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Transcriptional Analysis of Ty1 Deletion and Inversion Derivatives at *CYC7*

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One class of Ty insertion mutation in *Saccharomyces cerevisiae* activates expression of adjacent structural genes. The *CYC7-H2* mutation, in which a Ty1 element is inserted 5′ to the iso-2-cytochrome *c* coding region of *CYC7*, causes a 20-fold increase in *CYC7* expression. Deletion analysis of *CYC7-H2* has shown that distal regions of the Ty1 element are not essential for the transcriptional activation at *CYC7*. In this report, we have analyzed Ty1 and *CYC7* RNA from two *CYC7-H2* deletion derivative genes to determine whether a direct correlation exists between transcription of Ty1 and transcription of the adjacent gene. Assuming that all Ty1 elements in the genome are transcribed equally, amounts of *CYC7-H2* deletion derivative Ty1 RNA were found to be at least fivefold lower than the amount estimated for the average Ty1 element. These same Ty1 deletion derivatives caused a 20-fold increase in adjacent *CYC7* expression. This finding suggests that the mechanism by which Ty1 activates adjacent gene expression does not require normal levels of Ty1 transcription. Two inversion derivatives of the *CYC7-H2* Ty1 have also been analyzed. These derivatives did not produce any iso-2-cytochrome *c* or any normal *CYC7* mRNA. Instead they were found to produce a Ty1-*CYC7* fusion RNA. Consistent with our findings on *CYC7-H2* Ty1 transcription, the amount of the fusion RNA was very low. In addition, the Ty1 inversion derivatives produced a new RNA that mapped to sequences upstream from the inverted Ty1 segment. Similar to Ty1 insertions that activate transcription, the new RNA was found to be transcribed away from Ty1.

Genomic DNA rearrangements involving mobile genetic elements in eucaryotic organisms can cause alterations in gene expression. Well-known examples of this phenomenon include the effects of controlling elements on gene expression in maize (25), retrovirus activation of oncogenes by proviral DNA insertion in vertebrates (53), and transposable element insertion mutations in *Drosophila melanogaster* (4, 35, 38). A number of regulatory mutations in *Saccharomyces cerevisiae* have been caused by insertion of the *S. cerevisiae* transposable element Ty. Some Ty insertions cause loss or reduction in gene expression (10, 16, 43, 46), while other Ty insertions activate gene expression (2, 20, 28, 36, 55). Some cases have been observed in which different Ty elements inserted at the same site in a given locus have opposite effects on expression of the affected gene (44, 45).

Ty1 elements are a family of dispersed repetitive elements present at about 30 copies per haploid yeast genome (8). These elements are related to provirus forms of vertebrate retroviruses in their structure and gene organization (8, 11, 32). They consist of two 0.33-kilobase (kb) direct terminal repeat sequences, delta, and a 5.3-kb internal domain, epsilon. The Ty elements are transcribed into a major RNA ~5.7 kb in size and a minor RNA ~5.0 kb in size (18). The major transcript initiates in the "promoter delta" at a position 92 to 95 base pairs (bp) from the epsilon junction and terminates in the opposite delta at a position 20 to 40 bp from the flanking DNA sequences (17). This transcript specifies two open reading frames. One encodes a protein similar to sequence-specific DNA-binding proteins of *Escherichia coli*, and the other encodes a protein similar to retroviral reverse transcriptase (11, 32). It has been shown that transposition of Ty1 occurs by a mechanism involving reverse transcription of Ty1 RNA (5). Additional evidence demonstrating a Ty-encoded reverse transcriptase and Ty1-associated viruslike particles has been presented (26, 33).

The *CYC7-H2* mutation in *S. cerevisiae* was caused by insertion of a Ty1 element in the noncoding region of the iso-2-cytochrome *c* structural locus, *CYC7* (20). The Ty1 element is inserted at position −184 with respect to the ATG initiation codon (24, 29). Recent evidence indicates that the normal *CYC7* control region consists of both positive and negative sites that are centered at positions −240 and −300, respectively (58, 59). Hence, the Ty1 element in *CYC7-H2* is between the coding sequence and the normal *CYC7* upstream control sequences. The effect of the Ty1 insertion is to place iso-2-cytochrome *c* production under the control of regulatory signals that normally determine the cell type-specific functions in yeast of mating and sporulation (20, 22). In **a** and α haploid cells, the *CYC7-H2* mutation causes a 20-fold overproduction of iso-2-cytochrome *c*. In **a**/α diploid cells, *CYC7-H2* expression is repressed 10-fold (20, 22, 47). The cell type regulation of iso-2-cytochrome *c* in the *CYC7-H2* mutant is the same as the cell type control of Ty RNA production (13, 18). Sequences homologous to the **a**/α control sites identified at *MAT*α (49) and *HO* (34) are found in the *CYC7-H2* Ty1 (23). Taken together, these observations suggest that the Ty1 element contains regulatory sequences that replace normal *CYC7* transcriptional controls.

Although the actual mechanism by which Ty1 controls adjacent gene expression is not known, it appears that the inserted element is not providing a new promoter and a new transcription start site. When Ty elements activate gene expression, elements are found to be inserted in the same orientation within a region 600 to 125 bp upstream from the respective coding sequences (28, 29, 36, 54). Transcription of the element and the affected gene are divergent. The 5′ map position of the adjacent gene mRNA has been determined for Ty mutations at two different loci and shown to be the same as that from the corresponding wild-type strains

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(36, 54). Hence, Ty1 inserted at variable upstream positions activates transcription of an adjacent gene from its normal site. This property is similar in certain respects to the action of enhancer sequences (27).

It has been suggested that there should be a correlation between the transcriptional ability of a given Ty and its ability to enhance transcription of an adjacent gene (45). DNA sequence analysis of the *CYC7-H2* Ty1 suggested to us that the process of Ty1 transcription per se may not be required for adjacent gene activation. The *CYC7-H2* "promoter" delta is truncated. It lacks two-thirds of the normal delta sequences that correspond to the "upstream" promoter region and the Ty1 RNA start site (17, 24). In this report, we test for the presence of RNA derived specifically from the Ty1 element inserted at *CYC7*. These studies address whether the normal level of Ty1 transcription is necessary for its activation of the adjacent gene. In the course of these studies we characterized two transcripts other than *CYC7* mRNA from the *CYC7* region. One transcript was present in all strains analyzed and mapped 3′ to the *CYC7* coding region. The other transcript became activated by inversion of *CYC7-H2* Ty1 sequences and was homologous to *CYC7* upstream sequences.

MATERIALS AND METHODS

Yeast strains and alleles. The strains used in these studies were derived by transformation of either *S. cerevisiae* E480-1D (*MAT***a** *cyc1-363 cyc7-28 ura3-52 leu2-3,112*) or E724-7A (*MAT***a** *cyc1-11 cyc7-67 gal2 can1 trp1-289 ura3-52*). These recipient strains are iso-1-cytochrome *c* deficient because of mutations at the *CYC1* structural gene. The *cyc1-363* allele is a deletion of the *CYC1* structural gene (50). The *cyc1-11* allele is a 2-bp substitution that changes the CAA codon at amino acid position 76 to a UAA nonsense codon (19). The recipient strains are also iso-2-cytochrome *c* deficient because of mutations at the *CYC7* structural gene. The *cyc7-28* allele is a UGA mutation corresponding to amino acid position 39 within the *CYC7* coding region (T. Cardillo, cited in reference 21). The *cyc7-67* allele is a 0.4-kb deletion of *CYC7* sequences that includes 0.14 kb of 5′ noncoding sequences and 0.26 kb of coding sequences (T. Cardillo and K. Zaret, cited in reference 23). Each transformed strain derived from these has a different plasmid construction integrated at the *CYC7* locus. The structures of the resulting *CYC7* alleles are illustrated in Fig. 1. The construction and identification of all except *CYC7-P1103* (see below) have been described previously (21). Expression of the various plasmid *CYC7* alleles in the transformed strains was compared with the *CYC7-H2* strain E378-1A (*MAT***a** *cyc1-363 CYC7-H2 cry1 his1 lys2*), the *CYC7*$^+$ strain B544 (*MAT***a** *cyc1-49 CYC7*$^+$ *his1 lys2 trp2*), or the *CYC7-H3* strain GM105-15A (*MAT***a** *cyc1 CYC7-H3 his1 trp2*).

The *CYC7-P1103* allele is an integration of the plasmid designated pNC11 at the *CYC7* locus. pNC11 contains an inversion of a Ty1 *Xho*I fragment from the plasmid designated pAB50 (21). (The *CYC7-H2* region of pAB50 has the structure shown for *CYC7-P54* in Fig. 1.) The inversion was constructed by methods described for the plasmid designated pAB56 (21). (The *CYC7-H2* region of pAB56 has the structure shown for *CYC7-P112* in Fig. 1.) Strain E724-7A was transformed with pNC11 plasmid DNA by previously described procedures (21). Integrations of pNC11 at the *CYC7* locus were identified by Southern blot analysis of genomic DNA isolated from representative transformed strains. On the basis of size and number of *CYC7*-hybridizing *Hin*dIII and *Bam*HI restriction fragments, strain E724-P1103 was shown to contain two tandem copies of pNC11 integrated at *CYC7*. The integrated structure is given the allele designation *CYC7-P1103*. Conditions for yeast genomic DNA isolation and restriction endonuclease and hybridization analysis have been described previously (21).

Hybridization probes. The DNA fragments used as hybridization probes in various experiments were derived from the *CYC7*$^+$ plasmid designated pAB25 (31), the *CYC7-H2* plasmid designated pAB35 (20), or pBR322 (6). The *CYC7* mRNA probe designated mCYC7 was provided to us by D. Pietras. The probe consists of a 349-nucleotide (nt) *Sau*3A-*Hpa*II fragment from the *CYC7* transcribed region that was cloned into the *Bam*HI-*Acc*I site of M13mp9 (30). Other strand-specific probes were prepared by insertion of specified DNA fragments into M13 vectors. DNA fragments with two different restriction site ends were used so as to yield defined orientations with respect to viral sequences.

Plasmid DNA was prepared by a CsCl banding method (37). Double-stranded DNA fragments from appropriate plasmids were isolated from agarose gels (12). Single-stranded recombinant phage DNA was isolated by the methods given in reference 3. Double-stranded DNA fragments and M13mp8 replicative form (RF) DNA (no insert) were labeled with [α-^{32}P]dCTP (New England Nuclear Corp.) by the nick-translation reaction (42).

Yeast RNA preparation and Northern hybridization analysis. Cells were grown in YPD (1% [wt/vol] yeast extract [Difco Laboratories], 2% [wt/vol] peptone [Difco], 2% [wt/vol] dextrose) medium to 1×10^7 to 2×10^7 cells/ml. Total RNA from each strain was isolated (7) and enriched for polyadenylated [poly(A)$^+$] RNA by oligodeoxythymidylate [oligo(dT)] selection (1). Glyoxal-denatured RNAs were fractionated on 1% agarose slab gels and transferred to nitrocellulose sheets (9, 52). Hybridization and washing conditions were as described in reference 8. The sizes were deduced from the mobility of acridine orange-stained standards, consisting of lambda *Hin*dIII and ϕX174 *Hae*III DNA fragments. These DNA standards were denatured by the glyoxal procedure used for RNA (9).

Determination of transcript polarity from the *CYC7* region was done by RNA transfer from agarose gels as described above. Unlabeled virus (+)-strand DNA from the strand-specific M13 recombinants was hybridized to duplicate RNA nitrocellulose filters for 24 h at 65°C. The filters were briefly washed in hybridization buffer equilibrated at 65°C. They were then hybridized for 24 h at 65°C to radioactively labeled M13mp8 RF DNA (5×10^6 cpm). Hybridization and washing conditions were as described in reference 8.

Dot-blot sandwich hybridization procedure. A dot-blot adaptation of a sandwich hybridization procedure was used to detect RNA homologous to two nonoverlapping DNA probes (14, 40). This procedure permits detection of any RNA that has sequences homologous to two nonoverlapping DNA probes. The first DNA probe, which was not radioactively labeled, was immobilized on nitrocellulose to select homologous RNA species. From 0.1 to 10 μg of denatured double- or single-stranded DNA was spotted onto strips of nitrocellulose which had been equilibrated with 20× SSC (0.3 M sodium citrate, 3 M sodium chloride, pH 7.4) and allowed to dry at room temperature. If the probe consisted of double-stranded DNA, denaturation was accomplished by heating 500 μg of linearized DNA per ml in H_2O to 100°C for 10 min and then quickly chilling the solution on ice. An equal volume of 1 M NaOH was added. After incubation at room temperature for 20 min, the DNA sample was neutralized by

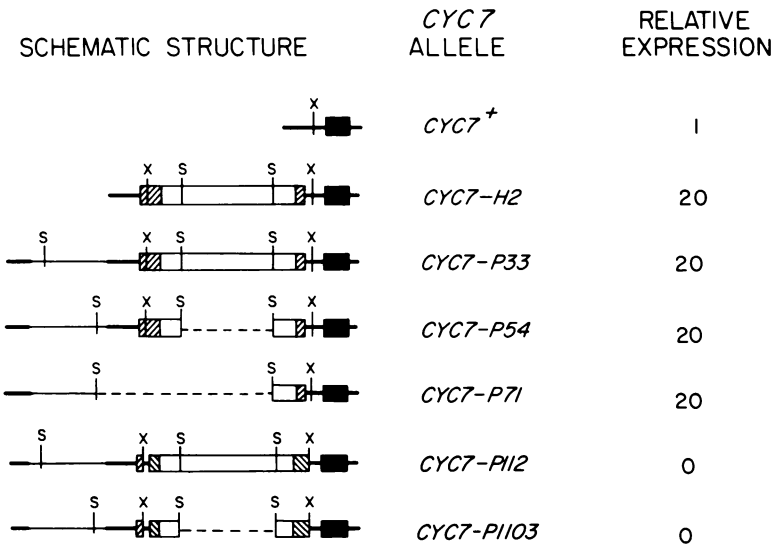

FIG. 1. Schematic structures and iso-2-cytochrome *c* phenotype of different *CYC7* alleles. The solid box represents *CYC7* coding sequences, and thick lines represent *CYC7* flanking sequences. Open boxes represent epsilon sequences of Ty1. Hatched boxes represent delta sequences. Thin lines represent pBR322 sequences. *Sal*I (S) and *Xho*I (X) sites are included to indicate the endpoints of deletion or inversion fragments. The *CYC7-P112* and *CYC7-P1103* alleles contain a tandem duplication of the corresponding integrated plasmids. A reversal in the direction of the hatchmarks indicates the inverted orientation of Ty sequences in *CYC7-P112* and *CYC7-P1103*. Expression of each allele is given in terms of the relative amount of iso-2-cytochrome *c* produced. The amount produced by a standard *cyc1 CYC7*$^+$ strain is assigned a value of 1. The cytochrome *c* content of intact cells was estimated by comparing c_α band absorption intensities with those for strains having known amounts of iso-2-cytochrome *c* (21).

addition of an equal volume of 1 M NaCl–0.3 M sodium citrate–0.5 M Tris, pH 8.0 (39). If the probe consisted of single-stranded DNA, denaturation was accomplished by simply heating 500 μg of DNA per ml in 0.3 M NaCl to 100°C for 10 min and then quickly chilling the solution on ice. After spotting the denatured DNA samples, the air-dried nitrocellulose strips were washed twice with 6× SSC, air-dried again, and incubated for 2 h at 80°C under vacuum. Hybrid selection of RNA homologous to the immobilized DNA was accomplished by incubation of the filters with 60 μg of poly(A)$^+$ RNA per ml of hybridization solution for 36 h at 65°C. The filters were washed 10 times with hybridization buffer at 65°C. Detection of retained RNA was accomplished by hybridization to a second, radioactively labeled DNA probe (5×10^6 cpm) for 24 h at 65°C. Hybridization and washing conditions were as described for Northern hybridization analyses.

Primer extension procedure. The 5′ map position of a new RNA species found in Ty1 inversion derivative strains was determined by primer extension. Procedures were essentially those described by Reeder et al. (41). A synthetic oligonucleotide, 5′-TCTTTTCCCACCTTCTCAAA, was prepared with an Applied Biosystems model 380A synthesizer. The oligonucleotide was end labeled with [γ-^{32}P]ATP (New England Nuclear) and T4 polynucleotide kinase (New England Biolabs). RNA was annealed with 1 pmol of end-labeled primer in 30 μl of buffer (250 mM KCl, 10 mM Tris, pH 8.0, 1 mM EDTA) at 58°C for 1 h. After the annealing reaction, 50 μl of a reverse transcriptase reaction mix was added. The reaction mix contained 24 mM Tris, pH 8.3, 16 mM $MgCl_2$, 8 mM dithiothreitol (DTT), 100 μg of actinomycin D per ml, 0.4 mM each dATP, dCTP, and dTTP, 0.8 mM dGTP, and 10 U of avian myeloblastosis virus (AMV) reverse transcriptase (Bethesda Research Laboratories). The primer extension reaction was carried out at 41°C for 1 h. After the extension reaction, RNA was hydrolyzed by addition of 16 μl of 1 N NaOH and incubation at 100°C for 3 min. The samples were neutralized by addition of 16 μl of 1 N HCl. Extension products were ethanol precipitated in the presence of 5 μg of tRNA carrier. The resulting pellets were washed five times with 80% ethanol and were then suspended in formamide dye mix (0.1% xylene cyanol FF, 0.1% bromophenol blue, 10 mM EDTA, 98% deionized formamide). The samples were subjected to electrophoresis on buffer gradient DNA-sequencing gels prepared as described in reference 3.

Size standards for the RNA template extension products were provided by a DNA sequence ladder. DNA sequencing procedures by the chain termination method with [^{35}S]dATP were performed as described by Bankier and Barrell (3). An M13mp8 subclone of a 468-bp *Pst*I-*Bgl*II fragment encompassing the upstream Ty1 *CYC7* junction region from *CYC7-H2* has been described previously and was used as a template (24). The synthetic oligonucleotide specified above was used as a primer but was not end labeled.

RESULTS

***CYC7* transcription.** To determine whether there is a direct correlation between transcription of Ty1 and the adjacent gene, we first determined the size and amount of *CYC7* mRNA from strains with different *CYC7* alleles. The structures and corresponding iso-2-cytochrome *c* phenotypes of these alleles are shown in Fig. 1. Standard *CYC7*$^+$ and *CYC7-H2* strains provide references for these comparisons. A control for the presence of plasmid sequences is provided by the *CYC7-P33* allele, which consists of an integrated plasmid carrying the *CYC7-H2* gene. The *CYC7-P54* and *CYC7-P71* alleles represent different deletions of distal Ty1 regions. Neither deletion prevented Ty1-associated overproduction of iso-2-cytochrome *c*. The *CYC7-P51* and *CYC7-P72* alleles are not shown in Fig. 1 but consist of tandemly

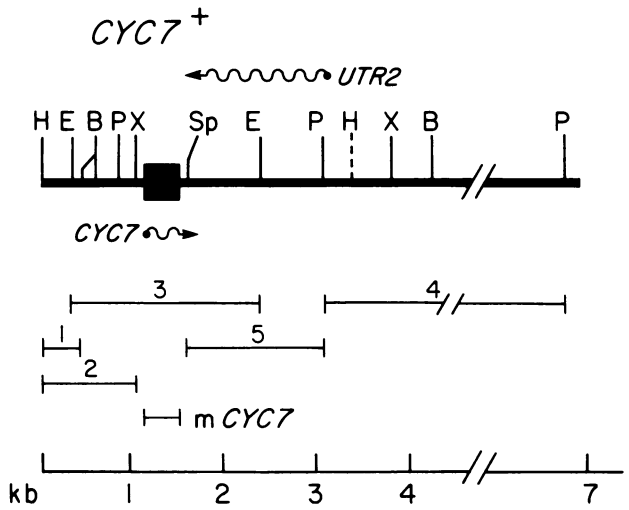

FIG. 2. Restriction sites, DNA hybridization probes, and transcription map of the *CYC7* region. The restriction sites that were determined by McKnight et al. (31) and in this study are designated as follows: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; Sp, *Sph*I; and X, *Xho*I. (The *Hin*dIII site indicated by the broken line is present in some strains but absent in others.) The horizontal lines below the restriction map indicate segments used as hybridization probes; each segment is designated by number. The solid box indicates the translated portion of the *CYC7* locus. The arrows indicate the 5′ to 3′ direction of transcription of the designated genes.

integrated copies of the deletion plasmids corresponding to the structures shown for *CYC7-P54* and *CYC7-P71*, respectively. The *CYC7-P112* and *CYC7-P1103* alleles are Ty1 inversion derivatives of *CYC7-P33* and *CYC7-P54*, respectively. Both inversion derivatives abolish iso-2-cytochrome *c* production.

RNA from strains with the different *CYC7* alleles was prepared and analyzed by the Northern blot method. Figure 2 shows a restriction map of the genomic *CYC7*$^+$ region and indicates the different DNA fragments used as hybridization probes for the experiments to be described. The autoradiogram illustrated in Fig. 3 shows the results with the *CYC7* fragment 3 as a probe for RNA blots. We observed a 0.6-kb hybridizing species that had previously been identified as the *CYC7* mRNA (36). This same RNA size was observed for *CYC7*$^+$ and *CYC7-H2* strains. The strains that overproduced iso-2-cytochrome *c* also overproduced *CYC7* mRNA. The Ty1 inversion derivative strains *CYC7-P112* and *CYC7-P1103*, which did not produce any cytochrome *c*, showed no detectable *CYC7* mRNA even after very long exposure times. A novel 1.1-kb RNA was produced by the *CYC7-P112* strain. In addition to the *CYC7* mRNA, 1.4-kb transcript was detected for all strains studied. Experiments to characterize the 1.1- and 1.4-kb RNAs are presented later in the results.

***CYC7-H2* Ty1 transcription.** Because there are approximately 30 Ty1 elements per haploid genome, measurement of transcripts from any one element requires "marking" it in some fashion. Strains with different *CYC7-H2* derivative alleles that have deletions or inversions of Ty1 were used to determine whether the *CYC7-H2* Ty1 is transcribed. These modifications would produce a characteristic RNA species that could be readily distinguished from normal Ty1. Experiments with each derivative are described.

The *CYC7-P71* allele carries a modified Ty1 in which 3.7 kb of distal Ty1 sequences have been deleted and the remaining Ty1 sequences are joined directly to pBR322 DNA. A fused RNA consisting of Ty1 and pBR322 sequences was predicted if the Ty1 of *CYC7-P71* is transcribed into a stable RNA. A dot-blot adaptation of a sandwich hybridization method was used to test for the predicted fusion RNA. This procedure permits detection of any RNA that has sequences homologous to two nonoverlapping DNA probes. Results provided qualitative evidence for the predicted Ty1 fusion RNA.

Denatured, unlabeled, double-stranded pBR322 DNA was immobilized on nitrocellulose filters. The immobilized pBR322 DNA was used to hybrid-select homologous poly(A)$^+$ RNA from the *CYC7-P72* strain, which has 4 to 5 tandem copies of the *CYC7-P71* structure. References for signal background were provided by performing parallel experiments with no RNA and with poly(A)$^+$-enriched RNA from the *cyc7-28* recipient strain and from the *CYC7-P51* control strain, which has 8 tandem copies of the *CYC7-P54* structure. The filters were then hybridized to radioactively labeled Ty1 DNA. This second hybridization allowed the detection of homologous RNAs retained on the filter by hybridization to the first probe. A Ty-pBR322 fusion RNA was detected in the *CYC7-P72* strain but not in the control strains (Fig. 4A). However, this result does not indicate whether the fusion transcript started in Ty1 and continued into pBR322 or vice versa.

Filters with strand-specific probes were prepared for sandwich hybridization to determine the polarity of the Ty1-pBR322 fusion RNA. Strand-specific probes containing a 1.4-kb *Sal*I-*Pst*I fragment from pBR322 were prepared by an M13 cloning strategy (Fig. 4C). Single-stranded DNA from two recombinant phage, designated mp8-pBR and mp9-pBR, were immobilized on nitrocellulose filters. Separate filters were then hybridized to RNA from the *CYC7-P71* strain. References for signal background were provided by perform-

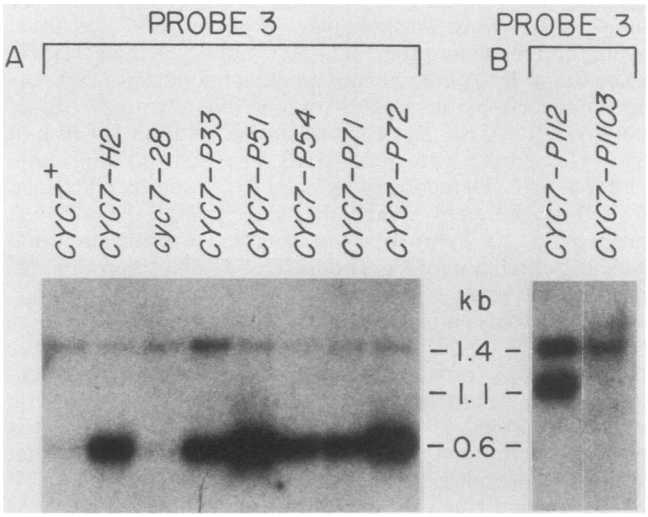

FIG. 3. Autoradiograms of Northern blots showing transcripts from the *CYC7* region of different *CYC7* alleles; 4.0 μg of *CYC7*$^+$, 3.5 μg of *CYC7-H2*, 2.5 μg of *cyc7-28*, 2.0 μg of *CYC7-P33*, 1.0 μg of *CYC7-P51*, 2.5 μg of *CYC7-P54*, 2.5 μg of *CYC7-P71*, 1.0 μg of *CYC7-P72*, 2.0 μg of *CYC7-P112*, and 4.0 μg of *CYC7-P1103* oligo(dT)-selected RNA was used in each of the corresponding lanes. Panels A and B show filters transferred from separate gels. The DNA probe was *CYC7*$^+$ fragment 3, shown in Fig. 2. The sizes of hybridizing bands are shown (in kilobases) between the autoradiograms. The 0.6-kb RNA from *CYC7*$^+$ and *cyc7-28* is not easily seen in reproduction but was clearly distinguished in the original autoradiograms.

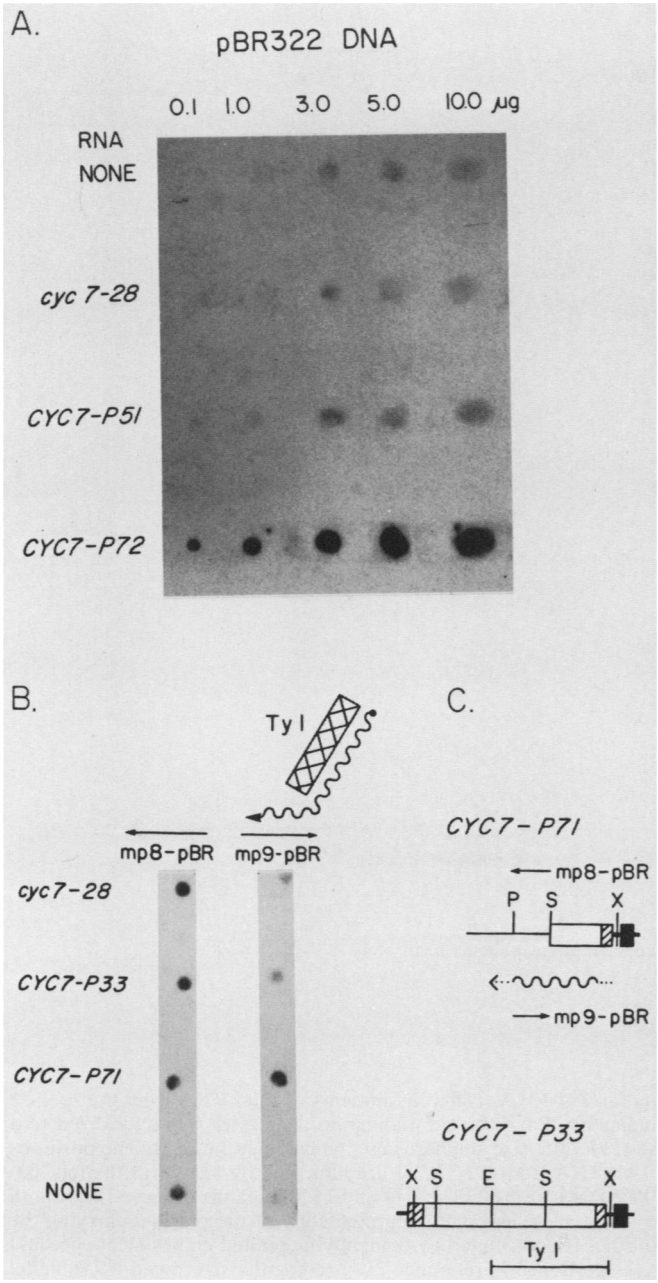

FIG. 4. (A) Autoradiogram showing the presence of a Ty1-pBR322 fusion RNA from the *CYC7-P72* allele; 0.1, 1.0, 3.0, 5.0, and 10 µg of denatured, double-stranded pBR322 DNA was immobilized on four identical nitrocellulose strips. Unlabeled oligo(dT)-selected RNA from the *cyc7-28* recipient strain, the *CYC7-P51* control strain, and the *CYC7-P72* test strain was hybridized to the filters as indicated to the left of the autoradiogram. (The *CYC7-P51* and *CYC7-P72* alleles consist of tandemly integrated copies of the Ty1 deletion plasmids corresponding to the structures shown for *CYC7-P54* and *CYC7-P71* in Fig. 1.) The pBR322-Ty1 fusion RNA was detected by hybridization to the radiolabeled Ty1 DNA probe indicated in panel C. (B) Autoradiogram of dot-blots showing polarity of the Ty1-pBR322 fusion RNA from the *CYC7-P71* allele; 0.1 µg of the unlabeled probes designated mp8-pBR and mp9-pBR per dot was immobilized on nitrocellulose filters. Unlabeled oligo(dT)-selected RNA from each of the indicated strains was hybridized to the mp8-pBR and mp9-PBR filters. The pBR322-Ty1 fusion RNA was detected by a second hybridization to the radiolabeled Ty1 DNA probe indicates in panel C. (C) Diagrams indicating DNA probes and Ty1-pBR322 fusion RNA. The probes designated mp8-pBR and mp9-pBR consist of the 1.4-kb *Pst*I (P)-*Sal*I (S) fragment from pBR322 cloned into the corresponding sites in M13mp8 and M13mp9, respectively. The *CYC7-P33* and *CYC7-P71* diagrams are drawn according to the conventions described in the legend to Fig. 1. Arrows above the *CYC7-P71* diagram indicate the position of the mp8-pBR and mp9-pBR probes with respect to the *CYC7-P71* structure and specify the polarity of each probe in the 5′-to-3′ direction. The radiolabeled Ty1 probe was prepared from an isolated *Xho*I (X)-*Eco*RI (E) Ty1 DNA fragment indicated by the bar below the *CYC7-P33* diagram. The fusion RNA is indicated by a wavy arrow. Dots indicate that the size and endpoints of the RNA are not known.

ing parallel experiments with no RNA and with RNA from the *cyc7-28* recipient and the *CYC7-P33* control strain. Ty1-pBR322 fusion RNA retained on one or the other filter was then detected by hybridization to radioactively labeled Ty1 DNA. Figure 4B shows results with mp8-pBR and mp9-pBR filters that were hybridized to the same Ty1 DNA probe and exposed on the same autoradiogram. RNA from the *CYC7-P71* strain gave a hybridization signal on the mp9-pBR filter that was clearly above the background amount for no RNA and for RNA from the two control strains. The hybridization signals were the same for no RNA and for all the RNA samples tested in the parallel hybridizations to the mp8-pBR filter. There was no obvious explanation for the high background observed with the mp8-pBR DNA filter. It was higher than observed for filters in which the same amount of either double-stranded pBR322 DNA or mp9-pBR DNA was immobilized. Nonetheless, the results clearly showed that a Ty1-pBR322 fusion RNA could be detected by the dot-blot sandwich method. The polarity of this RNA was consistent with its initiation in Ty1 sequences and termination in pBR322 sequences.

Even though we could detect the Ty1-pBR322 fusion RNA by the above method, we were unable to detect the fusion RNA from the *CYC7-P71* structure or from the related multicopy *CYC7-P72* structure by Northern blot analyses with either radiolabeled pBR322 or Ty1 DNA probes (results not shown). One possibility to account for the negative results in Northern blot experiments is that the fusion RNA is heterogeneous due to lack of proper termination signals in the pBR322 region.

The *CYC7-P54* allele carries a modified Ty1 in which 3 kb of epsilon sequence has been deleted. The normal Ty1 termination site is present, so that the *CYC7-P54* structure was expected to produce a discrete transcript that is 2.7 kb in size. This 2.7-kb RNA should be observed in Northern blot experiments with a Ty1 DNA probe. However, the predicted RNA was not detected from the *CYC7-P54* strain or from the related multicopy *CYC7-P51* strain. In contrast to the above experiments with the Ty1-pBR322 fusion RNA, it is unlikely that the negative results with the 2.7-kb Ty1 RNA were due to lack of proper termination and heterogeneous RNA populations.

Comparisons of RNA from the *CYC7-P51* strain and the *cyc7-28* control strain gave an indication of the upper limit for steady-state RNA produced from the *CYC7-H2* Ty1. The experiments shown in Fig. 5 compared the ability to detect the 5.7-kb RNA from 30 copies of Ty1 and the 2.7-kb RNA from 8 copies of the modified Ty1 of the *CYC7-P51* allele. If the modified Ty1 elements from *CYC7-P51* produced RNA in steady-state amounts equivalent to that for an average Ty1, the 2.7-kb signal is expected to be 25% as intense as the 5.7-kb signal. The number of modified Ty1 elements present

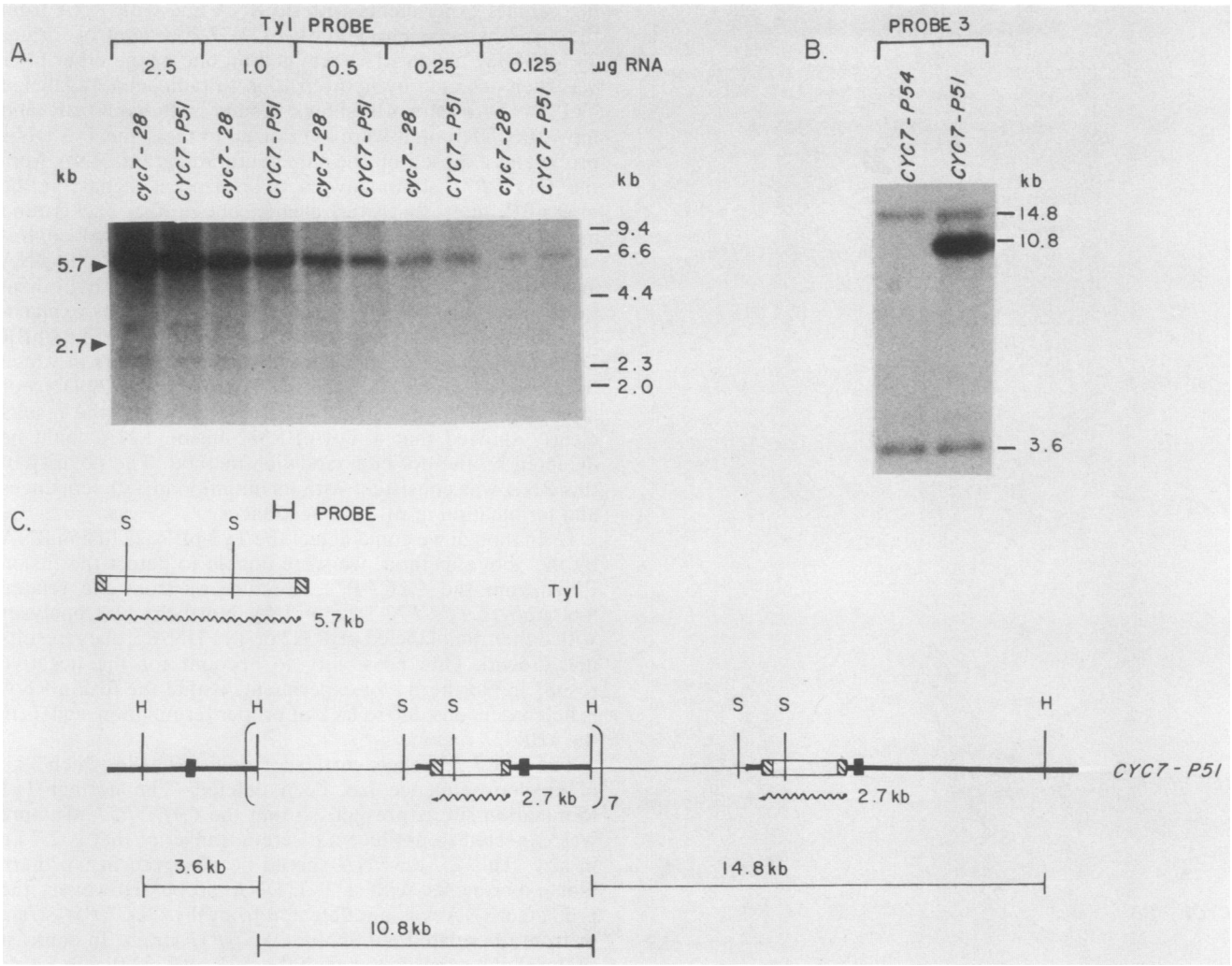

FIG. 5. (A) Autoradiogram of a Northern blot showing detection limits for Ty1 RNA. Different amounts of total RNA from the *cyc7-28* control strain and the *CYC7-P51* strain were loaded into each lane as indicated above the autoradiogram. The filter was hybridized to a radiolabeled Ty1 DNA probe (indicated in panel C). The positions of lambda *Hin*dIII size standards are indicated at the right. The positions of the 5.7-kb RNA from endogenous Ty1 elements and the predicted 2.7-kb RNA from *CYC7-P51* are indicated by arrows at the left. (B) Autoradiogram of genomic DNA *Hin*dIII fragments that hybridized to *CYC7* DNA probe 3. The 3.6- and 14.8-kb bands observed with both *CYC7-P51* and *CYC7-P54* DNA are contributed by the recipient strain *cyc7-28* allele and one integrated copy of the plasmid carrying the *CYC7-H2* deletion derivative. The 10.8-kb band observed with *CYC7-P51* DNA is contributed by tandemly integrated copies of the plasmid (see panel C). Procedures and conditions for Southern blot analysis were as described in reference 21. A computer-interfaced LKB model 2202 laser densitometer programmed to measure relative peak areas from bands on autoradiograms was used to determine the relative band intensities. (C) Diagram of a typical Ty1 and of the *CYC7-P51* allele. The positions of *Hin*dIII (H) and *Sal*I (S) restriction endonuclease cleavage sites are indicated. The *Hin*dIII fragments corresponding to the 14.8-, 10.8-, and 3.6-kb bands in panel B are indicated by bars below the diagram. The bar above the Ty1 diagram indicates the *Sau*3A-*Pst*I fragment used as the hybridization probe in panel A. The *CYC7-P51* and Ty1 RNA and the predicted 2.7-kb Ty1 RNA from the *CYC7-P51* allele are indicated by wavy arrows.

in DNA prepared from the *CYC7-P51* strain was determined by densitometric measurements of *Hin*dIII fragments that hybridized to a radioactively labeled *CYC7* DNA probe (Fig. 5B). The diagram in Fig. 5C shows that the 14.8- and 10.8-kb hybridizing *Hin*dIII fragments were contributed by integrated copies of the *CYC7-H2* deletion plasmid and the 3.6-kb hybridizing *Hin*dIII fragment was contributed by the single *cyc7-28* gene at the site of integration. The relative intensity of the 14.8-, 10.8-, and 3.6-kb signals as measured by densitometry was 0.7:7.6:1. The autoradiogram for a Northern blot experiment (Fig. 5A) shows that a signal for the 5.7-kb Ty1 RNA was detected with as little as 0.125 µg of RNA. No signal at the 2.7-kb position was detected even when 20 times more RNA from the *CYC7-P51* strain was used. Weak bands, approximately 3.5 and 2.3 kb in size, were observed (Fig. 5A, lanes 1 through 4). Because they were present at the same intensity in both the *cyc7-28* control strain and the *CYC7-P51* strain, neither could be attributed to transcripts from the *CYC7-P51* Ty1. This comparison indicated that the 2.7-kb RNA from the internally deleted Ty1 was present in an amount that was at least five times lower than that of the 5.7-kb RNA from the average Ty1.

In both the *CYC7-P112* and *CYC7-P1103* derivative genes,

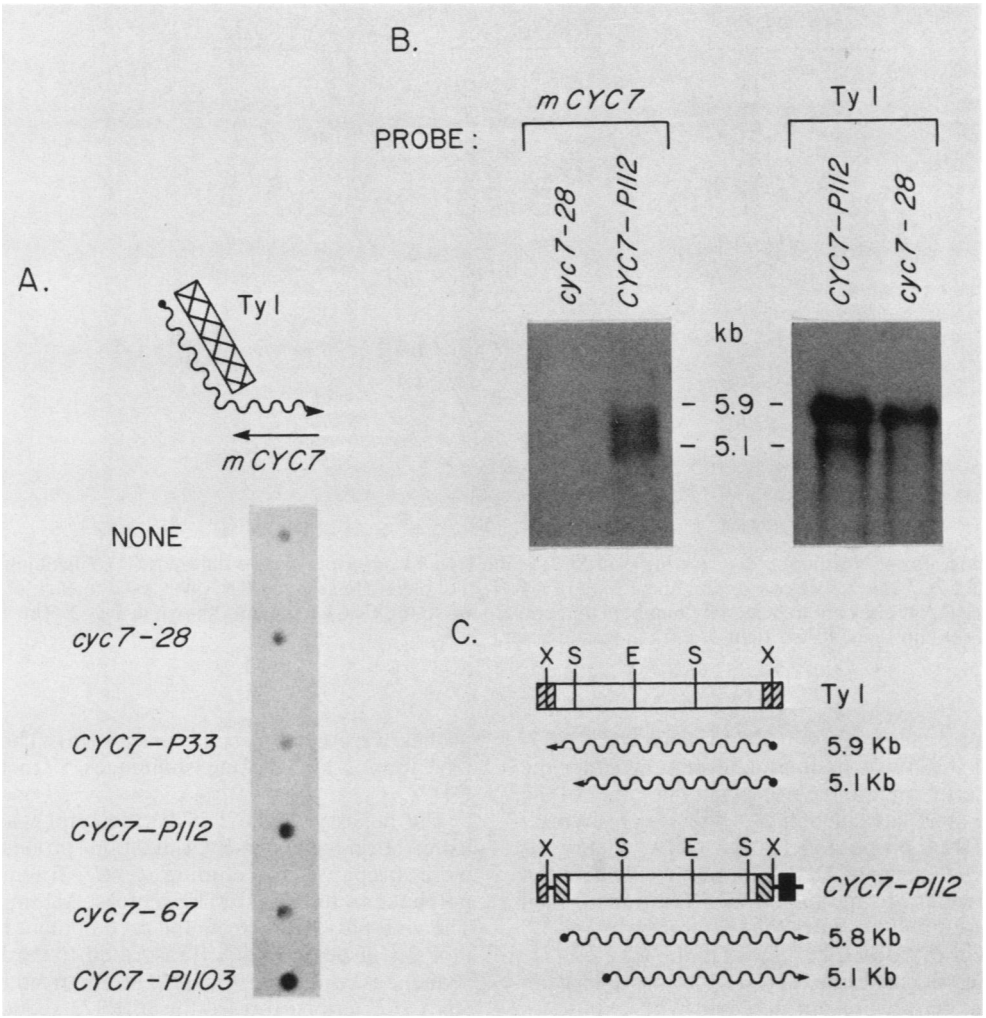

FIG. 6. (A) Autoradiogram of dot-blots showing a *CYC7*-Ty1 fusion RNA. The *CYC7* template probe designated mCYC7 was immobilized on nitrocellulose (1 μg per dot). Oligo(dT)-selected RNA from the indicated strains was hybridized to the immobilized mCYC7 DNA probe. The fusion RNA was detected by hybridization to radiolabeled *Xho*I (X)-*Eco*RI (E) Ty1 DNA fragment (see Fig. 4C). (B) Autoradiogram of Northern blots showing the size of the *CYC7*-Ty1 RNA; 10 and 3.6 μg of oligo(dT)-selected RNA from the *CYC7-P112*-transformed strain and *cyc7-28* recipient strain, respectively, was used per lane for the duplicate filters. One filter was hybridized to radiolabeled Ty1 DNA and the other to mCYC7 DNA. (C) Diagrams of Ty1 and Ty1-*CYC7* fusion RNA. Ty1 and *CYC7-P112* structures are shown according to the conventions described in the legend to Fig. 1. The normal Ty1 RNAs are indicated by wavy lines below the Ty1 structure. The postulated species corresponding to the Ty1-*CYC7* fusion RNAs are indicated by wavy lines below the *CYC7-P112* structure. The direction of the arrows indicates the 5′-to-3′ polarity of the RNAs.

a Ty1 *Xho*I fragment has been inverted. One fragment end is 5′ to *CYC7* (outside of Ty1), and the other is within the distal delta element (Fig. 1). The consequence of these rearrangements is that the direction of Ty1 transcription is now toward the *CYC7* coding region. The normal site for Ty1 transcript termination has been removed, so that transcription could continue into *CYC7* sequences. Thus, we predicted that a *CYC7*-Ty fusion RNA would be produced in the *CYC7-P112* and *CYC7-P1103* strains if the *CYC7-H2* Ty1 were transcribed.

The sandwich hybridization procedure described above was used to test for the predicted Ty1-*CYC7* fusion RNA. The single-stranded M13 recombinant mCYC7 DNA (Fig. 2) was immobilized on nitrocellulose filters. The filters were then hybridized to poly(A)$^+$ RNA isolated from *CYC7-P112*, *CYC7-P1103*, and appropriate control strains. Finally, the filters were hybridized to radiolabeled Ty DNA. The autoradiogram (Fig. 6A) shows that the predicted *CYC7* Ty1 fusion RNA was present in *CYC7-P112* and *CYC7-P1103* strains.

The *CYC7-P112* fusion RNA was expected to be a discrete transcript that began at one of the known Ty1 initiation sites and ended at the *CYC7* termination site. A major 5.7-kb and a minor 5.0-kb Ty1 RNA have been detected by Northern blot analysis of RNA from normal laboratory yeast strains (18). It has been shown that the 5′ end of both size Ty RNAs map to the same sequences within the promoter delta but terminate at different sites (17). The RNAs are diagrammed in Fig. 6C to show their relationship to Ty1 DNA sequences. Winston et al. (56) have found that *spt3* mutant strains of *S. cerevisiae* fail to initiate Ty transcription in the promoter delta. Instead, Ty transcription initiates within epsilon sequences approximately 800 bp downstream from the normal site.

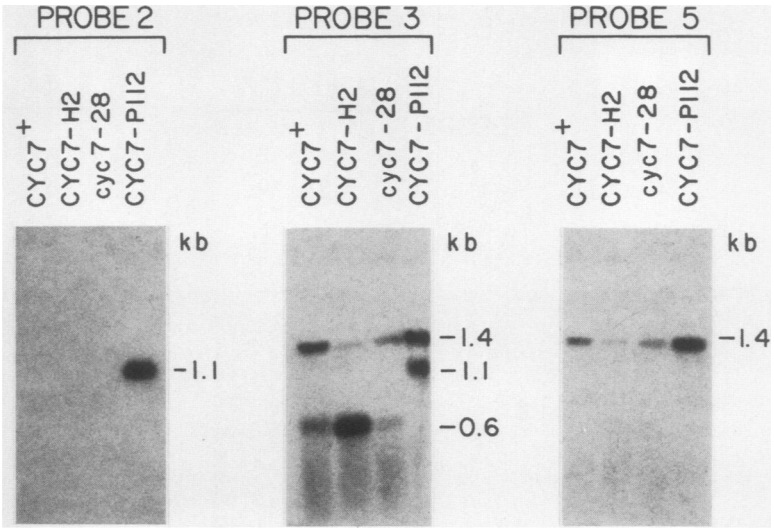

FIG. 7. Autoradiograms of Northern blots showing that the 1.1- and 1.4-kb transcripts map to different *CYC7* flanking sequences; 8.0 μg of *CYC7*$^+$, 3.5 μg of *CYC7-H2*, 2.5 μg of *cyc7-28*, and 1.5 μg of *CYC7-P112* oligo(dT)-selected RNA was used in each of the corresponding lanes. The different DNA probes are indicated by numbers that correspond to the *CYC7* fragments shown in Fig. 2. The sizes of hybridizing bands are indicated (in kilobases) to the right of each autoradiogram.

A Northern blot experiment with RNA from the *CYC7-P112*-transformed strain was done to determine whether the readthrough transcript initiated in the vicinity of either of the known Ty1 RNA start sites. Duplicate filters were hybridized to mCYC7 DNA probe and to Ty1 DNA. Figure 6B (right panel) shows the size of Ty1 RNA that we detected in the *cyc7-28* recipient strain and the *CYC7-P112*-transformed strain. Figure 6B (left panel) shows the two transcripts of 5.8 and 5.1 kb that hybridized to *CYC7* DNA in the *CYC7-P112*-transformed strain. Because the Ty-*CYC7* transcripts were of low abundance, they were not detected with exposures similar to that used in Fig. 3. However, by use of poly(A)$^+$ RNA and long exposure times, they could be detected. (They were not detectable under any conditions when total RNA was used.) An estimate of the predicted size for the *CYC7-P112* fusion RNA can be made from the measured 5.2-kb size of the *CYC7-P112 Xho*I fragment and the 0.7 kb of flanking *CYC7* sequence. The observed size of the 5.8-kb RNA is in good agreement with the interpretation that the transcript initiates ~100 bp from the *Xho*I inversion junction. This region is in the vicinity of the normal Ty1 initiation site. By the same reasoning, the 5.1-kb RNA could start within the epsilon sequence in the vicinity of the *spt3*$^-$ Ty initiation site. Because they were detected with a *CYC7* probe, both transcripts must continue through *CYC7* sequences and would presumably utilize the *CYC7* termination site. The two fusion RNA species are diagrammed in Fig. 6C.

Characterization of a novel RNA from Ty1 inversion derivatives. Several probes prepared from different *CYC7* regions as identified in Fig. 2 were used in Northern blot experiments to localize the 1.1-kb transcript produced by the *CYC7-P112* strain. The *CYC7-H2* plasmid allele, *CYC7-P33*, provided a control for these analyses. The 1.1-kb RNA hybridized to probes prepared from fragments 3 and 2, which include sequences 5′ to the iso-cytochrome *c* coding region, but not to fragment 5, which includes only 3′ flanking sequences (Fig. 7). No hybridization was detected when the more-distal 5′ fragment 1 or the mCYC7 coding region probes were used (results not shown). These results localized the 1.1-kb RNA to sequences 5′ to the *Xho*I site of *CYC7*.

The polarity of the 1.1-kb transcript was determined by using strand-specific hybridization probes. These probes were prepared by cloning *CYC7* fragment 2 into the polylinker site of two M13 vectors, M13mp8 and M13mp9. The viral plus strand from the recombinant phage designated mp8-2 will detect RNA transcribed toward Ty1 and *CYC7* sequences, while the viral plus strand from mp9-2 will detect RNA transcribed toward the pBR322 sequences. RNA prepared from *CYC7-P33* and *CYC7-P112* strains was separated by size on denaturing gels, transferred to duplicate nitrocellulose filters, and hybridized to each strand specific probe. The direction of transcription of the 1.1-kb RNA was toward the pBR322 sequences and away from the Ty1 and *CYC7* sequences (Fig. 8). These observations indicate that the inversion of Ty1 in the *CYC7-P112* construction caused activation of a transcription unit upstream from the inversion junction.

One puzzling result was that a 1.1-kb transcription unit was not observed by Northern blot analysis of RNA from the *CYC7-P1103* allele, which contains the same inversion junction as the *CYC7-P112* allele. However, using a dot-blot method of analysis, we could detect RNA from both *CYC7-P1103* and *CYC7-P112* strains that hybridized to the *CYC7* 5′ flanking region probe designated mp9-2 (results not shown). In the same experiment, no hybridization was detected to RNA from the recipient strain or from the *CYC7-P33* control strain.

The 5′ map position(s) for the new RNA from the *CYC7-P112* and *CYC7-P1103* strains was determined by the primer extension method. The inversion junction region which was common to both alleles is diagrammed in Fig. 9A. A 20-base oligonucleotide with the sequence 5′-TCTTTTCCCACC-TTCTCAAA was synthesized. This sequence corresponds to *CYC7*$^+$ DNA sequence positions −271 to −252 (36). The position of the primer is indicated by the solid bar in Fig. 9A. Control RNA was obtained from the *cyc7-28* and *cyc7-67*

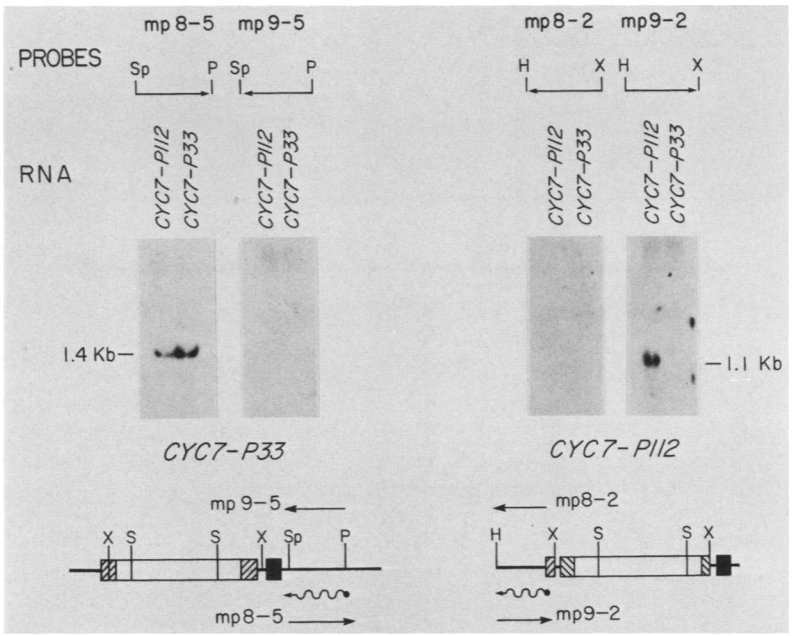

FIG. 8. Autoradiograms of Northern blots showing strand-specific hybridization of the 1.1- and 1.4-kb transcripts from the *CYC7* region; 20 μg of total RNA from strains with the indicated alleles was used for each lane. Separate filters were hybridized to the strand-specific probes indicated above the corresponding panels. The probes designated mp8-5 and mp9-5 were constructed by repairing the *Sph*I end of *CYC7*$^+$ fragment 5 (Fig. 2) and then cloning it into the *Hin*cII-*Pst*I sites of M13mp8 and M13mp9 vectors, respectively. The probes designated mp8-2 and mp9-2 were obtained by cloning *CYC7*$^+$ fragment 2 (Fig. 2) into the *Sal*I-*Pst*I sites of M13mp8 and M13mp9 vectors, respectively. The diagrams show the structure of the *CYC7-P33* and *CYC7-P112* alleles according to the conventions described in the legend to Fig. 1. The different strand-specific probes prepared from *CYC7* DNA are positioned above the diagrams for reference. The wavy line below the diagrams represents the 1.4- and 1.1-kb RNAs, respectively. Arrows indicate the polarities of RNA and single-stranded DNA in a 5′-to-3′ direction.

strains, which were the recipient strains for plasmid transformations to generate the *CYC7-P112* and *CYC7-P1103* alleles, respectively. Additional controls were provided by analysis of RNA from an unrelated *CYC7*$^+$ strain and a *CYC7-H3* strain. The *CYC7-H3* allele is a 5-kb deletion of *CYC7* upstream sequences (31). The proximal deletion endpoint is at position −222 from the *CYC7* ATG initiation codon (29), so that the *CYC7-H3* deletion encompasses the primer-binding site for these experiments.

The 5′-end-labeled oligonucleotide primer was annealed to RNA from the specified strains and extended by reverse transcriptase-catalyzed reaction with all four deoxynucleotide triphosphates. The lengths of extended DNA fragments were determined on DNA sequencing gels. A reference ladder was obtained by coelectrophoresis of DNA chain termination reaction products obtained with the synthetic oligonucleotide as a primer and an appropriate *CYC7-H2* M13 subclone as the DNA template. Figures 9B and C show autoradiograms from these experiments. The same primer extension product sizes were observed with RNA from the *CYC7-P112* and *CYC7-P1103* strains. The major extension products from both mapped to positions 120, 123 to 125, and 131 to 132 nucleotides from the 5′ end of the primer. A number of minor products were also visible at positions 73, 74, 108, 109, 112, 134, and 147 nucleotides from the 5′ end of the primer. These products were not observed with RNA from any of the control strains. However, the autoradiogram also shows that several bands were present with all RNAs examined, including the *CYC7-H3* control that lacks *CYC7* upstream sequences homologous to the oligonucleotide primer. Therefore, the oligonucleotide appears to prime DNA synthesis from RNAs that map elsewhere in the genome. Three conclusions could be made from these comparisons. (i) The two Ty1 inversion derivatives produced a new RNA with the same polarity and with the same 5′ map positions. (ii) The initiation sites for these RNAs mapped to sequences upstream from the Ty1 inversion (Fig. 9A). The major sites were within delta sequences, but none of these corresponded to the normal start site for Ty1 RNA. (iii) Based on the intensity of signals for different amounts of total RNA used, the RNA from the *CYC7-P1103* strain appeared to be about five times less abundant than that from the *CYC7-P112* strain.

The ability of primer extension experiments but not Northern blot experiments to detect the upstream RNA from *CYC7-P1103* species can be most easily explained if we assume that the RNA in question is very heterogeneous at its 3′ end. One difference between the *CYC7-P1103* and the *CYC7-P112* structures could account for heterogeneous 3′ termini with *CYC7-P1103* RNA. The *CYC7-P1103* allele was generated by integration of a plasmid with a deletion of a 0.7-kb *Bam*HI to *Hin*dIII region that was present in the plasmid used to generate the *CYC7-P112* allele (Fig. 9A). The additional *CYC7* sequences in *CYC7-P112* may be important for proper transcript termination and subsequent mRNA stability. For example, Zaret and Sherman (60) have shown that greatly reduced levels of *CYC1* mRNA are associated with a mutation at the transcription termination site of the corresponding gene.

Characterization of the 1.4-kb RNA from the *CYC7* region. Transcripts larger than the *CYC7* mRNA have been observed by others studying *CYC7* transcription (57, 58). In the course of these studies, we defined the location and polarity of this transcription unit. Only the 0.6-kb *CYC7* mRNA was detected in Northern blot experiments with a probe consisting of a single-stranded M13 recombinant phage, designated mCYC7. The mCYC7 probe contains the template strand from the *CYC7* coding region (Fig. 2). This result demon-

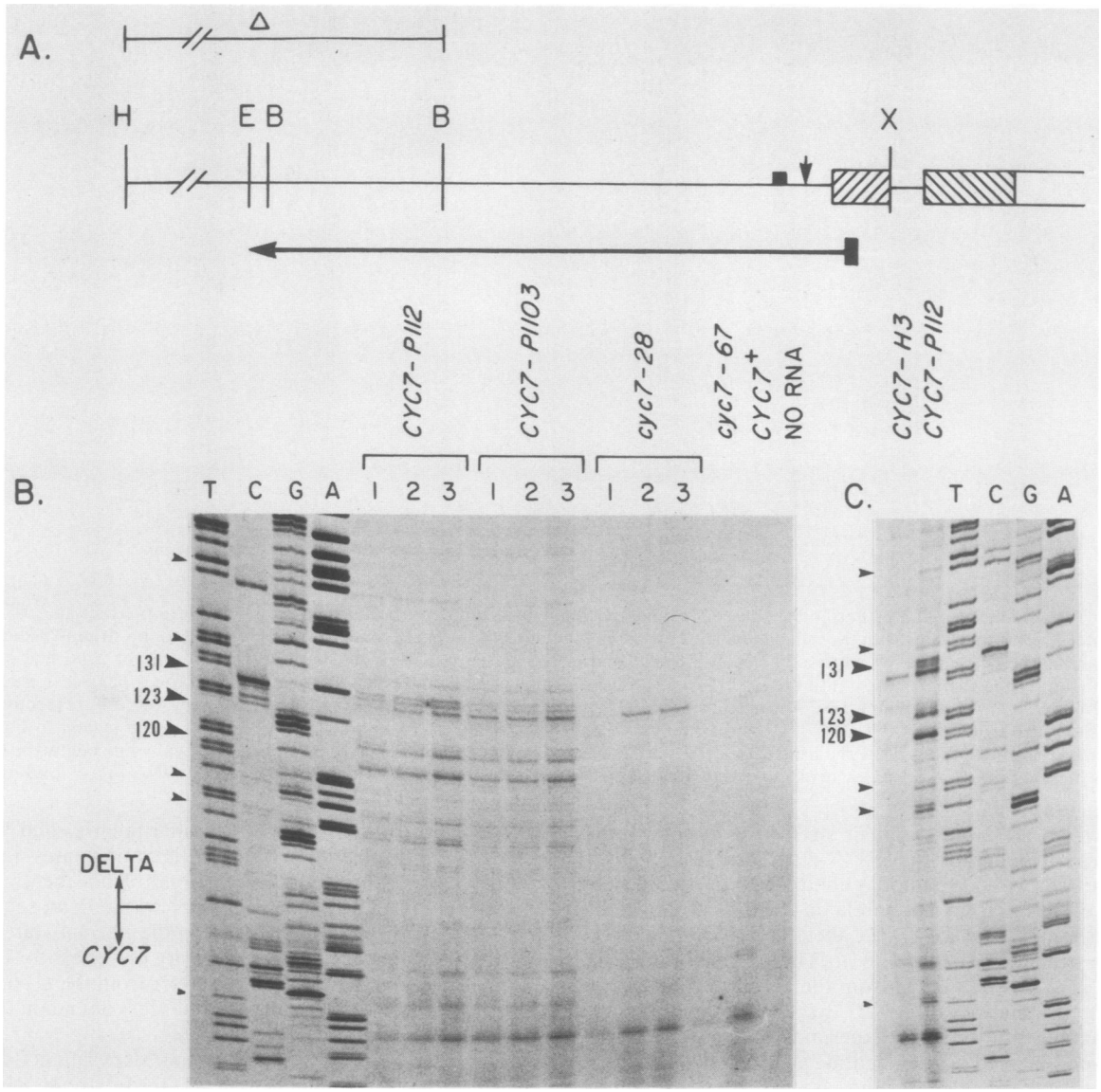

FIG. 9. (A) Schematic structure of the *CYC7-P112* inversion junction. The diagram of *CYC7-P112* is drawn according to the conventions described in the legend to Fig. 1. *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), and *Xho*I (X) restriction sites are shown. The solid box placed on the *CYC7-P112* DNA diagram shows the primer-binding site for the synthetic oligonucleotide used in the primer extension experiments shown in panels B and C. The downward-pointing arrow shows the proximal deletion endpoint in the *CYC7-H3* allele that provides the control RNA for the primer extension analysis shown in panel C. The arrow below the *CYC7-P112* diagram indicates the 5′-to-3′ direction and extent of transcription of the 1.1-kb RNA. The *CYC7-P1103* structure is similar to that shown for *CYC7-P112* except that the region indicated by the bar marked Δ above the diagram is deleted. (B and C) Autoradiograms of primer extension products analyzed on DNA sequencing gels. Amounts of total RNA in primer extension reactions for samples shown in panel B were: *CYC7-P112*-2, 10 μg; *CYC7-P112*-3, 20 μg; *CYC7-P1103*-1, 20 μg; *CYC7-P1103*-2, 40 μg; *CYC7-P1103*-3, 60 μg; *cyc7-28*-1, 2 μg; *cyc7-28*-2, 20 μg; *cyc7-28*-3, 60 μg; *cyc7-67*, 20 μg; and *CYC7*$^+$, 20 μg. The reaction products run on the lane marked *CYC7-P112*-1 were from reactions with 5 μg of *CYC7-P112* poly(A)$^+$ RNA. The lane marked NO RNA was a mock reaction that included 20 μg of tRNA. Amounts of total RNA for samples shown in panel C were: *CYC7-H3*, 20 μg; and *CYC7-P112*, 20 μg. Lanes marked T, C, G, and A display the extension products from the corresponding ddTTP, ddCTP, ddGTP, and ddATP chain termination reactions. Arrows to the left of the autoradiograms indicate the positions of extension products specific for the *CYC7-P112* and *CYC7-P1103* RNA. The sizes of the three major extension products are specified (in nucleotides) from the 5′ end of the primer.

strates that the 1.4-kb transcript is not a larger precursor of the *CYC7* mRNA. Other *CYC7* regions as identified in Fig. 2 were used as hybridization probes to localize the transcription unit for the 1.4-kb RNA. The 1.4-kb RNA hybridized to DNA probes prepared from fragment 3 and fragment 5, both of which include sequences 3′ to the iso-2-cytochrome *c* coding region (Fig. 7). No hybridization was observed with probes prepared from the 5′ noncoding region fragment 2 (Fig. 7) or the more-distal 3′ flanking region fragment 4 (results not shown). Southern blot experiments have shown that DNA fragments 3 and 5 hybridize uniquely to *CYC7* DNA sequences (see references 20 and 31). Therefore, we conclude that the transcription unit is localized 3′ to the iso-2-cytochrome *c* region. In addition, these results establish the *Pst*I site 3′ to the *CYC7* coding region as the most distal limit for one end of the transcript (Fig. 2).

Experiments were performed to determine the polarity of the 1.4-kb transcript. Strand-specific probes were prepared by cloning *CYC7* fragment 5 into the polylinker site of two M13 vectors, M13mp8 and M13mp9. The viral plus strand from the recombinant phage designated mp9-5 includes the same strand as the iso-2-cytochrome *c* template, while the recombinant phage designated mp8-5 includes the opposite strand. The 1.4-kb transcript from strains with the *CYC7-P33* and *CYC7-P112* alleles was detected by hybridization to the strand carried by the mp8-5 phage but not by the mp9-5 phage (Fig. 8). This result demonstrates that the 1.4-kb RNA is transcribed toward the *CYC7* gene. Hence, its polarity is opposite to that of the *CYC7* mRNA (Fig. 2).

DISCUSSION

***CYC7-H2* Ty1 transcription.** There has been little evidence to define the actual relationship between Ty1 transcription and Ty1 effects on adjacent genes. Elder et al. (18) have shown that diploid repression of Ty RNA is identical to diploid repression of *CYC7-H2* and other Ty1 insertion mutations (20, 51). It has also been shown that mutant alleles of *STE7* prevent overproduction associated with Ty1 insertion mutations (20, 51). Dubois et al. (13) have shown that the abundance of Ty1 RNA is similarly decreased in an *ste7* mutant background. The parallel regulation of Ty1 RNA and Ty1-controlled genes suggests that sequences within the Ty1 element are controlling transcription of its own RNA and that of the adjacent gene.

One model to account for these effects is that Ty1 transcription determines adjacent gene transcription. Another model is that *cis*-acting regulatory elements within Ty1, such as enhancers, control adjacent gene expression whether or not Ty1 is itself transcribed. Current views on control of eucaryotic mRNA synthesis suggest that mutliple DNA sequence elements are required. These elements include DNA sequences that specify sites for transcript initiation, as well as upstream control sequences and enhancer regions that regulate the efficiency of transcription (15). Enhancer elements are unusual because they are known to activate transcription from positions more than several hundred base pairs away. Proximal promoter sequences are activated in preference to more-distal ones. In the absence of normal sites, enhancers will activate transcription at substitute sites (see reference 27 for a review.) The first model for Ty1 activation of adjacent gene expression requires all components of the Ty1 transcriptional control region to be functional. The second model requires the activating Ty1 element to have functional enhancerlike components but not functional initiation sites for its own transcription. To ask whether transcription of Ty1 itself is necessary for its activation effects on *CYC7* gene expression in the *CYC7-H2* mutation, we undertook studies to test specifically for *CYC7-H2* Ty1 RNA. Four derivatives of *CYC7-H2* were used to distinguish the *CYC7-H2* Ty1 RNA from that of other Ty1 elements in the genome.

The *CYC7-H2* derivatives denoted by *CYC7-P71* and *CYC7-P54* have different Ty1 deletions. Both Ty1 deletions activate *CYC7* expression to the same extent as observed for *CYC7-H2*. The *CYC7-P71* deletion was predicted to produce a Ty1-pBR322 fusion RNA. A dot-blot sandwich procedure provided qualitative evidence for the existence of the postulated RNA. However, Northern blot experiments failed to detect the Ty1-pBR322 fusion RNA. The *CYC7-P54* deletion was predicted to produce a discrete Ty1 RNA that is 3.0 kb smaller than the normal 5.7-kb Ty1 RNA. The postulated 2.7-kb RNA could not be detected by Northern blot analysis even when RNA from a strain that contained 8 copies of the deletion derivative was used. In comparison with our ability to detect the full-length 5.7-kb Ty1 RNA, we estimated that the *CYC7-H2* Ty1 derivative is transcribed at least five times less efficiently than a single, average Ty1 element. In *CYC7-H2* and its Ty1 deletion derivatives, *CYC7* expression was 20-fold higher than $CYC7^+$ expression. $CYC7^+$ mRNA was detectable in our experiments, but the 2.7-kb RNA from the *CYC7-H2* deletion Ty1 was not. These observations suggest that the amount of steady-state *CYC7-H2* Ty1 RNA is at least 20 times less than that of the adjacent gene it activates.

The rearrangements represented by *CYC7-P112* and *CYC7-P1103* can be viewed as inversions that place Ty1 sequences in the same transcriptional orientation as *CYC7*. They have the additional modification that the site of Ty1 RNA termination in the downstream delta is removed from the transcription unit. A dot-blot sandwich procedure provided qualitative evidence for the predicted Ty1-*CYC7* fusion RNA. Although signals were reproducibly detected for each fusion RNA, they were not greatly above the background signal. The Ty1-*CYC7* fusion RNA from one of the inversion derivatives was further characterized by Northern blot experiments. These experiments demonstrated the presence of two very low abundance but discrete Ty1-*CYC7* fusion RNAs. The sizes were consistent with the interpretation that each started within one of the known Ty1 initiation sites and continued through to the *CYC7* termination site. One known Ty1 initiation site is within the delta element (17). The other site is observed in $spt3^-$ strains and is within epsilon sequences approximately 800 bp downstream from the normal site (56). The observation of two different-sized RNAs transcribed from a single Ty1 element suggests that both Ty1 RNA initiation sites can be utilized in an $SPT3^+$ genetic background. This situation may occur because transcription from the delta site of the *CYC7-H2* Ty1 is not efficient.

Neither of the *CYC7-H2* inversion derivative strains produced any iso-2-cytochrome *c* or any normal *CYC7* mRNA. The above observations with the Ty1-*CYC7* fusion RNA demonstrated that readthrough transcription occurred. Its occurrence may have contributed to the inhibition of iso-2-cytochrome *c* production in these two Ty1 inversion derivatives. Because it appears that the readthrough transcript is not efficiently produced, other factors that contribute to the inhibition need to be considered. For example, the normal *CYC7* control elements were displaced by several kilobases in both inversion derivatives, and the enhancer region of Ty1 may be unable to work over the distance required in the inverted orientation.

We cannot completely rule out the possibility that instability of the modified transcripts we measured accounts for their low steady-state abundance. However, two arguments suggest that instability is not the major cause of the low steady-state abundance we observed. First, three of the four derivative structures we analyzed included normal termination sites, so that the transcripts they produced should terminate normally and should be poly(A)$^+$. The demonstration of discrete transcripts from poly(A)$^+$ selected RNA for the Ty1-*CYC7* fusion structure is consistent with this assumption. Second, the *CYC7-H2* 5′ delta was abnormal. It had no sequences corresponding to the upstream promoter region and initiation sites of a normal delta (24). This finding strongly suggests that the low steady-state abundance for the various modified Ty1 RNAs we measured reflects poor transcript initiation of the *CYC7-H2* Ty1. Together these

findings indicate that normal levels of Ty1 transcription are not required for its activating effects on an adjacent gene.

Activation of an upstream transcript in Ty1 inversion derivatives of *CYC7-H2*. An unexpected observation was the presence of an abundant 1.1-kb transcript that mapped to *CYC7* sequences 5′ to the Ty1 insertion junction in the *CYC7-P112* strain. The 5′ map positions for the RNA were localized to sequences that are upstream from the Ty1 inversion junction in this construction (Fig. 8). The major map positions were in 3′ delta sequences upstream from the inversion junction. However, these start sites do not correspond to the major 5′ map position for Ty1 RNA determined by Elder et al. (17). One interpretation of our results is that the inverted Ty1 element brings enhancer sequences in proximity to the 3′ delta element. Enhancerlike sequences have been identified in the appropriate region of Ty1 (23, 45). In this orientation, Ty1 sequences activate substitute initiation sites that are not used in the normal Ty1 configuration. Activation of a "promoterless" *HIS3* by Ty1 has been reported and may reflect a similar phenomenon (48). The direction of transcription of the 1.1-kb RNA is away from Ty1 sequences. Therefore, the orientation of the Ty1 element with respect to the polarity of the novel upstream transcript is similar to that observed for all Ty1 insertion mutations known to activate gene expression.

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