicate the sequences missing in Y'-shorts. For both YP1 and Y55, the restriction enzymes shown above the line are conserved in all Y's. Those shown below the line are polymorphic within the strain. In 3/11 of the marked YP1 Y's, the *Pvu*I site was missing. One Y' of Y55 with pHinfI1–6 homology is missing the telomere proximal *Asp*718 site, one is missing the *Eco*RI site and one is missing the *Eco*RV site. These may all occur in the same Y' or different Y's. (B) The restriction fragment length variation for fragments resulting from sites flanking the probe sequence are shown. The *Asp*718 and *Hin*dIII fragments are only representative of Y'-longs while the *Pst*I fragments are representative of both Y'-longs and Y'-shorts. The number of Y's with each of the *Asp*718 and *Hin*dIII fragment sizes are given. *Bgl*II fragment variation is similar to the *Pst*I fragment variation and is not shown.

four classes may include fragments from the degenerate Y's.

Restriction site variation in other sequences: Assessment of divergence between the two strains for other sequences, both single copy and other repeated sequence families can be made by Southern blot analysis. This assessment may yield information on the rate of Y′ evolution relative to other sequences. DNA from YP1 and Y55 digested with the same restriction enzymes above, was probed with Ty, rDNA, *MET14*, *MAT* distal and *TRP1* sequences. DNA fragments from the two strains probed with either *MET14*, MAT distal or *TRP1* indicate five restriction site differences from the total of 60 sampled (ten enzymes cleaving two sites for each fragment at three different locations). There was one restriction site difference each for *MET14* (*Xba*I) and *TRP1* (*Sal*I) probes and three restriction site differences for the *MAT* distal probe (*Bam*HI, *Hin*dIII and *Pst*I). Each restriction enzyme used recognizes a unique 6-bp sequence so that the five restriction site differences could be attributed to at least 5-bp differences out of 360 sampled. This is similar to the 9/2700 bp differences found between strains Y55 and S288C for the *PMA1* gene (PERLIN *et al.* 1989) and for the 10/1170 bp differences between *URA3* sequences in strains D4 and FL100 (ROSE, GRISAFI and BOTSTEIN 1984). S288C has the same Y′ restriction fragment pattern as YP1 for several restriction enzymes (data not shown). YP1 is closely related to S288C as it is derived from a strain backcrossed to S288C at least ten times (HIETER *et al.* 1985).

The rDNA genes are a tandem repeat family on chromosome *XII* and are homogeneous within a strain (PETES 1980). The Ty sequence families are comprised of dispersed copies throughout the genome (ROEDER and FINK 1983). Figure 6, A and B, shows YP1 and Y55 genomic DNA digested with several different restriction endonucleases and probed with rDNA and Ty specific sequences. For the rDNA array, there is homogeneity of restriction fragment sizes within each strain but a restriction fragment length difference between the strains (arrows in lanes 1 and 4 Figure 6A). The majority of Ty specific fragments varied in size considerably between the two strains for most restriction enzymes used. This is expected for location differences between the strains and the lack of most of these sites within Tys. For Ty917s, none of the restriction enzymes recognized internal sites (data not shown). There are *Xho*I sites in most of the Ty-associated δ sequences flanking the Tys which resulted in a major band of unit Ty size. This major band was the same size in both strains (data not shown). For Ty1s, three enzymes [*Eco*RV (lanes 2), *Sal*I (lanes 7) and *Bgl*II (lanes 9) Figure 6*B*] recognized internal sites. The major band for each of these digestions is the same size for both strains. As with Ty917s, *Xho*I sites are present in most of the Ty-associated δ sequences flanking Ty1s (lanes 6 Figure 6B). For the majority of Tys that have the internal restriction sites, there are no restriction fragment length differences between the strains.

Y's marked with *SUP11*: Thirty-five independent transformants of Y's in YP1 marked with *URA3*, *SUP11* and pBR322 were obtained via gene transplacement (see MATERIALS AND METHODS). At least 11 different marked Y's at ten chromosome ends were obtained and these are shown in Figure 3. Several of the marked Y's fell into the middle of one of the tandem arrays and were not all analyzed further. Many Y's were marked several times independently whereas others were not marked. Each marked Y′ was characterized in terms of restriction site presence, presence of unmarked adjacent Y's, and location. Independent transplacements into the same Y′ were identical in terms of unmarked adjacent Y's. Marked Y's that were in the same chromosome band were tested for allelism by crossing each to a *MATα* strain with subsequent crossing of segregants from these diploids.

Restriction maps of marked Y's: DNA from strains bearing each marked Y′ was digested with each of *Asp*718, *Bam*HI, *Eco*RI, *Hin*dIII, *Pvu*I, *Pvu*II and *Xho*I. The separated fragments were then probed with pBR322 which is specific for the marked Y′. There are virtually no restriction site differences internal to Y's even between the long and short versions except for a *Pvu*I restriction site polymorphism. Three out of the 11 marked Y's were *Pvu*I$^-$ (Figure 5). The *Pvu*I site is adjacent to the 36-bp repeats and the variation in presence or absence of the *Pvu*I site may be related to variation in the 36-bp repeats. This site is also at the end of the sequence used in the transplacement, so that this polymorphism observed could be a transplacement artifact. Two of the marked Y's that reside on different chromosomes (ELT2.3 and ELT2.5) have identical *Xho*I and *Pvu*II restriction fragment sizes consistent with shared sequences adjacent to Y′. These shared sequences could include the X region as well as possible additional centromere-proximal sequences up to 5 kb from the end of the Y′. These were further analyzed with *Eco*RV, *Nco*I, *Pst*I and *Dra*I and were found to be identical in fragment sizes for these sites as well. These two Y's are indicated on Figure 3.

Cloning of marked Y's: The insertion of the origin of replication from pBR322 along with *URA3* and *SUP11* into individual Y's makes direct cloning of Y's into *E. coli* possible. DNA from strains with a marked Y′-long (ELT2.3 at end E5) and a marked Y′-short (ELT2.21 at end E4) was digested with *Xho*I and then ligated. pyrF$^-$ *E. coli* was transformed using electroporation and *URA3* function was selected. The result-

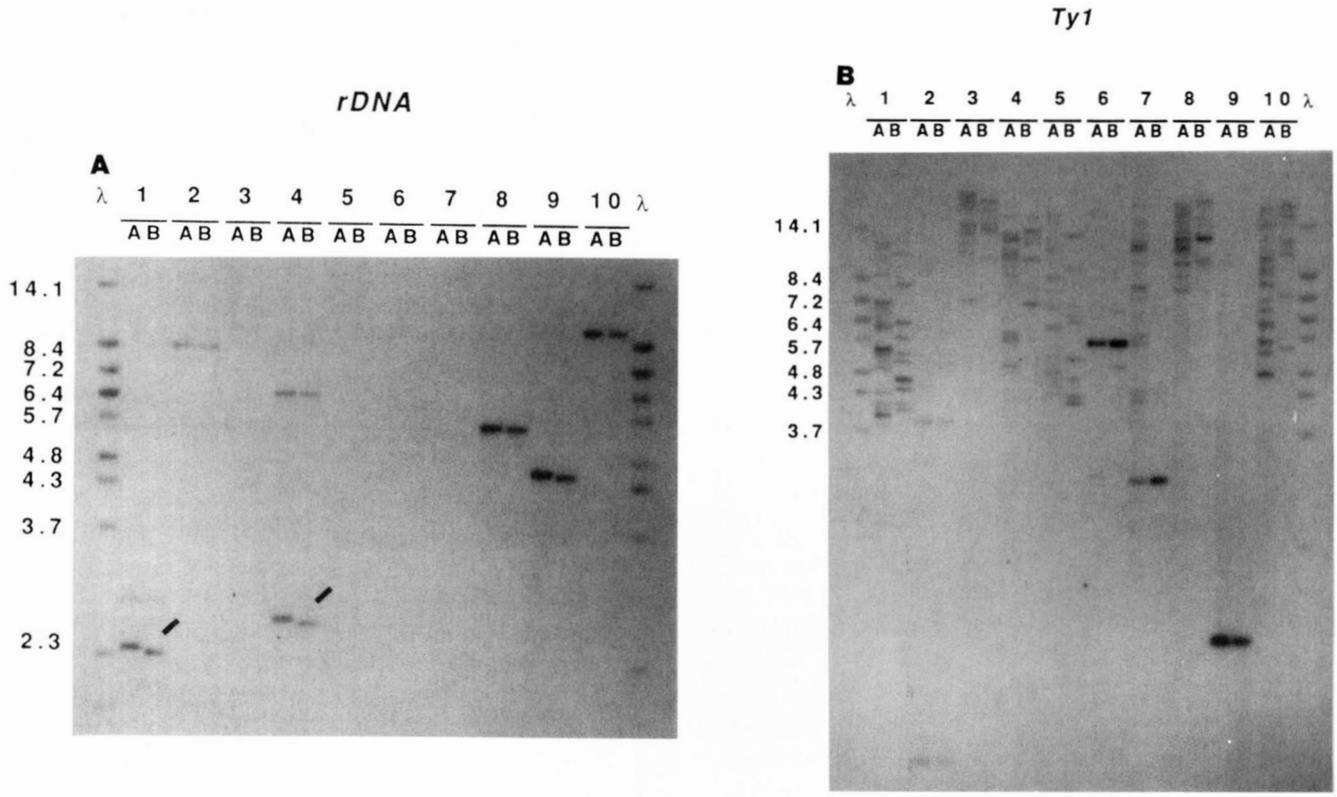


FIGURE 6.—Heterogeneity within other repeated sequences. DNA from strains YP1 (lanes A) and Y55 (lanes B) cut with each of *Eco*RI (1), *Eco*RV (2), *Bam*HI (3), *Hin*dIII (4), *Pst*I (5), *Xho*I (6), *Sal*I (7), *Xba*I (8), *Bgl*II (9) and *Asp*718 (10) is probed with both rDNA sequence (A) and Ty1 sequence (B) in order to assess the heterogeneity of these repeated sequence families within and between the strains. The arrows in A indicate a small restriction fragment length differences between the two strains in the rDNA genes for *Eco*RI and *Hin*dIII digestions. These differences are explicable by a single short insertion-deletion difference between the rDNA units of the two strains. Within each strain the arrays are homogeneous as there is only the single fragment size for digestion with *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I, *Bgl*II and *Asp*718. The other enzymes used recognize sites outside of the rDNA array and result in fragments too large to analyze by standard gel electrophoresis. In Figure B, a great deal of variation is seen in restriction fragments with Ty homology. Most of these are due to restriction sites (*Eco*RI, *Bam*HI, *Hin*dIII, *Pst*I, *Xba*I, and *Asp*718) outside of the Ty element. The location differences of Tys between the two strains explain this variation. For DNA digested with *Eco*RV, *Sal*I and *Bgl*II, which recognize sites within Tys there is a major band that is the same size in both strains. Most of the Ty1s in the two strains are homogeneous with respect to the presence of these sites. The *Xho*I restriction sites are found in the Ty-associated δ sequences and are present in most of the Tys of both strains as there is a single major *Xho*I band.

ing plasmids have Y′ homology by Southern blot analysis and have the appropriate restriction maps. The clones contain Y′ sequence from the telomere proximal *Xho*I site to the next *Xho*I site. For ELT2.3 this next site is in X or unique sequence internal to the marked Y′. For ELT2.21, this next site is in the adjacent internal Y′ of the tandem array. Restriction mapping of the Y′ internal sites confirms the conservation of sites between the long and short versions on both sides of the insertion-deletion difference (*i.e.*, both have a *Sal*I site in the appropriate position as well as the other sites analyzed).

DISCUSSION

The Y′ family of *S. cerevisiae* is a typical repeated sequence family. It consists of one to four tandem copies of a conserved sequence that is dispersed to several chromosome ends. No evidence was found for Y′ sequences at other than telomeric locations. Every Y′ in both strains studied, had restriction fragments characteristics of being at a chromosome end for most, if not all, of the ten restriction enzymes tested. Strains vary with respect to copy number and location. This is evident in the two strains characterized here which have only four to seven Y′s in allelic positions. Over time, Y′s must have the ability to move to new locations and/or be lost from resident locations in order to account for strain differences. The current Y′ families could be the result of different sets of losses from an original family in which most or all chromosome ends had Y′s. They could also be the result of dynamical movement of Y′s. The existence of other telomere-associated repeats, X and $(C_{1-3}A)_n$ (CHAN and TYE 1983; WALMSEY *et al.* 1984), as well as autonomously replicating circular forms (HOROWITZ and HABER 1985) may provide the vehicles for movement to and from locations. Such movement of telomere associated sequences is thought to account for *SUC* gene family evolution (CARLSON, CELENZA and ENG 1985). Other than *SUC2*, which is located in unique

chromosomal sequence, the other members of the *SUC* gene family reside between X and Y′ sequences. The locations of these vary between strains.

The structures of the Y′ families in the two strains have interesting features. Several of the Y′ bearing chromosome ends in YP1 have tandem copies whereas none of the Y′s in Y55 are tandemly arrayed. None of the tandem arrays in YP1 are mixed with respect to the short and long versions. Given the number of short and long versions of Y′s in YP1 and the number in tandem arrays, a random distribution of Y′s should yield mixed arrays. None of the tandem arrays appear mixed for any of the observed Y′-long restriction fragment length variations, whereas Y′-longs at different locations had *Asp*718 fragment variation (Figure 3). The observed distribution of long and short versions in the tandem arrays is analogous to primate rDNAs which are tandemly arrayed near the telomeres of six chromosomes. These arrays exhibit within-array homogeneity and between-array heterogeneity (ARNHEIM 1983).

There is apparent "concerted" evolution of Y′s in that there is conservation of restriction sites among Y′s within each strain. For all the Y′s of YP1 and the ten complete Y′s of Y55, ten restriction sites were checked (Figure 5A). In addition, the 11 marked Y′s of YP1 were individually mapped for at least six of these enzymes. The ability to observe a single site missing in one Y′ on a genomic Southern is seen in the *Asp*718 fragments of individual Y′-shorts. Among these sampled restriction sites only a *Pvu*I polymorphism was found in marked YP1 Y′s. For Y55, there is at least one Y′ that is missing one of the *Asp*718 sites and the *Eco*RI and *Eco*RV sites. These three sites could all be in one variant Y′ or three different Y′s could each be missing one site.

This minimal one base pair difference for the *Pvu*I polymorphism in YP1 is equivalent to that found for single copy sequence divergence between strains. This divergence is for the entire family of 26 to 30 Y′s, however, any pair of which would be expected to vary by roughly 1% if they evolved independently based on the unique sequence differences. A Y′-long and a short version have been cloned and analyzed to confirmed restriction site conservation on both sides of the insertion-deletion polymorphism. Sequencing of these clones will yield more definitive information on sequence homogeneity as well as an understanding of the insertion-deletion polymorphism and the observed homology of Y′-short sequence to sequences internal to the deletion.

There was very little variation in restriction fragment length for internal restriction sites among YP1 Y′s. Most of the differences can be attributed to different copy numbers of the 36-bp repeats (HOROWITZ and HABER 1984). Direct evidence of variation in copy number of the 36-bp repeats comes from sequence data. HOROWITZ and HABER (1984) sequenced a 12 copy 36-bp region and in another Y′ clone, found 15 copies (H. HOROWITZ and J. E. HABER, unpublished results). The Y′ sequences in Y55 differ from those in YP1 in restriction fragment length and there is more fragment length variation within Y55 than within YP1. The seven Y′-longs fall into four restriction fragment size classes for 36 bp repeat homologous *Asp*718 fragments. This contrasts with the same number of size classes found for the two to three times as many Y′-longs of YP1. These differences are not all attributable to variation in the 36-bp repeat region. Additional fragment length variation is found in the telomere distal side of Y55 Y′s (Figure 5B).

There are also degenerate Y′s in Y55 that have homology with only one of the two Y′ probes used in this analysis. This degeneracy is likely to be due to missing sequences rather than few copies of the 36-bp repeats as a 1-kb probe telomere proximal to the 36-bp region also fails to hybridize to these degenerate Y′s. There are many possible explanations for the structural differences in the two Y′ families. The differences may reflect different evolutionary histories. The divergent subsets of Y′s in Y55 may represent "escapes" from the overall homogenization processes (WALSH 1987) though within subsets, homogenization may still be occurring. The Y′s of YP1 may not have had enough time to accumulate the differences necessary for this "escape."

The Y′ repeated sequence family is similar to other repeated sequence families in *S. cerevisiae*. Y′s share the dispersed nature of Tys and in some strains the tandem nature of rDNA genes. There is internal homogeneity within all three repeated sequence families. The Y′s vary in copy number and location as do the dispersed Tys. Tandem Y′s are similar to rDNAs in that they are homogeneous within an array. It is not possible to compare the rates of divergence of the different repeat families between strains with the data presented.

In the accompanying paper (LOUIS and HABER 1990), recombinational interactions among Y′s in YP1 are studied. These interactions may explain the homogenization of Y′s within a strain, even at different chromosomal locations as well as the copy number and location differences between strains. The well characterized Y′ family in YP1 along with the short term recombinational interactions among Y′s presented in the accompanying paper provide the opportunity to predict long term consequences of recombination among Y′ repeats which can then be tested by observing long term Y′ dynamics in long term cultures. An understanding of Y′ structure and evolu-

tion, as well as repeated sequence evolution, will come out of this combined short- and long-term approach.

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