Isolation and characterization of a Ty element inserted into the ribosomal DNA of the yeast *Saccharomyces cerevisiae*

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ABSTRACT

The yeast *Saccharomyces cerevisiae* has about 30 to 50 copies of a transposable element Ty. Most of these elements are located at the 5' ends of protein coding sequences and are flanked by a 5 bp duplication. We report below an insertion of a Ty element into one of the repeated ribosomal RNA (rRNA) genes of yeast. The element is located between the 3' ends of the divergently transcribed 37S and 5S rRNA's and is not flanked by a 5 bp duplication. In addition, one end of the Ty insertion is contiguous with a 306 bp deletion of the sequences of the rRNA gene. We find that this insertion, unlike most Ty insertions, is mitotically unstable.

INTRODUCTION

The transposable element Ty is found in about 30 to 50 copies in most strains of *Saccharomyces cerevisiae* (1). Ty elements have a structure similar to that observed in retroviruses, with a central element of 5 kb (epsilon) flanked by direct repeats of 338 bp (delta repeats) (1,2). Insertion of the Ty element into a new site is associated with a 5 bp duplication of the target sequence (3,4). Although most new insertions of Ty elements are located at the 5' end of genes outside the coding region, a few examples of insertion of Ty elements into structural genes have recently been found (5-7).

Most Ty insertions characterized thus far have been in single-copy yeast sequences. As described below, we have found an insertion of a Ty element into one of the repeated ribosomal RNA genes. In yeast, there are about 100 copies of these repeats (8). Each repeat is about 9.5 kb in size and encodes four species of rRNA (25S, 17S, 5.8S and 5S transcripts) (9,10); the three largest rRNA species are transcribed as part of a 37S precursor RNA that is processed to yield the mature transcripts (11,12). Unlike most eukaryotes, in yeast, most of the 5S rRNA coding sequences are located within the same repeat as the other rRNA species but are transcribed from the opposite strand (13,14,15). The 9.5 kb rRNA genes are located in a single tandem array located on chromosome XII (16). In some yeast strains, there are a small number of a different type of rRNA gene, a 3.6 kb repeat containing 5S but not 37S coding sequences (17).

Among the common lab strains of yeast, two different types of 9.5 kb rRNA genes exist (18). The most common type of repeat contains 7 EcoRI sites per repeat (form I rDNA). In the

other type (form II), one of the EcoRI sites is missing although the position of the remaining six sites is conserved. Within a single haploid strain, the repeats are usually either form I or form II variants rather than a mixture of the two types of genes. When a diploid strain that is heterozygous for the two types of repeats undergoes meiosis, the two forms usually segregate 2:2, indicating that the 100 rRNA genes are tightly linked genetically (19).

We describe below an insertion of a Ty element into one of the 9.5 kb rRNA genes. We have used the two variant forms of the rDNA to demonstrate that this insertion is within the rRNA gene cluster on chromosome XII. Although our study is the first direct demonstration of an insertion within a 9.5 kb repeat, Oyen *et al* (20) have previously shown that the satellite DNA which contains the yeast rRNA genes hybridizes to a Ty hybridization probe. In addition, Piper *et al* (21) have found a Ty element associated with the variant 3.6 kb 5S rRNA repeats. The Ty insertion that we describe is unusual in four ways: 1) the insertion is located at the 3' end of the coding sequences rather than at the 5' end, 2) the insertion is not flanked by a 5 bp duplication of the target sequence, 3) the insertion abuts a 306 bp deletion of the rRNA gene into which it is inserted and 4) the insertion is mitotically unstable.

MATERIALS AND METHODS

Strains

The yeast strains used in this study were: A364A(W) (a *ade1 ura1 lys2 tyr1 his7 ade2 gal1* form I rDNA), A364A(P) (same genotype as A364A(W) but separated in different labs for about 10 years) and 2262 (*α leu2 his5 lys11 ade1 ura1 gal1* form II rDNA). The strain A364A(W) was provided by J. Woolford (Carnegie-Mellon University). This strain was used for construction of a recombinant bacteriophage library by J. Woolford. DNA from this strain was partially digested with EcoRI and inserted into the lambda vector Charon 4A (22). The *E. coli* host for the library was LE392 (23). The *E. coli* host for plasmids used in this study was HB101 (24). Standard yeast genetic analysis and tetrad dissection techniques were used in this study (25).

Plasmid Constructions

The A1 and A2 EcoRI fragments of the recombinant bacteriophage AV 4.5 (Figure 1) were sub-cloned into the EcoRI site of pBR329 (26). The conditions of ligation were standard (27). The resulting DNA fragments were transformed into the *E. coli* strain HB101 (28) and ampicillin-resistant, chloramphenicol-sensitive transformants were analyzed. The plasmid containing the A1 fragment was pAV3 and the plasmid with the A2 fragment was pAV4. By a similar procedure, we also sub-cloned the EcoRI B fragment of the rDNA repeat from a recombinant bacteriophage AV 7.3 (derived from the Woolford bank) that had a complete form I rRNA gene; the resulting plasmid was pAV16.

Isolation and Hybridization Analysis of DNA

The procedures for plasmid and bacteriophage DNA isolations were those described by

Davis *et al* (27). Yeast DNA was purified using CsCl gradients containing the fluorescent dye Hoechst 33258 (18). Yeast DNA minipreparations were isolated as described by Sherman *et al* (29). We treated the DNA with restriction enzymes using the conditions specified by the supplier (New England Biolabs). The resulting fragments were separated by agarose gel electrophoresis and transferred to either nitrocellulose (30) or Gene Screen (New England Nuclear). Plasmid DNA molecules used as hybridization probes were labeled by nick translation (31). Plaque hybridization was done as specified by Benton and Davis (32).

DNA Sequence Analysis

DNA sequencing was performed as described by Maxam and Gilbert (33). The fragments were 5' end-labeled for sequence analysis using T4 polynucleotide kinase. DNA polymerase was used to end-label the 3' ends of the fragments.

RESULTS

Isolation of a recombinant bacteriophage containing a Ty element within an rRNA gene

In order to identify rRNA genes that were different from the normal 9.5 kb repeat, we analyzed a collection of recombinant lambda bacteriophage constructed by J. Woolford (Carnegie-Mellon). This bank was constructed by inserting a partial EcoRI digest of DNA isolated from the haploid yeast strain A364A(W) into the lambda vector Charon 4A. We identified those phage that contained rRNA genes by hybridizing plaques to labeled DNA derived from the 9.5 kb repeat. The hybridization probe used in this experiment was the EcoRI B fragment of the repeat; this 2.5 kb fragment contains a portion of the 37S and 5S coding sequences as well as most of the non-transcribed spacer (34). DNA was isolated from plaques that hybridized to this fragment. We did a Southern analysis on EcoRI digests of DNA from each isolate, using the EcoRI B fragment as a hybridization probe. Although most of the recombinant bacteriophage contained a fragment identical to EcoRI B in size, two independent recombinant phage, AV 4.5 and AV 19.3, contained EcoRI fragments of 4.2 kb that hybridized to the EcoRI B fragment. AV 19.3 contained a subset of the EcoRI fragments present in AV 4.5; therefore AV 4.5 was used for all future analysis.

A restriction map of AV 4.5 is shown in Figure 1. The normal 9.5 kb rRNA gene of A364a contains 7 EcoRI restriction sites (18). The seven EcoRI fragments derived from the rDNA are labeled A through G, A being the largest and G the smallest. The order of these fragments within the 9 kb repeat is: (BEFADCG)_N (35,9,18). As shown in Figure 1, the recombinant bacteriophage AV 4.5 had EcoRI fragments that co-migrated with A, F, E, G and C. In addition, the yeast DNA insert contained three fragments A1, A2, and H (4.2, 4.0 and 0.2 kb in size, respectively) that did not co-migrate with any of the known rDNA EcoRI fragments. The A1 fragment hybridized very strongly to EcoRI B. From the order of these fragments within the restriction map, it appeared that an insertion of approximately 6 kb had occurred within the EcoRI B fragment of a 9.5 kb repeat. Since the Ty repeat is 5.6 kb, we tested the possibility that

Figure 1. Restriction maps of AV 4.5 insert and yeast rDNA. EcoRI fragments of the yeast rDNA insert cloned in lambda are labeled alphabetically based on size. Fragments A through G correspond to the normal form I rDNA fragments (18). Fragments A1, A2, and H result from the insertion of a Ty element containing two EcoRI sites into an rDNA EcoRI B fragment. Restriction enzymes are abbreviated as follows: EcoRI (R), HindIII (H), KpnI (K), SacI (Sc), XhoI (X), BstEII (B), SalI (SI).

the insertion was a Ty element by Southern analysis. An EcoRI digest of AV 4.5 DNA was hybridized to a labeled plasmid (pS13) that included a previously characterized Ty element (36). Fragments A1 and A2 hybridized strongly to this Ty element. Most Ty elements contain one XhoI site in each delta sequence, resulting in a characteristic Ty-specific XhoI fragment of about 5.3 kb. As shown in Figure 1, AV 4.5 had XhoI sites at the positions expected if the Ty element within the rRNA repeat is intact. Thus, our restriction analysis of the yeast DNA insertion in AV 4.5 indicated that a Ty element was inserted within the EcoRI B fragment of a 9.5 kb repeat. From the comparison of the restriction map in Figure 1 with the published restriction maps of the 9.5 kb rRNA gene and the Ty element, we deduced that the insertion was within the non-transcribed spacer approximately 400 bp to the right of the EcoRI restriction site separating EcoRI B from EcoRI E. To confirm these conclusions and to determine the exact nature of the junction between the Ty element and the rDNA sequences, we sequenced the point of insertion of the Ty repeat.

DNA sequence analysis of the Ty insertion within the rRNA gene

The EcoRI fragments of AV 4.5 (A1 and A2) expected to contain the junctions between the Ty element and the rRNA gene sequences were sub-cloned into the EcoRI site of pBR329, producing plasmids pAV3 (containing A1) and pAV4 (containing A2). In the same manner, we also sub-cloned an EcoRI B fragment of an rRNA gene repeat isolated from A364A(W) that did not have an insertion, producing the plasmid pAV16. The source of the EcoRI B fragment in

Figure 2. DNA sequencing strategy for fragments from pAV3, pAV4, and pAV16. A 450 bp HindIII-XhoI fragment and a 450 bp XhoI-BstEII fragment containing the Ty-rDNA junctions from pAV3 and pAV4, respectively, were subjected to Maxam and Gilbert DNA sequencing as described in Materials and Methods. The corresponding region in a typical rDNA EcoRI B fragment was also sequenced from pAV16. Both strands of all three fragments were sequenced. Each arrow represents the extent of DNA sequence determined from either the 5' or 3' end of the restriction enzyme site as marked. Restriction enzyme sites are abbreviated as follows: BstEII (B), HindIII (H), HpaI (P), MspI (M), Sau3A (S), XhoI (X).

pAV16 was a recombinant bacteriophage (AV 7.3) containing one complete 9.5 kb repeat from A364A(W); this phage was isolated from the same collection of recombinant phage as AV 4.5.

The EcoRI insertions in pAV3 and pAV4 were mapped with various restriction enzymes in preparation for sequence analysis. A 450 bp HindIII-XhoI fragment and a 450 bp XhoI-BstEII fragment were isolated and sequenced from pAV3 and pAV4 respectively. We chose to sequence from the XhoI sites since we assumed that these sites were within the delta sequences of the Ty insertion whereas the HindIII and BstEII sites were likely to be in the rDNA. We also sequenced the corresponding HindIII-BstEII fragment from the rRNA gene without an insertion (pAV16). Figure 2 indicates the sequencing strategy used for each fragment. The DNA

A.

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5' HindIII
CTTCCCGAGCGTGAAAGGATTGGCCGGACAGTTTGCTTCATGGAGCAGTTTTTCCGCACCATCAGAGCGGCAACATGAGTGCTTGATAAGTTTGA
GAATTGAGAAAAGCTCATTTCCTATAGTTAACAGGACATGCCTTTGATATGAAAAAAATACTACGAACTACGATTTTACCAAGAAAGATGTAAGAGACA
AGTGAACTGTTGGAATAAAAAACAACATATCATCTACTAACAAGTATTTACGTTACTAGTATATTATCATATACGGTGTTAGAAGATGACGCAAAATGATGA
GAAATAGTCATCTAAATTAGTGGAAGCTGAAACGCAAGGATTGATAATGTAATAGGATCAATGAATATTAAACATATAAAACGATGATAATAATTTATA
GAATTGTGTAGAAATGAGATTCCCTTTTATGGATTCTAAATCC--Ty--CGAGGAGAACTTCTAGTATATTCTGTATACCTAATATTATAGCCTTTAT
XhoI XhoI
CAACAATGGAAATCCCAACAATTATCTCAACATTCACATATTTCTCAGGAAGGAATAAGAAGCAACTAAACGAGGGTGAGAAAAGACGAAGAGGAAAGT
ATTAACCGTAGAAGAGAGGGAATGGAGGAAGAGACAATAACCGAGTGTTTTTTTTTTTTTTTTTTTGTAGGAATATCGAGGAGAATATTGTTTGTACA
TATCAAGTAGTAGCAACCAATGAGCATATATGGAGTGCTTAACCTTCAGAAGAAGAGTGCAGCTGGATAGTGCGAATTTTCTGAATCGAATGGTAGGT
TAGTTATGGGATTTAGCATAGGAAGCCAGAACTAGAAAAAAGAAATAAAGATTGCAGCACCTGAGTTTCGCGTATG
BstEII 3'

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B.

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5' HindIII
CTTCCCGAGCGTGAAAGGATTGGCCGGACAGTTTGCTTCATGGAGCAGTTTTTCCGCACCATCAGAGCGGCAACATGAGTGCTTGATAAGTTTGA
GAATTGAGAAAAGCTCATTTCCTATAGTTAACAGGACATGCCTTTGATATGAAAAAAATACTACGAACTACGATTTTACCAAGAAAGATGTAAGAGACA
AGTGAACAGTGAACAGTGAACAGTGGGGACATTTTTTTTTTTTTTTTTTTTAAAGTAAATGGCAGTTTCTAGGGAATGATGATGGCAAGTTCAGAGAG
GCACGCTAAAGAGTAGGCTACTGGGAAGAAGAAAGAGAAAAAGTGCAAGATGAATAGCCAGTGCATATATACATGTATCTTAACAGATATGGAATG
GTTGGCGAAGTAAATTTTTTGGCGACGCGCGACGCGCGGATGCGGAGTGTAAAGTGTACTACGATCATATAGTGAATGCGGCAAAATGTTA
GTGCAGGAAGCGGGAAGGAATAAGAAGCAACTAAACGAGGGTGAGAAAAAGACGAAGAGGAAGTATTAACCGTAGAAGAGAGGGAATGGAGGGAAG
AGAACAAAACCGAGTCTTTTTTTTTTTTTTTTGTAGGAATATCGGAGGAGAATATTGTTGTACATATCAAGTAGTAGCAACCAATGAGCATAATGG
AGTGCTTAACCTTCAGAAGAAGAGTGCAGCTGGATAGTGCGAATTTTCTGAATCGAATGGTAGGTAGTTATGGGATTAGCATAGGAAGCCAGAA
CTAGAAAAAAGAAATAAAGATTGCAGCACCTGAGTTTCGCGTATG
BstEII 3'

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Figure 3. DNA sequence of Ty-rDNA junction fragments and corresponding region in a normal rDNA B fragment. The DNA sequence of only one strand of each fragment is represented, written 5' to 3'. Part A of Figure 3 contains the sequences of the Ty-rDNA junctions contained in the HindIII-XhoI and XhoI-BstEII fragments from pAV3 and pAV4, respectively. The underlined letters indicate delta sequences as identified from comparison with known delta sequences (1,2). Part B is the sequence of the HindIII-BstEII fragment from pAV16 which contains a normal rDNA EcoRI B fragment. Asterisks mark the endpoints of the 306 bp rDNA sequence deleted in the Ty clone, AV 4.5. The endpoints of the deletion are precisely at the ends of the deltas of the Ty element. The endpoints of the Ty element and the deletion are 207 bp from the HindIII site and 354 bp from the BstEII site. The rDNA sequences surrounding the point of the Ty insertion and rDNA deletion are identical to sequences found in normal rDNA.

sequences obtained are shown in Figure 3.

Although the sequence analysis confirmed the existence of a Ty element integrated into the rDNA, there were several unexpected properties of the insertion. First, unlike previously studied Ty insertions (3,4), the element was not flanked by a 5 bp duplication of the target sequence. A second (and presumably related) observation is that the Ty insertion was associated with a 306 bp deletion of the rDNA sequences contiguous to the element. The deletion directly abuts the Ty element. It is likely, therefore, that the event that deleted the 306 bp of the rDNA also deleted one of the copies of the target sequence. Except for the deletion, the rDNA sequences that flank the element are identical to those present in a normal rRNA gene.

Another unusual feature of the insertion is that the element is located between the 3' ends of both rRNA transcripts. The element is 326 bp from the 3' end of the 5S transcript (34) and approximately 470 bp from the 3' end of the 37S transcript (37). Most Ty elements are located upstream of coding sequences, although a few examples of insertions of a Ty element within a coding sequence have been observed (6,7). As with other Ty elements (2), the sequences flanking the element are AT-rich. The 100 bp regions of the control rRNA gene that flank the position of insertion are 70% and 58% AT. Since the rRNA genes are approximately 54% AT, these regions are quite different from the average rRNA gene sequence(38).

Identification of the Ty insertion in genomic rDNA sequences

The analysis described above was done with DNA cloned from the haploid yeast strain A364A(W). To confirm that the Ty insertion was also present in genomic DNA, we did a Southern analysis of DNA isolated from three strains: A364A(W), A364A(P) and 2262. The A364A(W) and A364A(P) strains are, in theory, identical in genotype although the strains have been separated in two different labs for about ten years. A364A(W) was obtained from the lab of John Woolford (Carnegie-Mellon University) and was used as a source of DNA for construction of the recombinant bacteriophage bank from which AV 4.5 was derived. The strain A364A(P), originally obtained from L. Hartwell (University of Washington) has been in our lab for the past ten years. The strain 2262 contains form II rDNA (18,19). In order to detect the Ty insertion in the rDNA, we hybridized the EcoRI B fragment of the rDNA to yeast DNA that had been treated with the restriction endonuclease HindIII. This enzyme cleaves the 9.5 kb rDNA repeat into two fragments, approximately 6.5 kb and 2.9 kb. Since one of the restriction sites for HindIII is within the EcoRI B fragment, both of these fragments hybridize to EcoRI B. The HindIII fragment of AV 4.5 that contains the Ty insertion and hybridizes to EcoRI B is about 9 kb in size.

Equal amounts of DNA from the three yeast strains described above were examined by gel electrophoresis (Figure 4). In addition, the gel contained five samples of HindIII treated DNA from the recombinant bacteriophage AV 4.5. The amounts of AV 4.5 DNA in each gel lane were equivalent to 0.25, 1, 5, 10 and 25 copies of the 9 kb fragment per haploid yeast genome. When the gel was examined by Southern analysis using the EcoRI B fragment as a hybridization probe,

Figure 4. Presence of Ty element in A364A(W). Genomic DNA from A364A(W), 2262, and A364A(P) was digested with HindIII (H) and HindIII-SalI (HS) as indicated and run on a horizontal 0.7% agarose gel. Bacteriophage AV 4.5 DNA was also digested with HindIII (H) and HindIII-SalI (HS) as a control and loaded onto the gel in the ratios indicated. After transfer to Gene Screen, the DNA was hybridized with ^{32}P -labeled pBRB (a plasmid that contains the EcoRI B fragment of the rDNA). The arrow indicates the band present in a HindIII digest of A364A(W) due to the Ty element. This band is cut in a HindIII-SalI double digest of A364A(W) to produce a new band that comigrates with the corresponding control band from an AV 4.5 HindIII-SalI double digest.

we found that only the A364A(W) strain had a band that co-migrated with the 9 kb fragment of AV 4.5. This band had the intensity expected for single-copy DNA. To confirm that this band represented the same sequences found in AV 4.5, we also did a Southern analysis on yeast DNA that had been treated with both HindIII and SalI. SalI does not cut yeast rDNA but does cut within the Ty element in AV 4.5. We found that in HindIII-SalI treated samples of A364A(W) DNA, the 9 kb band disappeared and a new band of about 6 kb was observed that co-migrated with a fragment derived from a HindIII-SalI digest of AV 4.5 DNA. In addition, we found that when the radioactive EcoRI B hybridization probe was stripped from the filter and the filter re-hybridized with a probe containing a cloned Ty element (pS13), the 6 kb fragment hybridized to Ty. Thus, the Ty element characterized in AV 4.5 is still present in the strain from which it was cloned (A364A(W)) but is not present in a strain of the same genotype (A364A(P)) that has been sub-cultured separately.

In addition to the 9 kb HindIII fragment representing the Ty insertion in the rDNA of A364A(W), all three strains had a band representing a DNA fragment of about 9.5 kb. This

band is likely to represent either an rDNA repeat that lacks one of the two HindIII sites present in most repeats or a junction between rDNA and other chromosomal sequences.

Genetic mapping of Ty insertion in the rDNA of A364A(W)

Although most of the yeast rRNA genes are located in a single tandem array on chromosome XII (16), the possibility of a small number of rRNA genes on other chromosomes has not been ruled out. To determine whether the Ty element represented by the AV 4.5 clone was located within the array of rRNA genes on chromosome XII, we crossed the haploid strain A364A(W) (a strain that contains the Ty element in the rDNA and has form I rDNA sequences) with the haploid 2262 (a strain that has form II rDNA and is devoid of the Ty insertion represented by AV 4.5). The resulting diploid strain was sporulated and tetrads were dissected. DNA was isolated from 20 spore cultures derived from 5 complete tetrads. We examined EcoRI digests of each DNA sample to determine whether the rDNA was form I or form II (18). In addition, the DNA was treated with HindIII and examined by Southern analysis in order to detect the presence of the Ty insertion within the rDNA. For all five tetrads analyzed, the Ty insertion in the rDNA co-segregated with the form I rDNA. This result indicates that the Ty insertion is within or very close to the tandem array of rRNA genes located on chromosome XII.

Mitotic loss of the Ty insertion from the rDNA of A364A(W)

Since the rDNA of A364A(W) contained a Ty insertion whereas the theoretically isogenic strain A364A(P) did not, we examined the mitotic stability of the Ty insertion in A364A(W). We vegetatively sub-cultured eight separate colonies of A364A(W) for approximately 100 cell generations. After the last sub-culturing, we isolated DNA from each of the eight cultures and looked for the presence of the Ty element in the rDNA by the Southern analysis already described. We found that the Ty element in the rDNA was retained in seven of the eight isolates but was absent in one isolate. Thus, the Ty insertion in the rDNA is mitotically unstable. One mechanism that would cause instability of the insertion is unequal crossing-over within the rRNA gene tandem array. This type of exchange is responsible for the loss of selectable genes inserted into the yeast rDNA in both meiotic and mitotic cells (39,40,41).

DISCUSSION

We have characterized an unusual Ty element inserted within the tandem array of yeast rRNA genes. The location of the element, between the 3' coding sequences for two rRNA transcripts, is unusual. In previous studies, Ty elements have been identified that are located within or at the 5' end of yeast genes. Since most previous studies have been done with Ty elements that affect gene expression, it is not clear that Ty elements have a bias against insertion into 3' regions. We do not know whether the Ty element that we have characterized has any effect on the expression of the adjoining rRNA genes. The region between the 3' ends of the rRNA coding sequences is particularly A-T rich and Oyen and Gabrielsen (20) have suggested that this region would be a good target for Ty transposition.

A second unusual feature of the Ty element is the lack of a 5 bp repeat of the target sequence flanking the element. The lack of this repeat is presumably the result of the 306 bp deletion that is contiguous with the element. In other studies, deletions contiguous to Ty elements have also been detected (42,43). There are two mechanisms that could give rise to the deletion. First, the deletion may have arisen as the result of a recombination event between two Ty sequences (or a Ty sequence and a delta sequence) located in different rRNA genes at positions 306 bps apart. Second, the deletion may be the result of a mistake made during the integration of the DNA copy of the Ty element; although the Ty element is known to transpose through a reverse transcription process (44), the details of the integration event are not understood. Whatever caused the deletion, our result indicates that it is unwise to make conclusions about the mechanisms of transposition based on a single sequenced insertion.

The Ty element that we examined was mitotically unstable. Since Ty elements in single-copy genes do not excise (6), we believe that the reversion of the element in the rDNA is the result of its insertion within repeated sequences. Unequal crossing-over, which has been detected within yeast rRNA genes in both meiosis and mitosis (39,40,41), could result in deletion of the Ty element from one daughter cell associated with duplication of the element in the other daughter. The instability of the element is one indication of the difficulty of maintaining isogenic strains in the laboratory.

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REFERENCES

1. Roeder, G. S. and Fink, G. R. (1982) in *Mobile Genetic Elements*, Shapiro, J. A. ed., pp299-328, Academic Press, New York.
2. Williamson, V. M. (1983) *Int. Rev. Cytol.* **83**, 1-25.
3. Farabaugh, P. J. and Fink, G. R. (1980) *Nature* **286**, 352-356.
4. Gafner, J. and Philippsen, P. (1980) *Nature* **286**, 414-418.
5. Eibel, H. and Philippsen, P. (1984) *Nature* **307**, 386-388.
6. Rose, M. and Winston, F. (1984) *Mol. Gen. Genet.* **193**, 557-560.
7. Simchen, G., Winston, F., Styles, C. A. and Fink, G. R. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2431-2434.
8. Schweizer, E., MacKechne, C. and Halvorson, H. O. (1972) *J. Mol. Biol.* **40**, 261-277.
9. Bell, G. K., DeGennaro, L. J., Gelfand, D. H., Bishop, R. J., Valenzuela, P. and Rutter, W. J. (1977) *J. Biol. Chem.* **252**, 8118-8125.
10. Philippsen, P., Thomas, M., Kramer, R. A. and Davis, R. W. (1978) *J. Mol. Biol.* **123**, 387-404.
11. Trapman, J. and Planta, R. J. (1976) *Biochim. Biophys. Acta* **442**, 265-274.
12. Udem, S. A. and Warner, J. R. (1972) *J. Mol. Biol.* **65**, 227-242.
13. Maxam, A. M., Tizard, R., Skryabin, K. G. and Gilbert, W. (1977) *Nature* **267**, 643-645.
14. Valenzuela, P., Bell, G. I., Masiarz, F. R., DeGennaro, L. J. and Rutter, W. J. (1977) *Nature* **267**, 641-643.
15. Kramer, R., Philippsen, P. and Davis, R. W. (1978) *J. Mol. Biol.* **123** 405-416.

16. Petes, T. D. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 410-414.
17. McMahon, M. E., Stamenkovich, D. and Petes, T. D. (1984) *Nucleic Acids Res.* **21**, 8001-8016.
18. Petes, T. D., Hereford, L. M. and Skryabin, K. G. (1978) *J. Bacteriol.* **134**, 295-305.
19. Petes, T. D., Hereford, L. M., and Botstein, D. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 1201-1207.
20. Oyen, T. B. and Gabrielsen, O. S. (1983) *Fed. Euro. Biochem. Soc.* **161**, 201-206.
21. Piper, P. W., Lockheart, A. and Patel, N. (1984) *Nucleic Acids Res.* **10**, 4083-4096.
22. Blattner, F. R., Williams, B. G., Blechl, A. E., Denniston-Thompson, K., Faber, H. E., Furlong, L., Grunwald, D. J., Kiefer, D. O., Moore, D. D., Schumm, J. W., Sheldon, E. L. and Smithies, O. (1977) *Science* **196**, 161-169.
23. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) *Molecular Cloning*, p.504, Cold Spring Harbor Laboratory, New York.
24. Boyer, H. W. and Roulland-Dussoix, D. (1969) *J. Mol. Biol.* **41**, 459-472.
25. Mortimer, R. K. and Hawthorne, D. C. (1969) in *The Yeasts*, Rose, A. H. and Harrison, J. S. eds., pp286-460, Academic Press, New York.
26. Covarrubias, L. and Bolivar, F. (1982) *Gene* **17**, 79-89.
27. Davis, R. W., Botstein, D. and Roth, J. R. (1980) *Advanced Bacterial Genetics*, pp. 106-125, 138-9, Cold Spring Harbor Laboratory, New York.
28. Mandel, M. and Higa, A. (1970) *J. Mol. Biol.* **53**, 159-162.
29. Sherman, F., Fink, G. R. and Hicks, J. (1983) *Methods in Yeast Genetics Laboratory Manual*, p. 119B, Cold Spring Harbor Laboratory, New York.
30. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503-517.
31. Schatchat, F. W. and Hogness, D. S. (1973) *Cold Spring Harbor Symp. Quant. Biol.* **38**, 371-381.
32. Benton, W. D. and Davis, R. W. (1977) *Science* **196**, 180-182.
33. Maxam, A. M. and Gilbert, W. (1980) *Methods Enzymol.* **65**, 499-560.
34. Valenzuela, P., Bell, G. I., Venegas, A., Sewell, E. T., Masiarz, F. R., DeGennaro, L. J., Weinberg, F. and Rutter, W. J. (1977) *J. Biol. Chem.* **252**, 8126-8135.
35. Cramer, J. H., Farrelly, F. W., Barnitz, J. T. and Rownd, R. H. (1977) *Mol. Gen. Genet.* **151**, 229-244.
36. Cameron, J. R., Loh, E. Y. and Davis, R. W. (1979) *Cell* **16**, 739-751.
37. Veldman, G. M., Klootwijk, J., de Jonge, P., Leer, R. J. and Planta, R. J. (1980) *Nucleic Acids Res.* **8**, 5179-5192.
38. Cramer, J. H., Bhargava, M. M. and Halvorson, H. O. (1972) *J. Mol. Biol.* **17**, 11-20.
39. Szostak, J. and Wu, R. (1980) *Nature* **284**, 426-430.
40. Petes, T. D. (1980) *Cell* **19**, 765-774.
41. Petes, T. D., Smolik-Utlaut, S. and Zamb, T. (1981) *Molecular Genetics in Yeast, Alfred Benzon Symposium* **16**, 137-153.
42. Downs, K. M., Brennan, G., and Liebman, S. W. (1985) *Mol. Cell. Biol.* **5**, in press.
43. Liebman, S., Shalit, P. and Picologlou, S. (1981) *Cell* **26**, 401-9.
44. Boeke, J. D., Garfinkel, D. J., Styles, C. A. and Fink, G. R. (1985) *Cell* **40**, 491-500.