Mitotic Recombination Among Subtelomeric Y′ Repeats in *Saccharomyces cerevisiae*

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ABSTRACT

Y′s are a dispersed family of repeats that vary in copy number, location and restriction fragment lengths between strains but exhibit within-strain homogeneity. We have studied mitotic recombination between members of the subtelomeric Y′ repeated sequence family of *Saccharomyces cerevisiae*. Individual copies of Y′s were marked with *SUP11* and *URA3* which allowed for the selection of duplications and losses of the marked Y′s. Duplications occurred by ectopic recombinational interactions between Y′s at different chromosome ends as well as by unequal sister chromatid exchange. Several of the ectopic duplications resulted in an originally Y′-less chromosome end acquiring a marked Y′. Among losses, most resulted from ectopic exchange or conversion in which only the marker sequence was lost. In some losses, the chromosome end became Y′-less. Although the two subsets of Y′s, Y′-longs (6.7 kb) and Y′-shorts (5.2 kb), share extensive sequence homology, a marked Y′ recombines highly preferentially within its own subset. These mitotic interactions can in part explain the maintenance of Y′s and their subsets, the homogeneity among Y′s within a strain, as well as diversity between strains.

THE repeated sequence families ubiquitous among eukaryotic genomes are puzzling in several respects. Repeated sequences within a population or species generally share a greater degree of homogeneity than expected for independent evolution of members of the family. This "concerted" evolution has been attributed to homogenization caused by recombination (including gene conversion) between members of the repeated sequence family even at different chromosomal locations [see ARNHEIM (1983) for review]. The observed sequence homogeneity could also be due to rapid turnover of divergent copies via transpositional duplication and segregation (SELKER *et al.* 1981).

Reciprocal recombination and gene conversion between repeats in different chromosomal locations (ectopic), and unequal exchanges within tandem arrays of repeats have been observed in many organisms. Most of what is known about repeated sequence interactions comes from experiments with microorganisms [see PETES and HILL (1988) for review]. Unequal exchanges have been observed in the tandemly arrayed rDNA genes of *Saccharomyces cerevisiae* (PETES 1980; SZOSTAK and WU 1980) as well as in tandem arrays of *CUP1* (FOGEL, WELCH and LOUIS 1984). Ectopic gene conversions and exchanges have been observed in the dispersed Tys of *S. cerevisiae* (CHALEFF and FINK 1980; LIEBMAN, SHALIT and PICOLOGLOU 1981; ROEDER and FINK 1983) as well as dispersed tRNAs of *Schizosaccharomyces pombe* (MUNZ and LEUPOLD 1981; KOHLI *et al.* 1984; AMSTUTZ *et al.* 1985; HEYER *et al.* 1986; SZANKASI *et al.* 1986). Among artificially created duplications both intrachromosomal and interchromosomal ectopic gene conversions and exchanges have been observed (SCHERER and DAVIS 1980; JACKSON and FINK 1981; KLEIN and PETES 1981; SUGAWARA and SZOSTAK 1983; BORTS *et al.* 1984; KLEIN 1984; JACKSON and FINK 1985; JINKS-ROBERTSON and PETES 1985, 1986; BORTS, LICHTEN and HABER 1986; FASULLO and DAVIS 1987; LICHTEN, BORTS and HABER 1987; MALONEY and FOGEL 1987; WILLIS and KLEIN 1987; LICHTEN and HABER 1989).

The rates of various types of recombinational interactions during mitosis and meiosis in the above experimental systems have been measured. These experimental systems are limited, however, in that they can only monitor a subset of the possible interactions among the entire repeated sequence family, or they cannot yield information on long-term dynamics of recombinational interactions. In order to understand repeated sequence evolution, a system is needed with repeats that are easily manipulated experimentally, and in which recombination can be monitored over both short and long-term time scales.

The Y′ repeated sequence family adjacent to some telomeres of *S. cerevisiae* is used here as a model system

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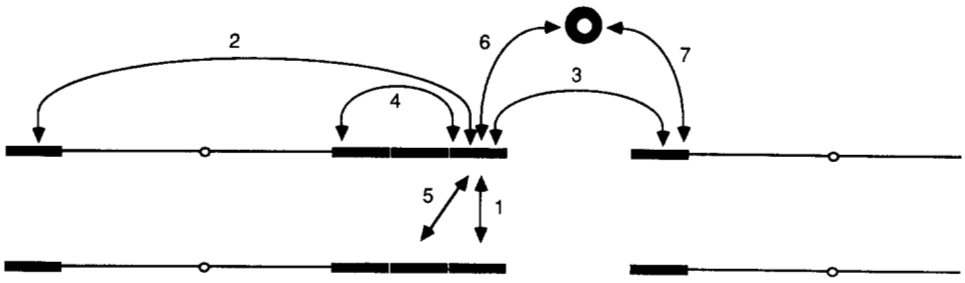

FIGURE 1.—The possible recombinational interactions between repeated sequences. (1) Allelic, (2) ectopic-intrachromosomal, (3) ectopic-interchromosomal, (4) unequal within an array-intrachromatidal, (5) unequal within an array-interchromatidal, (6) the generation of autonomously replicating circular forms, and (7) integration or transfer of sequence from circular free forms to resident chromosomal repeats.

for studying repeated sequence interactions and evolution. LOUIS and HABER (1990) characterized the following features of Y′ families in two yeast strains. (1) The two strains diverge greatly in terms of copy number, location and restriction fragment lengths of their Y′s. (2) Within each strain the Y′s are more homogeneous. Strain YP1 exhibits very little sequence or restriction fragment length variation among Y′s. The five tandem arrays of Y′s are either all Y′-longs or all Y′-shorts. Strain Y55 exhibits somewhat more heterogeneity in restriction fragment lengths and has at least one Y′ that varies at several restriction sites. Y55 also has at least four degenerate Y′s that are apparently missing distal sequences.

Ectopic recombination between Y′s has been observed both mitotically (DUNN *et al.* 1984) and meiotically (HOROWITZ, THORBURN and HABER 1984). Y′s have also been shown to be able to exist as autonomously replicating circles which can integrate into resident Y′s at different chromosome ends (HOROWITZ and HABER 1985).

As a first step toward understanding the means by which homogeneity of repeated sequence families is preserved, we have characterized the types of mitotic recombination events that can occur among Y′s. The possible types of Y′ interactions are represented in Figure 1. These include allelic and ectopic recombination, unequal interactions within a tandem array, and the generation and reintegration of autonomously replicating circular forms. Using genetically marked Y′s, recombinational interactions among Y′s are detectable and selectable. Here we describe the distribution of recombinational interactions among Y′s in a well characterized strain (LOUIS and HABER 1990) with the hope of formulating hypotheses to explain the evolution of Y′s and repeated sequences in general. Our goal is to test predictions of such hypotheses with long term cultures under controlled conditions.

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). SC media with a limiting concentration of adenine (5 μg/ml) was prepared as described in HIETER *et al.* (1985). Media containing 5-fluoro-orotic acid was prepared as described in (BOEKE, LACROUTE and FINK, 1984).

Plasmids and construction: Plasmids pHinfI1–6, pEL2, pEL16 and pHH4 are described in the accompanying paper (LOUIS and HABER 1990) and are illustrated in Figure 2. The plasmid pEL24 contains *URA3* and *LEU2* sequences flanking the 36 bp repeats of Y′ with additional Y′ sequence flanking the *LEU2* and *URA3* sequences. The *LEU2* sequence of pEL24 is *Cla*I$^-$. The *Cla*I digested ends were blunt ended with the large fragment of DNA polymerase I and ligated together to disrupt the coding sequence. This plasmid is derived from an *Asp*718 fragment from pHH4 containing Y′ sequences with *URA3* adjacent to the 36-bp repeats on the telomere proximal side. The 2.2-kb *Xho*I to *Sal*I fragment, with blunted ends, containing *LEU2* was inserted at the *Pvu*I site (blunt ended) of Y′ sequence adjacent to the 36-bp repeats on the telomere distal side. pRHB7, obtained from R. H. BORTS, contains 400 bp of *LEU2* sequence from *Kpn*I to *Eco*RI and is used as a marked Y′ specific probe. pYeMET14-27, containing *MET14* and *CEN11*, was also used as a probe (FITZGERALD-HAYES *et al.* 1982).

Strains and construction: All strains used are listed in Table 1. YP1 and YP3 are related strains (HIETER *et al.* 1985). ELT2.3, ELT2.5, ELT2.9, and ELT2.15 are transformants of YP1 (LOUIS and HABER 1990) and have single marked Y′s in different chromosomal locations (ends E5, E9, E21 and E11 respectively, Figure 3). Each has the *SUP11, URA3* and pBR322 insertion from pEL2 (Figure 2). The marked Y′s are solo Y′s, with no unmarked adjacent Y′s as determined by Southern analysis. ELT2.15 is a marked Y′-short whereas the other three are Y′-longs. EJL8-4B was obtained by successively backcrossing a *MATα* segregant of YP3 to YP1. The recessive drug resistance mutations in *CAN1* and *CYH2* were obtained in YP1 spontaneously and via UV mutagenesis respectively. The diploids EJL40, EJL41, EJL49 and EJL53 are heterozygous for each of ELT2.3, ELT2.5, ELT2.9 and ELT2.15 respectively and were made by crossing the transformant to the *MATα* congenic strain EJL8-4B. EJL77-7r is congenic with ELT2.3 and has the spontaneous antisuppressor *ASUx*. This strain was pBR322$^+$, Ura$^+$ but Ade$^-$. Genetic analysis showed that the phenotype was due to a single mendelian locus unlinked to the *SUP11*-bearing Y′. Further analysis with other ochre suppressors and other ochre mutations resulted in phenotypes similar to previously described anti-suppressors of *SUP11* (MCCREADY and COX 1973, 1976). *ASUx* is not centromere linked nor is it linked to any of the markers used in this study.

The presence of *SUP11* causes meiosis I nondisjunction (LOUIS and HABER 1989) and viability problems presumably due to mistranslation products. In order to measure some

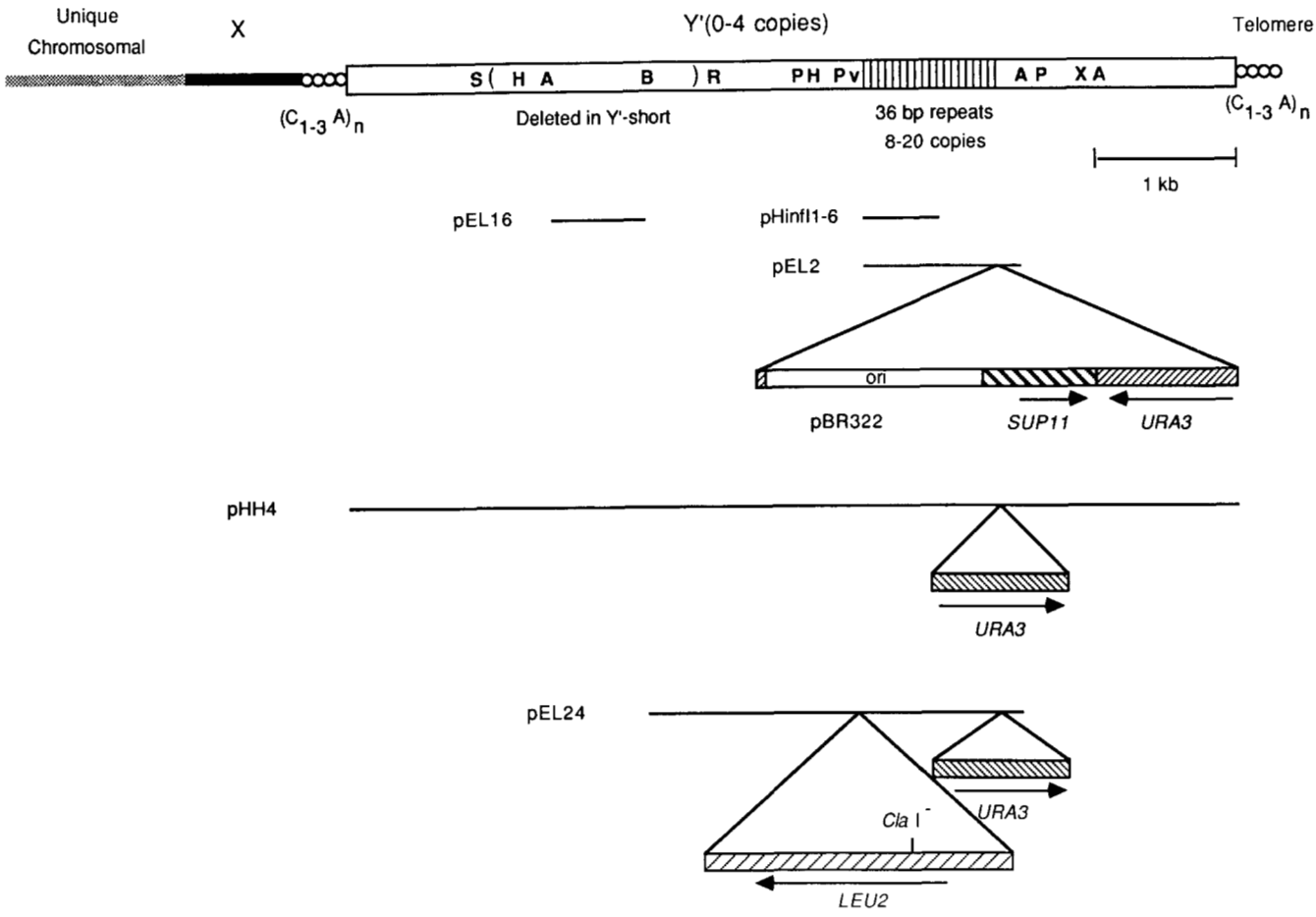

FIGURE 2.—Structure of the chromosome ends of *S. cerevisiae*. Adjacent to the unique sequences (shaded) at the end of a chromosome are X sequences (solid), which comprise a not very highly conserved repeated sequence family. Adjacent to X, on some chromosome ends are 1 to 4 tandem copies of Y′ sequences (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 telomere proximal *Asp*718 (A) sites of Y's. The sequences within 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. pHinfI1–6 contains Y′ sequence from the *Pvu*I site through six to seven 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. pHH4 contains an entire Y′ with *URA3* inserted at the telomere proximal end of the 36-bp repeats. pEL24 contains the *Asp*718 *URA3*-containing Y′ fragment from pHH4 with an insertion of *leu2-cla*I⁻ at the *Pvu*I site.

TABLE 1

Strains

Name	Genotype
YP1	*MAT***a** *ura3-52 ade2-101 lys2-801*
YP3	*MAT***a** *ura3-52 ade2-101 lys2-801* / *MATα ura3-52 ade2-101 lys2-801*
EJL8-4B	*MATα ura3-52 ade2-101 lys2-801 can1*^{R} *cyh2*^{R}
EJL363-6D	*MATα leu2-Δ ura3-52 ade2-101 lys2-r2 cyh2*^{R}
ELT24.12	EJL363-6D with *leu2-URA3* marked Y′ at chromosome *V* or *VIII*
ELT24.17	EJL363-6D with *leu2-URA3* marked Y′ at chromosome *IX*
EJL77-7r	*MAT***a** *ASUx ura3-52 ade2-101 lys2-801 can1*^{R} *cyh2*^{R} *SUP11 URA3* pBR322 marked Y′ at chromosome end E5
EJL40	YP1 × EJL8-4B heterozygous for *SUP11 URA3* pBR322 marked Y′ at chromosome end E5
EJL41	YP1 × EJL8-4B heterozygous for *SUP11 URA3* pBR322 marked Y′ at chromosome end E9
EJL49	YP1 × EJL8-4B heterozygous for *SUP11 URA3* pBR322 marked Y′ at chromosome end E21
EJL53	YP1 × EJL8-4B heterozygous for *SUP11 URA3* pBR322 marked Y′ at chromosome end E11

interactions without the effects of *SUP11*, the following strains with *URA3*-marked Y's were constructed. EJL363-6D is isogenic to YP1 and was constructed by mutagenesis and transformation as described in (LOUIS and HABER 1989). ELT24.12, and ELT24.17 were constructed by transplacement (ROTHSTEIN 1983) of resident Y′ sequences in EJL363-6D using the *Bam*HI (one site within the Y′ sequence and one site adjacent to the end of Y′ in pEL24) fragment of pEL24 and lithium acetate transformation (Ito *et al.* 1983).

Selection for increased dosage of marked Y's: Selection for two copies of marked Y's from strains with one copy was based on the phenotypes (displayed in Table 2) associated with the *SUP11* and *ASUx* marked strains. The haploid EJL77-7r is Ade$^-$, Ura$^+$ and has one copy of the marked Y′ in an *ASUx* background. Two copies of *SUP11* can overcome the antisuppressor effect of *ASUx*. This strain was subcloned onto non-selective, YEPD, media. Independent subclones were patched onto YEPD and after growth overnight at 30° were replica plated onto adenineless media. After 3–4 days, Ade$^+$ papillae were isolated for analysis as described below. These Ade$^+$ derivatives should include strains with two copies of the *SUP11*-marked Y′.

The diploid strains heterozygous for a *SUP11*-marked Y′, EJL40, EJL41, EJL49 and EJL53, are Ade$^-$ as well. As with the *ASUx* haploid, two copies of *SUP11* in a diploid make the strain prototrophic for adenine (Table 2). These four diploids were subcloned on YEPD media. Independent subclones were replicated to adenineless media and Ade$^+$ papillae were isolated for analysis as described below. The Ade$^+$ derivatives from these strains can include homozygoses of the marked Y′ via mitotic crossing over between the centromere and the chromosome end and subsequent segregation or mitotic conversion as well as marked Y′ duplications via the recombinational interactions (2)–(7) shown in Figure 1. Mitotic nondisjunction could also lead to $(n + 1)$ or $(2n + 1)$ Ade$^+$ papillae. These homozygoses and nondisjunctions can be screened for as described below.

Selection for marked Y′ loss: Mitotic losses of marked Y's were isolated in one of two ways. In haploid Ade$^+$ derivatives which had an ectopic second marked Y′ (as selected above), red Ade$^-$, Ura$^+$ derivatives of the pink Ade$^+$, Ura$^+$ strains were isolated after screening subclones on synthetic complete media with a limiting concentration of adenine. These derivatives were analysed for loss of one of the two marked Y's as described below.

Losses were also selected using 5-fluoro-orotic acid to select against *URA3* function. Haploids with a single copy of a Y′::*SUP11* and *URA3* were subcloned on YEPD media and replica plated to synthetic complete media. These were then replica plated onto media containing 5-fluoro-orotic acid to select for Ura$^-$ derivatives. 5-fluoro-orotic acid resistant papillae were isolated after 3–4 days. These Ura$^-$ derivatives were analyzed for presence of pBR322 sequence and for Y′ sequence at the originally marked chromosome end as described below.

Haploid strains with a single Y′::*URA3* and *leu2* were subjected to the same selection. These strains were used in order to circumvent any deleterious effects of *SUP11*. Independent Ura$^-$ subclones were analyzed for the presence of *LEU2* and *URA3* sequences and for Y′ sequences at the originally marked chromosome end.

Genetic analysis of Ade$^+$ derivatives: For the diploids EJL40, EJL41, EJL49 and EJL53, each Ade$^+$ derivative was sporulated and dissected. For the Ade$^+$ derivatives of EJL77-7r, each was crossed to a nonsuppressor strain and the resulting diploids were sporulated and dissected. The segregation of adenine and uracil prototrophy was followed and could be used to help distinguish between different types of events leading to adenine prototrophy. For example, the homozygosing of a marked Y′ in EJL40 would result in tetrads that were 4:0 Ade$^+$, Ura$^+$ and pink. An ectopic second copy of the marked Y′ would result in mixed 4:0, 3:1 and 2 Ade$^+$ Ura$^+$:2 Ade$^-$ Ura$^-$. One of the Ade$^+$ spores in the 3:1 case and both of the Ade$^+$ spores in the 2:2 case would be white rather than pink due to having two copies of *SUP11*. A tandem duplication at the marked end would result in tetrads with 2 Ade$^+$ Ura$^+$ white:2 Ade$^-$ Ura$^-$ red spores. Similar association of phenotypes with genotypes can be made for other possibilities and for the strains with *ASUx*. Not all possibilities are distinguishable by genetic analysis alone. A reversion of *ASUx* in an EJL77-7r derivative would yield the same segregation pattern as a tandem duplication upon test crossing.

Chromosome analysis of marked Y′ gains and losses: Chromosomes from 5-ml overnight cultures of strains with possible Y′ duplications and losses were prepared and analyzed on chromosome separating gels as described in a companion study (LOUIS and HABER 1990). Separated chromosomes were probed with pBR322, pEL16, pHinfI1–6 and pRHB7. pBR322 was used as a marked Y′-specific probe in strains with the *SUP11* marker system while pRHB7 was used as the marked Y′-specific probe in strains with the *leu2-URA3* marker system. Ectopic gains in dosage of the marked Y′ are identified by the presence of a new marked Y′-specific homologous chromosome band. Tandem duplications, ectopic events involving the other end of the same chromosome, homozygoses and mitotic nondisjunctions would have no new marker-specific homologous chromosome band. The presence or absence of unmarked Y's at a given chromosome was determined with pEL16 and pHinfI1–6 for the selected marked Y′ losses.

Southern analysis of marked Y′ gains and losses: Figure 4 displays the diagnostic restriction enzyme digestions for determining the presence and type of the marked Y′ gain. For the *SUP11* marker system, restriction enzyme digestion with each of *Pvu*II, *Xho*I, *Eco*RI and *Bam*HI along with the genetic and chromosome analysis information, uniquely identifies the type of marked Y′ gain present. Tandem duplications, ectopic second copies and marked Y′ circles can all be distinguished and the presence of adjacent unmarked Y's as well as size class can be determined. For example, a tandem duplication of a Y′-long would result in new *Xho*I, *Bam*HI and *Eco*RI fragments, each of the same 10.5-kb size (one unit marked Y′) along with the original fragments. The original *Pvu*II fragment would be replaced by one 10.5 kb larger. The generation of a Y′-circle would look the same except that the original *Pvu*II fragment would still be present and the new *Pvu*II fragment size would depend on the state of the circular DNA molecule. An ectopic duplication resulting in an unlinked new marked Y′-long would result in new *Xho*I and *Pvu*II fragments and possibly new *Eco*RI and *Bam*HI fragments depending on the presence of adjacent unmarked Y's (Figure 4). Other possibilities are also distinguishable. Cases of an internally adjacent unmarked Y′ cannot always be unambiguously determined as it depends on the new *Xho*I fragment size. A *Xho*I site in the unique or X sequences adjacent to the marked Y′ can result in a *Xho*I fragment of the same size as that of an adjacent unmarked Y′. Homozygoses and mitotic nondisjunctions would have no new marker specific bands upon Southern analysis.

Measurement of rates of Y′ interactions: Rates of mitotic Y′ gain and loss were measured using the median method of (LEA and COULSON 1948). Two day old independent colonies, grown non-selectively, from a given strain,

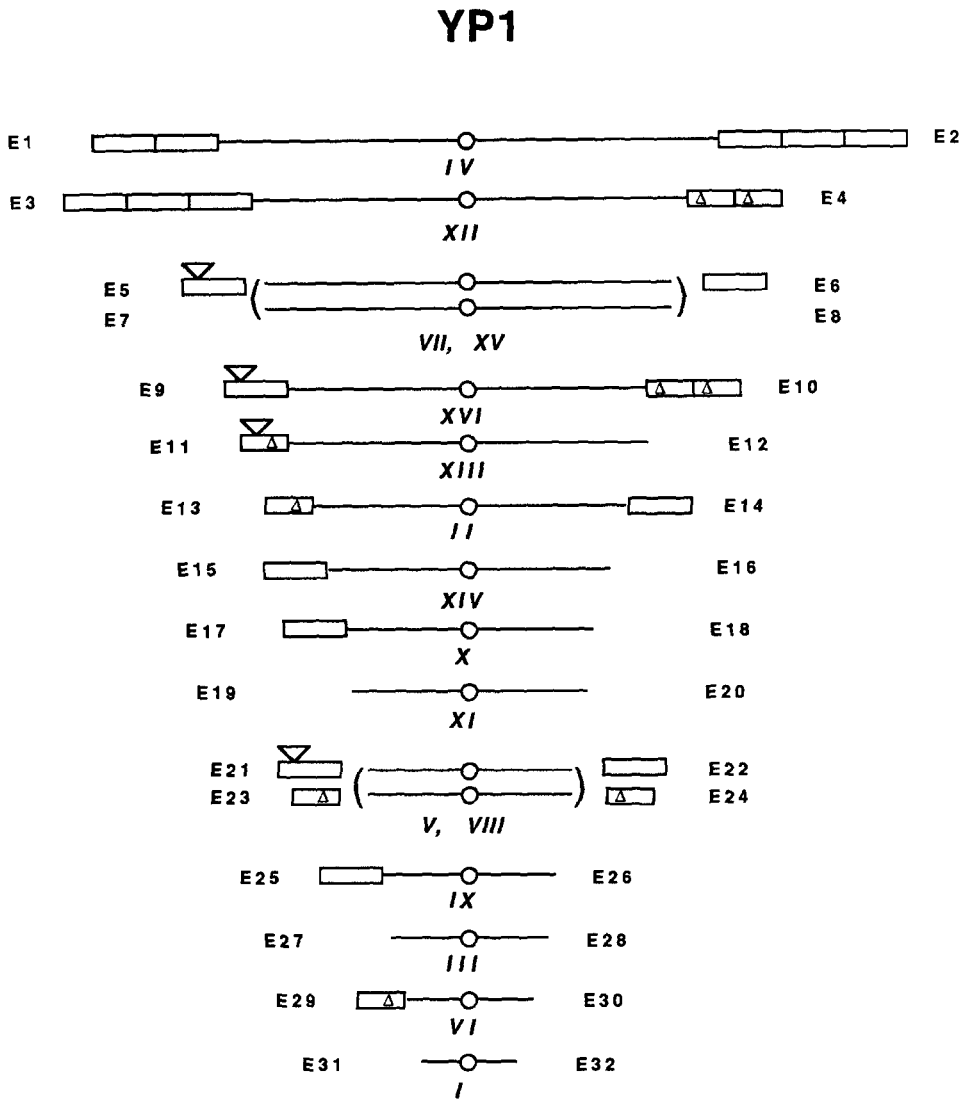

FIGURE 3.—The Y′ family of strain YP1. The locations of Y′-longs and Y′-shorts are shown. 19 of the 32 ends bear Y′s, five of these have tandem arrays. Each chromosome end is labelled E1 through E32. The left and right end designations for each end are not known though the two ends are distinguished by Southern analysis. The four marked Y′s in this study are shown. The marked Y′-long at end E5 is used in strains EJL77-7r and EJL40. The marked Y′-long at end E9 is used in strain EJL41. The marked Y′-short at end E11 is used in strain EJL53 and the marked Y′-long at end E22 is used in strain EJL49.

were resuspended and plated onto selective media. Dilutions were also plated in order to count the number of cells in the colony. The number of colonies on the selective media were used to measure the proportion of the colony that had the selective event (duplication or loss). Large differences in growth rates and viabilities of strains with different dosages of *SUP11* were noted (data not shown). These differences are consistent with the known selective disadvantage of increased copies of *tRNA* suppressors (HIETER *et al.* 1985). The measurement of mitotic loss of marked Y′s was carried out in nonsuppressor strains as well.

RESULTS

Marked Y′s can duplicate: The strain YP1 has Y′ sequences at 19 of the 32 chromosome ends. No evidence was found for Y′ sequences at other than telomeric locations in this strain (LOUIS and HABER 1990) nor has any Y′ sequence been found that is not associated with other telomere-associated sequences (SZOSTAK and BLACKBURN 1982; CHAN and TYE 1983; WALMSEY *et al.* 1984). The 32 chromosome ends are labeled E1–E32 for convenience (Figure 3). No distinction is made for left and right arms of each chromosome (LOUIS and HABER 1990), however, the two ends are distinguishable by restriction fragment lengths of marked Y′s.

Duplications of a single *SUP11* marked Y′ were selected on the basis of the ability of two copies of *SUP11* to overcome the adenine auxotrophy of a single copy, as described in MATERIALS AND METHODS. Four different marked Y′s at different locations (chromosome ends E5, E9, E11 and E21) were used in the analysis, and each was the only Y′ at that chromosome end (Figure 3). One of the marked Y′s (at end E11 in EJL53) was a Y′-short (5.2 kb), whereas the rest were Y′-longs (6.7 kb). There were 17 gains in marked Y′ copy isolated from the haploid EJL77-7r and a total of 72 isolated from the diploids EJL40, EJL41, EJL49 and EJL53. The 17 events from the haploid strain EJL77-7r were not different in distribution from the 24 events isolated from diploid strain EJL40, which has the same marked Y′ at chromosome end E5. The

TABLE 2

Selection for marked Y′ duplications and losses

Ploidy	Genotypes (all strains *ade2-101*, *ura3-52*) Copies of Y′::*SUP11 URA3*	*ASUx*	Phenotypes Auxotrophies	Color
Haploids	0	Yes/no	Ade⁻ Ura⁻	Red
	1	Yes	Ade⁻ Ura⁺	Red
	2	Yes	Ade⁺ Ura⁺	Pink
	3	Yes	Ade⁺ Ura⁺	White
Diploids	1	No	Ade⁻ Ura⁺	Red
	2	No	Ade⁺ Ura⁺	Pink

Selection of marked Y′ duplications:

Haploids: ASUx Y′::*SUP11 URA3* (1 copy) under selection for adenine prototrophy yields Y′::*SUP11 URA3* (2 copy) derivatives.

Diploids: Y′::*SUP11 URA3* (1 copy) under selection for adenine prototrophy yields Y′::*SUP11 URA3* (2 copy) derivatives.

Selection for marked Y′ losses in haploids:

1) Y′::*SUP11 URA3* (2 copies) under screen for reds yields Y′::*SUP11 URA3* (1 copy) derivatives.
2) Y′::*SUP11 URA3* (1 copy) under selection for 5-fluoro-orotic acid resistance yields zero marked Y′ derivatives.
3) Y′::*leu2 URA3* (1 copy) under selection for 5-fluoro-orotic acid resistance yields zero marked Y′ derivatives.

marked Y′ in EJL41 is at end E9 whereas the marked Y′ in EJL49 is at end E21.

Figure 5 shows the marked Y′ homologous chromosomes for some of the selected duplications from EJL7-7r. In each case the originally marked chromosome band (*VII* and *XV*) has pBR322 homology as does the newly marked chromosome. A summary of the selected duplication events in marked Y′s is given in Table 3.

In each case of diploid-selected events, the presence of two copies of marked Y′s could arise by mechanisms other than ectopic recombination and unequal exchange. Homozygosis of the marked Y′ via mitotic recombination or by mitotic nondisjunctions in fact accounted for 60–70% of the Ade⁺ papillae. The significance of similar rates of recombination among Y′s and mitotic homozygosis is discussed later. Mitotic homozygoses and nondisjunctions were screened away from the other Y′ duplications by genetic and Southern blot analysis and are not included in the data of Table 3.

In the haploid-selected events, approximately 50% of Ade⁺ papillae had no second copy of a *SUP11* marked Y′. Because these could be reversions of *ASUx* or some other modifying mutation, they were excluded from further analysis. Many previously described antisuppressors are unstable (McCready and Cox, 1973, 1976) and strains bearing them tend to accumulate modifying mutations (B. Cox, personal communication).

Single copy Y′s can become tandemly arrayed: In 21 of the 89 (24%) mitotic Y′ duplications, the second copy of the marked Y′ resulted from a tandem duplication as confirmed by genetic, chromosome and Southern analysis (data not shown). Such duplications most likely result from unequal sister chromatid exchange involving the ($C_{1-3}A$) repeats that flank all Y′s. In each case the original marked Y′ was not adjacent to any other Y′ sequence (Figure 3). In four of the cases the apparent duplication proved to be a tandem triplication of the marked Y′ as determined by Southern analysis.

Y′s can interact ectopically with other Y′s: In 68 of the 89 events (76%), the second marked Y′ was on another chromosome end. In all but three cases, discussed below, these second marked copies occurred at chromosome ends that previously had Y′s. In 29 of the 65 ectopic events involving another Y′-bearing chromosome, the new second copy was not adjacent to any other unmarked Y′ while in 29 cases the new marked Y′ was adjacent to unmarked Y′s. The 7 others could not be determined unambiguously, as the *Xho*I fragment size was consistent with either no adjacent unmarked Y′ or an internally adjacent unmarked Y′ (Figure 4). In 28 of the 29 cases in which the marked Y′ was adjacent to an unmarked Y′, the original recipient chromosome end had two or more Y′s. The remaining case may have involved actual duplication of Y′ sequences. The 29 cases with no adjacent unmarked Y′s all involved chromosome ends originally having only one Y′. Thus, in all but one case, there was no net gain or loss in Y′ copy number, only the gain of the *URA3-SUP11* insertion.

In some of the diploid-selected events, there was both an ectopic recombination and homozygosis of either the original marked chromosome end or the new marked chromosome end. In one case there were two ectopic recombination events and in one other the ectopic marked Y′ was tandemly duplicated.

Size and location dependence of Y′ recombinational interactions: The ectopic events exhibited a significant bias in terms of where the new second copy appeared. A marked Y′-long interacted with other Y′-longs for the most part (45 out of 49 cases) and similarly for Y-shorts (12 out of 16 cases). The observed frequency of 8 of 65 interactions between Y′s of different size classes is considerably less than expected for random size class interactions. A marked Y′-long has 16 to 20 other Y′-longs to interact with and 9 Y′-shorts. There should be minimally a 31% chance of interacting with a Y′ short. The 4 observed versus the 15 expected is significantly different ($P < 0.05$, by χ^2). Similarly a marked Y′-short should minimally have a 64% chance of interacting with a Y′-long if partner choice were random. The 4 observed versus the 10 expected is again significantly different ($P < 0.05$).

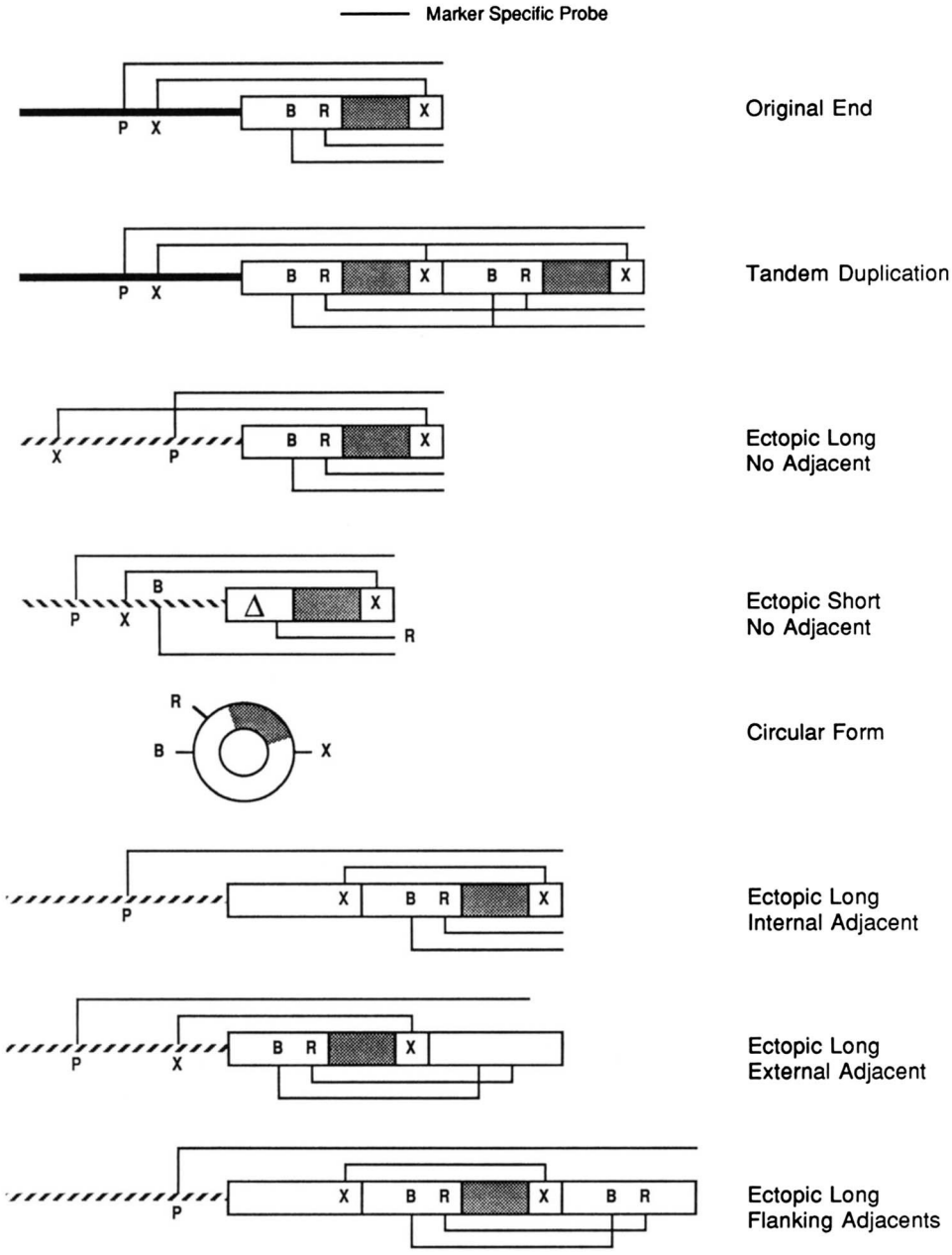

FIGURE 4.—Diagnosis of new copies of marked Y's. Many of the possible outcomes of Y′ recombination are displayed. Restriction digestion with each of *Eco*RI (R), *Bam*HI (B), *Xho*I (X) and *Pvu*II (P) and probing with pBR322 (marked Y′ specific) can help identify the type of recombination event that resulted in the second copy. In each case the originally marked Y′ is retained in the selection scheme so that the restriction fragment sizes associated with that end are also retained. A tandem duplication of a Y′ long will result in additional *Eco*RI, *Bam*HI and *Xho*I fragments all of the same unit marked Y′ size. The original *Pvu*II fragment is replaced by one that is one marked Y′ unit larger. An ectopic second copy that is now a Y′-short will result in additional *Xho*I, *Pvu*II and *Bam*HI fragments but no additional *Eco*RI fragment. Other events lead to different outcomes which along with the genetic and chromosomal analysis, uniquely define the location and context of the new marked Y′ (see text for details).

Y′s can move to new Y′-less locations: In three cases, the new second copy of the marked Y′ was located on one end of chromosome *XI*. Chromosome *XI* originally had no Y′ sequence as confirmed by probing with pHH4 which has an entire Y′ (Figure 6). In each case the novel Y′ had no adjacent unmarked Y′s and they all had the same restriction fragment sizes (data not shown). There were two different donors, at ends E5 and E21, both of which were marked Y′-longs (Table 3). The new marked end did not have the same *Xho*I and *Pvu*II restriction fragment sizes as the original donor ends (data not shown). The newly marked end from one of the three events was segregated away from the original marked copy for further analysis.

Y′-bearing chromosome ends can become Y′-less: Losses of marked Y′ information were obtained using 5-fluoro-orotic acid to select against *URA3* function as described in the MATERIALS AND METHODS. Chromosome *XI* was used in this analysis as it is easily separated on chromosome gels and has no other Y′s. A chromosome *XI* marked haploid strain was subjected to selection for loss of the Y′::*SUP11* and *URA3*. Each independent Ura$^-$ derivative was subjected to chromosome analysis. Separated chromosomes were probed first with a Y′-specific probe (Figure 7) and then with pBR322 (data not shown) to ask whether the marked Y′ was replaced with another unmarked Y′ and whether the uracil auxotrophy was due to something other than loss of the marker sequence. Figure 7 displays chromosomes from some of the

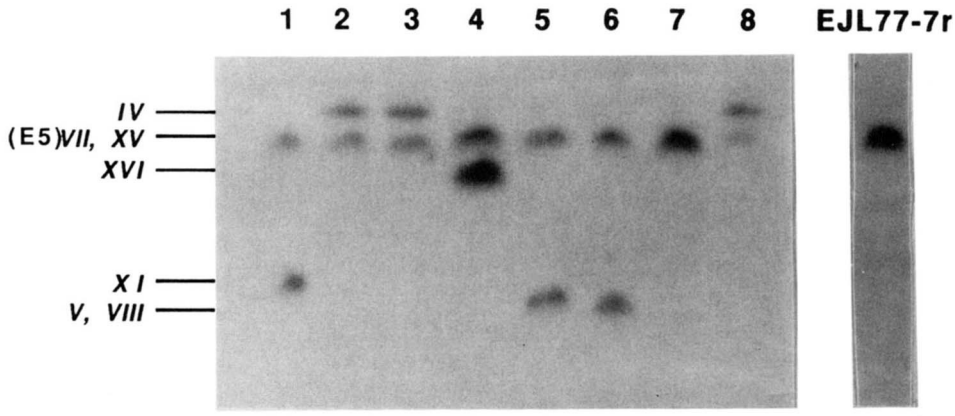

FIGURE 5.—Chromosome analysis of selected increases in marked Y′ copy number. Chromosomes of eight Ade$^+$ derivatives from EJL77-7r, originally having end E5 marked with *SUP11*, *URA3* and pBR322 are probed with pBR322 to determine the chromosomal location of the second copy of the marked Y′. All have pBR322 homology in the chromosome *VII*, *XV* band. In three of the events the second copy is at one or the other end of chromosome *IV* (lanes 2, 3 and 8). In two, the second copy is at one of the ends of chromosomes *V* or *VIII* (lanes 5 and 6). In one there is no new pBR322 homologous chromosome band but there is an ectopic second copy so it must reside at an end other than E5 in chromosome *VII* or *XV* (lane 7). In one case, the second copy is on chromosome *XVI* (lane 4). In one case to be discussed in detail in the text, the second copy is at one end of chromosome *XI*, a previously Y′-less location.

selected losses at chromosome *XI* which are probed with pEL16. In 9/76 (11.8%) cases in which the marker sequence was lost (no pBR322 homology), the chromosome end became Y′-less (lower arrow, Figure 7). The other 67 events had unmarked Y′s at that end. Two of the cases in which an unmarked Y′ remained at chromosome *XI* had coincident loss of Y′ sequence at chromosome *XIV* (see upper arrow, Figure 6).

A similar analysis was performed with ELT24.17 which has a single Y′ on chromosome *IX* marked with *leu2-URA3*. Again, 5/40 (12.5%) losses of the marked Y′ were now Y′-less at chromosome *IX* while the rest had unmarked Y′ sequence at the originally marked end.

The homology of Y′-shorts with pEL16, a probe derived from the sequences deleted in Y′-shorts (LOUIS and HABER 1990), precluded the determination of the size class of the unmarked Y′ that replaced the marked Y′. In two cases the Ura$^-$ derivative still had pBR322 and *URA3* sequence homology. This could have resulted from a mutation in the *URA3* gene of the marked Y′ or possibly conversion of it to *ura3-52* via interaction with the resident *ura3-52*. These possibilities were not distinguished.

Tandem duplications can expand and contract: A tandem duplication at end E5 from an Ade$^+$ derivative of EJL77-7r was subjected to the screen for losses of one of the two copies. Red derivatives had single-marked Y′s instead of the tandem duplication. Along with the red derivatives, there were smaller white derivatives. These were tandem triplications as confirmed by Southern analysis.

Rates of mitotic interactions: Use of the Lea and Coulson method for measuring rates of recombinational interactions assumes that the resulting selected derivatives have the same viability and growth rates. Differences in growth rates can be compensated for or can be ignored if the P_0 class in a fluctuation test is used (LURIA and DELBRÜCK 1943). Different dosages of *SUP11* in different backgrounds have differential viabilities as well as different growth rates (data not shown). The differential viabilities preclude the use of the fluctuation as well as the median methods of rate measurement. Consequently these measurements were performed in haploid strains with a Y′ marked with *URA3* but not *SUP11*. The median rates of loss of ten independent colonies for two different ends, in strains ELT24.12 and ELT24.17, are 1.6 and 3.1×10^{-6} respectively. If the recombinational interactions involved in marked Y′ gains and losses are the same, then this represents an estimate of the rate of recombinational interactions involving a particular Y′.

Some losses of marked Y′s are accompanied by changes at other chromosomes: In 6/67 of the marked Y′ loss events in which there was replacement of the marked Y′ with an unmarked Y′ at chromosome *XI* there were coincidental changes in chromosome size, either in *XI* or some other chromosome or both, indicative of translocation events not involving Y′s directly. In one of these, chromosome *II* is shorter, in another, chromosome *XVI* is no longer separable from some other chromosome band on a gel. When test crossed, all derivatives with changes in chromosome size yielded normal spore viabilities indicating

TABLE 3

Summary of Y′ duplication events

End	Chromosome	Y′[#]	EJL77-7r/ EJL40 E5	EJL41 E9	EJL53 E11	EJL49 E21
E1	*IV*	LONG [2–4]		(1)[a]	(1)[a]	1
E2	*IV*	LONG [3–4]	(12)[b]			1
E3	*XII*	LONG [3–4]				3
E4	*XII*	SHORT [2]		1	2	
E5	*VII, XV*	LONG [1]	10 TDs	1	(2)[c]	1
E6	*VII, XV*	LONG [1]	1			
E7	*VII, XV*					
E8	*VII, XV*					
E9	*XVI*	LONG [1]	7	2TDs,2TTs		2
E10	*XVI*	SHORT [2]	1		3	
E11	*XIII*	SHORT [1]			3TDs,2TTs	1
E12	*XIII*					
E13	*II*	SHORT [1]			3	
E14	*II*	LONG [1]	4			4
E15	*XIV*	LONG [1]			1	
E16	*XIV*					
E17	*X*	LONG [1]		1	1	
E18	*X*					
E19	*XI*		2			1
E20	*XI*					
E21	*V, VIII*	LONG [1]	(4)[d]	(2)[d]		2TDs
E22	*V, VIII*	LONG [1]				
E23	*V, VIII*	SHORT [1]			(3)[e]	
E24	*V, VIII*	SHORT [1]				
E25	*IX*	LONG [1]				1
E26	*IX*					
E27	*III*					
E28	*III*					
E29	*VI*	SHORT [1]				
E30	*VI*					
E31	*I*					
E32	*I*					
Totals:			41	10	21	17

TD = tandem duplication. TT = tandem triplication.

[a] These two events could be at either end E1 or E2.

[b] These locations of these twelve events could be at any of the chromosome ends E1, E2, E3, E4 or E6.

[c] These two events could be at any of ends E5, E6, E7 or E8.

[d] These six events are at either end E21 or E22.

[e] These three events are at either end E23 or E24.

that no deleterious deletions or duplications were produced. These will be further analyzed elsewhere. None of the 40 losses selected from chromosome *IX* were accompanied by changes in chromosome size. This is significantly different from the 6/67 losses selected from chromosome *XI* ($P < 0.05$).

DISCUSSION

Y′s can move to and from chromosome ends: Copy number and location differences between strains can be accounted for by the ability of Y′s to move to new originally Y′-less chromosome ends and the ability for a Y′-bearing chromosome end to become Y′-less. If a Y′-less end could not become Y′-bearing then an ancestral yeast population would have to have had Y′s at all chromosome ends bearing Y′s in existing strains.

Movement to new originally Y′-less locations may depend on other telomere-associated repeated sequences. Every chromosome end has $C_{1-3}A$ repeats at the very end and flanking Y′s. These may be sufficient for recombination between a Y′-less and a Y′-bearing chromosome end resulting in the transfer of a Y′ to the Y′-less end. The other telomere-associated repeated sequence family, X, may also be involved in the transfer of a Y′ to an originally Y′-less location in the same manner. Recombination involving X sequences is thought to be the explanation for the distribution of *SUC* genes in different strains of yeast (CARLSON, CELENZA and ENG 1985). In addition to direct chromosome end to chromosome end recombination, transfer of a Y′ to a Y′-less location could involve the integration of an autonomously replicating circular Y′ via recombination within the $C_{1-3}A$ repeats

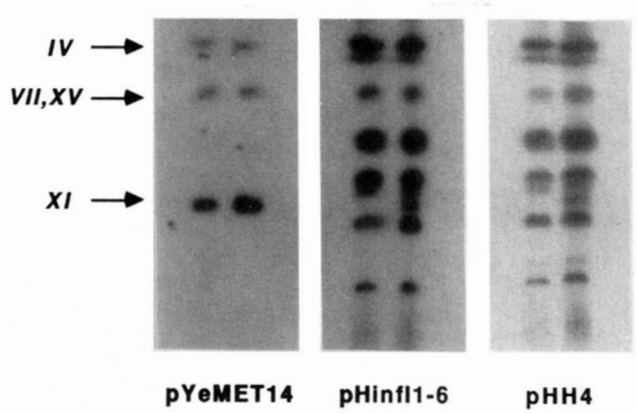

FIGURE 6.—Analysis of novel Y′ at chromosome *XI*. Chromosomes from EJL77-7r (left lanes in each panel) and an Ade$^+$ derivative (right lanes in each panel) with a second marked Y′ at chromosome *XI* are probed with each of chromosome *XI* specific sequence, pYeMET14, a 36-bp repeat sequence, pHinfI1–6, and an entire Y′-long, pHH4. The original chromosome *XI* has no homology to Y′ sequence as can be seen by the side by side comparisons.

or it could be due to some other duplication-integration mechanism.

The loss of Y′ sequence from a chromosome end could also result from recombination between $C_{1-3}A$ repeats or X repeats. The reciprocal product of a tandem duplication resulting from unequal exchange between flanking $C_{1-3}A$ repeats is a Y′-less end. Similarly the reciprocal product of ectopic exchanges between a Y′-bearing and a Y′-less chromosome end involving either the $C_{1-3}A$ repeats or X sequences is the loss of Y′ from the original end. In addition to interchromosomal and interchromatidal recombination, intrachromatidal recombination between the $C_{1-3}A$ repeats flanking a Y′ can result in the generation of an autonomously replicating Y′ circle and a Y′-less chromosome end. The presence of autonomously replicating Y′ circles in unselected cultures has been documented (HOROWITZ and HABER 1985). The instability of these circles (HOROWITZ and HABER 1985) and the deleterious effects of multiple copies of *SUP11* may have reduced the probability of their recovery in this study. In preliminary meiotic studies using the same system, a marked Y′ circle has been recovered along with a reciprocal loss of Y′ sequence at the originally marked chromosome end (E. J. LOUIS and J. E. HABER, manuscript in preparation).

Y′s interact via recombination: The duplication events observed here were either expansions of single copies into tandem arrays (24%) or ectopic duplications (76%). Expansions of single copy Y′s into tandem arrays presumably occur through unequal sister chromatid exchange involving the flanking $C_{1-3}A$ repeats. Tandem duplications also have the ability to regenerate single Y′ ends as seen in the loss of one copy of a marked Y′ within a tandem duplication. This is also presumably due to unequal exchange which now can involve the Y′ sequences as well. Unequal exchange is supported by the recovery of tandem triplications along with single copy Y′s from a tandem duplication.

The marked Y′ duplications that occur at ectopic locations are also most easily explained via homologous recombination. In 7 of 8 of the Y′-long with Y′-short interactions, the new marked Y′ was a recombinant between the recipient and the donor Y′ as they retained the recipient size class but obtained the donor marker sequence. In 65 out of 68 ectopic duplication events, the new marked Y′ occurred at a chromosome end that previously had a Y′. In all but one of these cases, there was no net change in Y′ copy number, only the duplication of the marker sequence.

These results are inconsistent with the integration of autonomously replicating Y′ circles as the predominant route by which duplications occur. If such integration were the predominant route of ectopic duplication, each integration of a marked Y′ circle into an unmarked Y′ would be expected to create an increase in the number of copies of Y′s at the recipient chromosome end, resulting in an unmarked Y′ adjacent to the marked copy. This is not the case.

Ectopic interactions among Y′s occur at rates comparable to other mitotic recombination events: The rate with which one Y′ mitotically recombines with another Y′ (about 2×10^{-6} per cell per generation) is similar to the rates found for other repeated sequences. KUPIAC and PETES (1988) found that the mitotic rate of ectopic Ty recombination was around 1×10^{-6}. These rates are also on the same order as the rate of mitotic recombination between dispersed heteroallelic copies of the 2.2-kb fragment containing the *LEU2* gene (LICHTEN and HABER 1989) which recombine to yield leucine prototrophs at a rate of 2×10^{-6} (LICHTEN and HABER 1989).

The rate of ectopic Y′ recombination is on the same order as mitotic recombination anywhere along the chromosome arm between the centromere and the marked Y′. From 30 to 40% of marked Y′ duplications in heterozygous diploids were ectopic or unequal Y′ interactions whereas up to 60–70% were the result of homozygosis along the chromosome arm. These chromosome arms can involve several hundred kilobases of sequence, as the chromosomes involved range in size from 600 to 1100 kb (CARLE and OLSON 1985). The fact that 6.7-kb Y′s undergo ectopic recombination at rates similar to mitotic recombination anywhere along the chromosome arm might be the result of preferential interactions of subtelomeric sequences. LICHTEN and HABER (1989) observed that mitotic recombination occurred as frequently between ectopic sites as between allelic sites on opposite homologues using the *LEU2* heteroalleles. The observed rate of Y′ recombination could reflect the fact that there are 29 other copies (about 180 kb) to interact with.

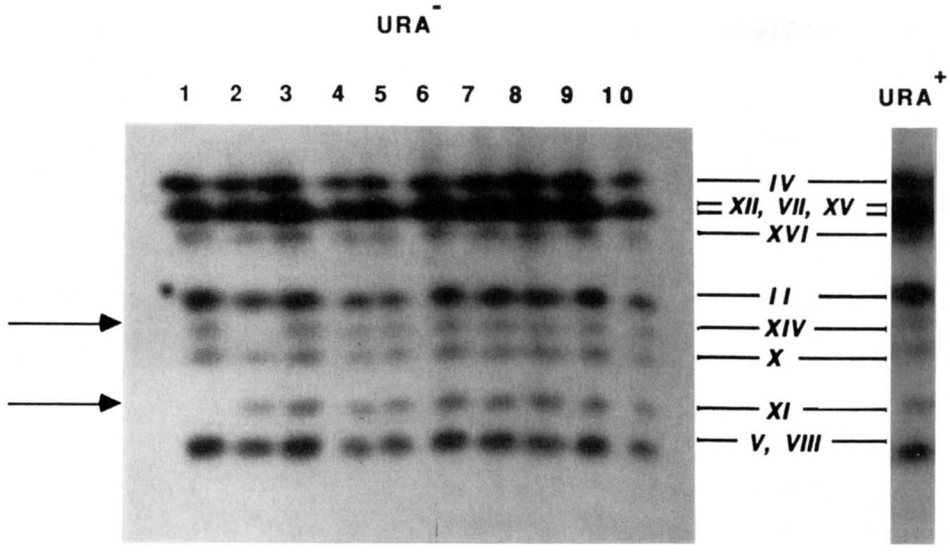

FIGURE 7.—Marked Y′ losses at chromosome *XI*. Chromosomes from Ura⁻ derivatives of a chromosome *XI* marked strain are probed with pEL16. In one case (lane 1) there is no Y′ sequence at chromosome *XI* while the rest retain Y′ sequence but have lost marker sequence (lower arrow). In one of these examples (lane 2), there is loss of Y′ sequence at chromosome *XIV* coincident with the loss of marker sequence at chromosome *XI* (upper arrow).

Evolutionary implications of the observed Y′ interactions: The expansion and contraction of tandem arrays may explain the observed homogeneity within tandem arrays. The tandem arrays of YP1 are more homogeneous than the Y′s as a whole in that they are not mixed for size class or for restriction fragment length polymorphisms (LOUIS and HABER 1990). Any new Y′, in a tandem array, with sequence differences would be rapidly lost or would replace the existing Y′ relative to the rate of introduction of the difference. Such an outcome is predicted (SMITH 1973; OHTA 1983) when the relative rate of unequal sister chromatid exchanges is high enough compared to introduction of new sequence via mutation or ectopic interaction. This observation is analogous to the primate rDNA gene variation seen among their six tandem arrays near the telomeres (ARNHEIM 1983).

The observed ectopic interactions indicate that Y′ can exchange sequence information even between the long and short size classes. These ectopic interactions may be sufficient to explain the observed homogeneity between the Y′s of YP1. There are virtually no restriction site differences among the Y′s of YP1 (LOUIS and HABER 1990). Theoretical models predict homogenization of repeats via ectopic recombination when the rate of recombination is sufficiently high relative to the rate of diversifying mutations (ARNHEIM 1983; OHTA 1983; OHTA and DOVER 1983; NAGYLAKI 1984). The mitotic rates of 1 to 3×10^{-6} of recombination involving a particular Y′ may be sufficiently high. Preliminary meiotic studies indicate that the rate of ectopic interaction involving a particular Y′ is on the order of 2% of meioses (E. J. LOUIS and J. E. HABER, manuscript in preparation) which should be well above diversifying mutation rates.

The maintenance of some variation among Y′s within a strain may be due to biases in the recombinational interactions that occur. Both the long and short classes coexist in YP1 and in other strains analyzed (HOROWITZ and HABER 1985; LOUIS and HABER 1990). The tendency for Y-longs to interact preferentially with other Y′-longs, and Y′-shorts to interact with other Y′-shorts may tend to homogenize each subset in terms of size class faster than one size class could replace the other via cross size-class interaction. This bias could be sequence homology dependent or location dependent. Ectopic recombination may be homology dependent such that the frequency of interaction is inversely proportional to the amount of sequence heterogeneity, precluding some Y′ interactions or enhancing others. If this were the case, a recombinant Y′ resulting from a rare cross size-class conversion would now interact preferentially with the donor size class rather than the recipient size class. This homology dependence would also allow for "escapes" from homogenization (WALSH 1987) for Y′s that have sufficiently diverged. Location dependence of ectopic interactions could result from nuclear localization of telomeres such that chromosome ends can only interact with the subset of ends in their vicinity. Under this hypothesis, a recombinant Y′ from a rare cross size-class conversion would interact preferentially with the recipient size-class Y′s with which it is physically associated rather than the donor size class with which it now shares more homology. Distinction of these possibilities will require further analysis with appropriately marked Y′s of different size classes at the same location.

Multiple interactions were recovered in several cases. Two losses of marked Y′s at chromosome *XI* were accompanied by losses of Y′ sequence at chromosome *XIV*. Many of the selected duplications had three copies of a marked Y′ rather than two. These include tandem triplications, tandem duplications

with an ectopic event, two ectopic events and an ectopic event with homozygosis of either the originally marked end or the newly marked end. These multiple duplications occur at a much higher frequency than expected for independent events. Coordinate multiple events have also been seen in Ty recombination (CHALEFF and FINK 1980; LIEBMAN, SHALIT and PICOLOGLOU 1981).

The recombinational interactions among Y's observed in this study provide some understanding of the processes that may be involved in Y' evolution. Most of the properties of the Y' repeated sequence families of the well characterized strains YP1 and Y55 described in the accompanying paper (LOUIS and HABER 1990) can be accounted for by the observed recombinational interactions. The copy number and location differences between strains is explicable by the ability of Y's to move to and from chromosome ends. The within-tandem array homogeneity is explicable by the high rate of expansion and contraction, relative to ectopic interaction, of tandem arrays via unequal sister chromatid exchange. The within-strain homogeneity is explicable by the ectopic interactions among Y's. There are biases in terms of which Y's interact together which may explain the distribution of the heterogeneity (long and short size classes) that is seen.

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