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Genetics

Transposable element sequences involved in the enhancement of yeast gene expression

(*Ty* element/mating type/enhancer/recombination)

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ABSTRACT **The *his4-917* mutation of yeast results from the insertion of a *Ty* element, *Ty917*, into the 5′ regulatory region of the *HIS4* gene. *Ty917* prevents *HIS4* transcription, thus rendering the cell histidine requiring. Recombination between *Ty917* and a *Ty* element elsewhere in the yeast genome can result in the replacement of part or all of the *Ty917* element by sequences from the other *Ty* element. Recombinant derivatives display a variety of phenotypes including His$^-$, weakly His$^+$, and strongly His$^+$. In most of the His$^+$ derivatives, the expression of *HIS4* is controlled by genes at the mating type locus. To identify the *Ty* sequences important in controlling the expression of an adjacent gene, we used *Ty* elements that have different effects on gene expression to construct hybrid *Ty* elements *in vitro*. The effects of these hybrid elements on *HIS4* expression were examined. These experiments indicate that the critical sequence differences between *Ty* elements that permit *HIS4* expression and those that prevent its expression lie in the rightmost (*HIS4*-proximal) 730 base pairs of the element. The DNA sequence of this region was determined for three elements: *Ty917*, which prevents *HIS4* expression; *Ty917(467)*, which confers a weak His$^+$ phenotype; and *Ty917(480)*, which confers a strong His$^+$ phenotype. Within this region, *Ty917(467)* differs from *Ty917* by a single base-pair change that is in the internal (ε) region of the *Ty* element. *Ty917(480)* differs from *Ty917* by this same base-pair change and by 10 changes in the terminal δ sequence. The sequence change common to *Ty917(467)* and *Ty917(480)* lies in a region of the *Ty* element that is homologous to the simian virus 40 enhancer of transcription.**

Haploid yeast cells contain ≈35 copies of a dispersed repetitive mobile element known as *Ty* (*t*ransposon *y*east) (1–3). Each element consists of an internal region referred to as epsilon (ε) flanked by direct terminal repeats known as deltas (δ). The ε region is ≈5.25 kilobase pairs (kbp) long and the δs are each ≈330 base pairs (bp) long.

Ty elements transpose preferentially into the regulatory regions at the 5′ ends of yeast genes (4, 5). Insertion of a *Ty* into the regulatory region of a gene often leads to overexpression of the adjacent gene and renders expression of the gene subject to control by genes at the mating type (*MAT*) locus (6). This *MAT* control causes the *Ty*-adjacent gene to be expressed at high levels in haploid cells and diploid cells homozygous (**a**/**a** or α/α) at *MAT* but at much lower levels in diploid cells heterozygous (**a**/α) at *MAT*. In all cases of *Ty*-controlled gene expression, the orientation of the *Ty* is such that the *Ty* element and the adjacent gene are divergently transcribed (2, 6–9). *Ty* transcription starts in the δ sequence proximal to the gene and terminates in the distal δ sequence (10). Transcription of the adjacent gene initiates at the normal transcription initiation site, but increased amounts of the transcript are made relative to wild-type strains (8, 11–13).

The *his4-917* mutation results from the insertion of a *Ty* element (known as *Ty917*) into the regulatory region at the 5′ end of the *HIS4* gene (14). Unlike the *Ty* insertion mutations described above, the *his4-917* mutation prevents HIS4 transcription, thus rendering the cell histidine requiring. We have previously described the construction of a yeast strain in which *Ty917* is genetically marked with the yeast *URA3* gene [*Ty917(URA3)*] (9). When Ura$^-$ derivatives were isolated from this strain, most resulted from recombination events between *Ty917(URA3)* and *Ty* elements elsewhere in the yeast genome. This recombination results in replacement of part or all of *Ty917(URA3)* by part or all of the other recombining *Ty* element via a gene conversion-like event. These gene conversion derivatives display a variety of phenotypes with respect to *HIS4* expression: His$^-$, weak His$^+$, strong His$^+$, and His$^+$ under *MAT* control. They differ from each other only in the sequence of the *Ty* element present at *HIS4*; the position and orientation of the *Ty* and the sequence of the flanking *HIS4* DNA are identical in every case. Thus, *Ty* elements of different DNA sequence can have radically different effects on the expression of an adjacent gene.

We have used these *Ty* variants at *HIS4* to identify some of the *Ty* DNA sequences important in controlling the expression of the adjacent gene. Our approach has involved the *in vitro* construction of hybrid elements from *Ty* elements having different effects on gene expression followed by the introduction of these hybrid elements at the *HIS4* locus and an examination of their effects on *HIS4* expression. These experiments indicate that a sequence contained within 730 bp of the *HIS4*-proximal end of the *Ty* acts as an activator of *HIS4* expression.

MATERIALS AND METHODS

Genetic Analysis. Methods of tetrad analysis and media used were as described by Sherman *et al.* (15).

Yeast Strains. S291 was derived from S277 (*MAT***a** *ura3-52*) by the introduction of *Ty917* (*URA3*) at the *HIS4* locus by substitutive transformation as described (9). S480 is a His$^+$ (Ura$^-$) revertant of S291. To obtain S455, S458, and S467, S291 was first crossed to an inositol-requiring haploid strain. Sporulation and dissection of tetrads yielded SR104-8D [*his4-917(URA3) ura3-52 ade2 inos1 inos4*]. S455, S458, and S467 were isolated from SR104-8D by inositol starvation (16) and selection for Ura$^-$ cells.

Yeast Transformations. Hybrid *Ty* elements were introduced into S277 as described by Roeder and Fink (9). The transforming plasmid carried a *Ty* element marked at the unique *Hin*dIII site by a 1170-bp *Hin*dIII fragment carrying

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Abbreviations: bp, base pair(s); SV40, simian virus 40.

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the *URA3* gene. The *Ty* sequences were flanked on both sides by *HIS4* DNA. Prior to their introduction into yeast, the plasmids were linearized by digestion with *Eco*RI, which cuts at one of the junctions between the pBR322 vector sequences and the *HIS4* DNA. These linear fragments were used to transform a *HIS4*$^+$ *ura3-52* strain and Ura$^+$ transformants were selected. Cells were transformed according to the method of Hinnen *et al.* (17).

Doubling Times. Cells were grown to saturation in YEPD medium, washed twice in water, and diluted 1:50 into liquid synthetic complete medium lacking histidine. Cultures were grown at 30°C with shaking and cell concentrations were determined by Klett meter readings taken at approximately 2-hr intervals. Readings taken within the 50–100 Klett unit range were used to calculate doubling times. The numbers in Fig. 1 (column iv) represent the averages (±SD) of three independent cultures. For the S291, S467, and S480 parental strains, the strains were streaked for single colonies and three colonies were examined. For strains constructed by transformation, three independent transformants were examined.

Tests for *MAT* Control. S291, S467, S480, and the S277 transformants containing hybrid *Ty* elements were mated to strain SR149-6B (*MATα his4-29 leu2-3 ura3-52 cry1*). The resulting diploids were sporulated and haploid *MATα cry1* spores carrying the *Ty* insertion mutation were isolated by tetrad dissection. These were mated to the original *MAT***a** haploids to generate diploids homozygous for the *Ty* insertion mutation. These diploids carry the *MATα cry1* alleles on one chromosome 3 and the *MAT***a** *CRY1* alleles on the other chromosome. (*MAT* and *CRY1* are very tightly linked.) These diploids are sensitive to cryptopleurine because the *cry1* mutation, which confers resistance to cryptopleurine, is recessive. From each diploid, several cryptopleurine-resistant mitotic recombinants were selected. More than 90% of these segregants were homozygous, α/α, at *MAT*. For each *Ty* insertion mutation, the original haploid parent, an **a**/α diploid and an α/α diploid, were grown in patches on YEPD plates and then replica-plated to synthetic complete medium lacking histidine. To obtain light replicas of patches, cells were taken from the second velvet copy of the patches. Plates were incubated at 20°C for 2 days (for S480 and its derivatives) or 4 days (for S467 and its derivatives).

DNA Sequence Analysis. *Ty* elements and flanking *HIS4* DNA were cloned as described (9, 18). DNA sequence data were obtained by the method of Maxam and Gilbert (19, 20). Restriction fragments with 5′ protruding ends were radioactively labeled by incubation with the Klenow fragment of DNA polymerase I and [α-^{32}P]dNTP as described by Maniatis *et al.* (21). Some fragments with 5′ protruding ends or blunt ends were dephosphorylated with calf intestinal phosphatase and 5′-end-labeled with T4 polynucleotide kinase and [γ-^{32}P]dATP as described by Maniatis *et al.* (21). In one case, an *Xho* I fragment was subcloned into the *Sal* I site of M13 mp8 and sequenced by the method of Sanger *et al.* (22, 23), using the universal M13 primer (24).

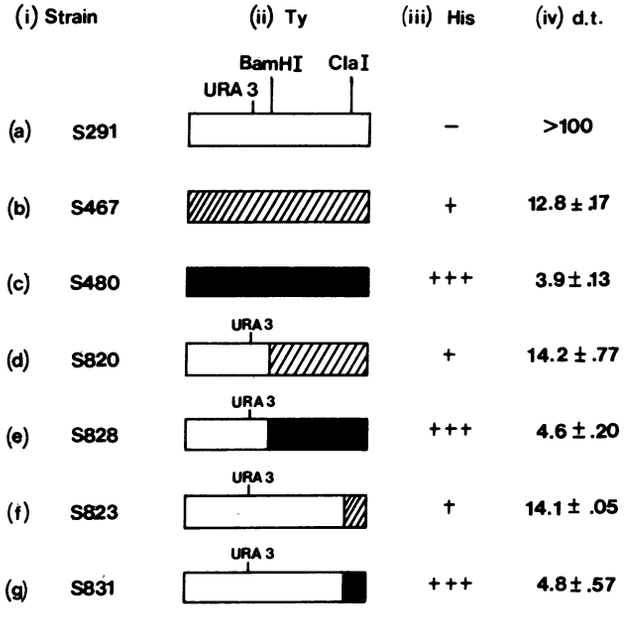

FIG. 1. Properties of yeast strains containing *Ty* elements constructed *in vitro*. The strain designation is indicated in column i. Column ii shows a diagrammatic representation of the *Ty* element present at the *HIS4* locus. *Ty917(URA3)* and sequences derived from it are represented by the open boxes. *Ty917(467)* sequences are represented by the hatched boxes. *Ty917(480)* sequences are represented by the solid boxes. The position of the *URA3* insert is indicated; this *Hin*dIII site is located ≈3700 bp from the right end of the element. The hybrid *Ty* elements indicated in *d* and *e* fuse *Ty917(URA3)* sequences to *Ty917(467)* or *Ty917(480)* sequences at a *Bam*HI site located ≈3300 bp from the right end of the element. The hybrids in *f* and *g* fuse sequences at a *Cla* I site located 730 bp from the right end. Column iii indicates the His phenotype of each strain, as indicated by replica-plating patches of cells to medium lacking histidine. –, His$^-$ phenotype; + and +++, cells grew to confluence in 3 days or 1 day at 30°C, respectively. Column iv indicates the doubling time (d.t.) in hours (±SD) in liquid medium lacking histidine. Wild-type *HIS4*$^+$ haploid yeast strains have a doubling time of ≈2.5 hr in the same medium.

RESULTS

Construction of Strains Carrying Hybrid *Ty* Elements. Two gene conversion derivatives of *Ty917(URA3)* were chosen for detailed study. These are *Ty917(467)* and *Ty917(480)*, derived from the yeast strains S467 and S480, respectively. S467 shows a weak His$^+$ phenotype, whereas S480 displays a strong His$^+$ phenotype; in both strains, *HIS4* expression is under *MAT* control. In the experiments described below, we constructed hybrid *Ty* elements consisting of varying amounts of *Ty917(URA3)* and *Ty917(467)* or *Ty917(480)* sequences. These three *Ty* elements are identical in their restriction maps; hybrid elements were constructed by fusing *Ty* sequences at homologous restriction sites, thus leading to full-length hybrid *Ty* elements. The hybrid *Ty* elements were genetically marked with the *URA3* gene and introduced into yeast at the *HIS4* locus by substitutive transformation as described in *Materials and Methods*.

In the course of these studies, a large number of hybrid *Ty* elements were constructed and analyzed. A subset of these is represented in Fig. 1 *d–g*. The *Ty* in S820 (Fig. 1*d*) contains the right (*HIS4*-proximal) half of *Ty917(467)* and the left (*HIS4*-distal) half of *Ty917(URA3)*. Similarly, the *Ty* in S828 (Fig. 1*e*) contains the right half of *Ty917(480)* and the left half of *Ty917(URA3)*. In the constructs shown in Fig. 1 *f* and *g*, the *Ty* elements introduced at *HIS4* contain only the rightmost 730 bp of *Ty917(467)* or *Ty917(480)*, respectively; all remaining *Ty* sequences are derived from *Ty917(URA3)*.

Hybrid *Ty* elements were introduced into yeast on restriction fragments carrying the *URA3*-marked *Ty* and flanking *HIS4* DNA as described in *Materials and Methods*. Ten to 12 Ura$^+$ transformants from each transformation were analyzed by Southern hybridization using the wild-type *HIS4* gene as probe. Approximately 90% of the Ura$^+$ transformants resulted from the replacement of the chromosomal *HIS4* gene by the *Ty*-containing *HIS4* gene carried by the transforming DNA molecule. All those transformants showing the correct pattern of restriction fragments at *HIS4* were analyzed for their His phenotype by replica-plating to medium lacking histidine. All the transformants obtained from any one transformation were identical in His phenotype. This identity

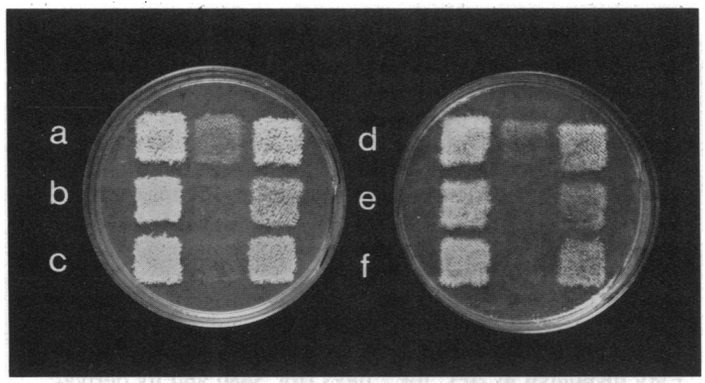

FIG. 2. Mating type control of *HIS4* gene expression. Patches of cells were replica-plated to medium lacking histidine. From left to right on each plate, the strains represented are *MATα* haploids, *MATα/MAT***a** diploids, and *MATα/MATα* diploids. All diploids are homozygous for the relevant *Ty* insertion at *HIS4*. The haploid yeast strains and their derivative diploids are as follows: (*a*) S480, (*b*) S828, (*c*) S831, (*d*) S467, (*e*) S820, (*f*) S823.

of transformants indicates that the transforming DNA molecules did not undergo a high frequency of rearrangement or mutation during transformation.

Measurement of *HIS4* Expression. In strains containing the parental *Ty* elements or the hybrid *Ty* elements constructed *in vitro*, *HIS4* expression was measured in two different ways. First, strains were grown in patches on complete medium and then replica-plated to medium lacking histidine. These results are summarized in Fig. 1 (column iii). Second, to obtain more precise quantitative measurements of *HIS4* expression, the doubling time in liquid medium lacking histidine was determined for each strain. These data are presented in Fig. 1 (column iv). As indicated in Fig. 1 *d* and *f*, strains carrying hybrid *Ty* elements consisting of *Ty917(URA3)* and *Ty917(467)* sequences are phenotypically identical to the S467 parent. Similarly, the *Ty917/Ty917(480)* hybrid *Ty* elements indicated in Fig. 1 *e* and *g* confer a His^+ phenotype as strong as that of the parental *Ty917(480)*.

***MAT* Control.** Tests were carried out to determine if the hybrid *Ty* elements described in Fig. 1 *d–g* confer the *MAT* control effected by *Ty917(467)* and *Ty917(480)*. For each of the parental and hybrid *Ty* elements, diploid strains were constructed that were homozygous for the *Ty* insertion mutation and either heterozygous (**a**/α) or homozygous (α/α) at *MAT*. These strains were then compared with each other and with their haploid parents. As seen in Fig. 2, for all *Ty* constructs, the α/α diploids grow almost as rapidly as their haploid parents on medium lacking histidine; however, the **a**/α diploids are phenotypically His^-.

DNA Sequence Analysis of *Ty917(467)* and *Ty917(480)*. The results presented above indicate that the sequence differences between *Ty917* and *Ty917(467)* [or *Ty917(480)*] that are responsible for the observed variations in *HIS4* expression lie within the rightmost 730 bp of the *Ty* element. This end of the element is proximal to *HIS4* and contains the *Ty* transcription initiation site. The DNA sequence of this 730-bp region from *Ty917*, *Ty917(467)*, and *Ty917(480)* was determined in order to identify the sequence changes. The strategy used to sequence these elements is indicated in Fig. 3*a*; the results are presented in Fig. 4. The *Ty917(467)* sequence differs from that of *Ty917* by a single base pair change at position −684, 612 bp from the end of the element. *Ty917(480)* differs from *Ty917* by this same base pair change and by 10-bp substitutions in the δ sequence.

DNA Sequence Analysis of *Ty917(455)* and *Ty917(458)*. To further analyze the DNA sequences responsible for a His^+

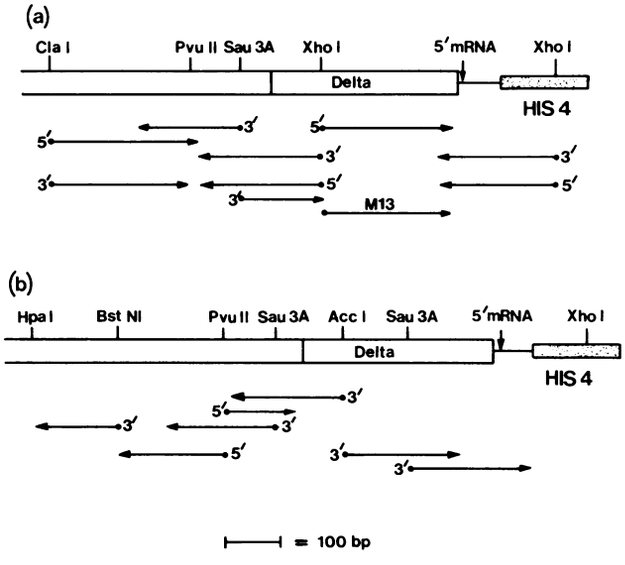

FIG. 3. Sequencing strategy for *Ty* elements. Open boxes represent *Ty* sequences. The δ sequence is labeled; the ε region extends to the left. Shaded boxes represent the *HIS4* coding region; solid line represents *HIS4* 5′ noncoding sequences. Vertical arrow indicates the *HIS4* transcription initiation site. Restriction endonuclease cleavage sites used to generate fragments for sequencing are shown. Arrows below the diagrams show the extent and direction of sequences obtained from the fragments indicated. Arrow marked M13 denotes the sequence obtained by the chain termination method of Sanger *et al.* (22, 23). All other fragments were 3′- or 5′-end-labeled (21) and sequenced by the Maxam and Gilbert method (19, 20). (*a*) Sequencing strategy for *Ty917*, *Ty917(467)*, and *Ty917(480)*. (*b*) Sequencing strategy for *Ty917(455)* and *Ty917(458)*.

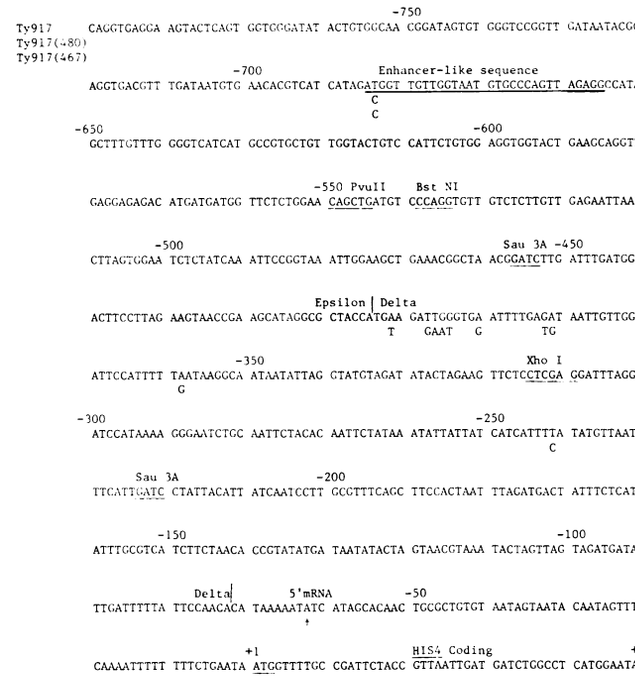

FIG. 4. DNA sequence of *Ty917*, *Ty917(467)*, and *Ty917(480)*. The sequence shown in its entirety is that of *Ty917*. The sequences of *Ty917(467)* and *Ty917(480)* are shown only where they differ from this sequence. Pertinent restriction sites are indicated. The sequence shown starts ≈10 bp from the *Cla* I site in the *Ty* (see Fig. 3*a*) and ends 50 bp inside the *HIS4* coding region. The sequence is written 5′ to 3′ in the direction of *HIS4* transcription; numbering is relative to the start of *HIS4* translation. The ATG codon at +1 is underlined, and the normal *HIS4* transcription initiation site at −63 is indicated by the arrow (25). Vertical lines mark the boundaries of the δ sequences. The enhancer-like sequence shown in Fig. 6*a* is indicated.

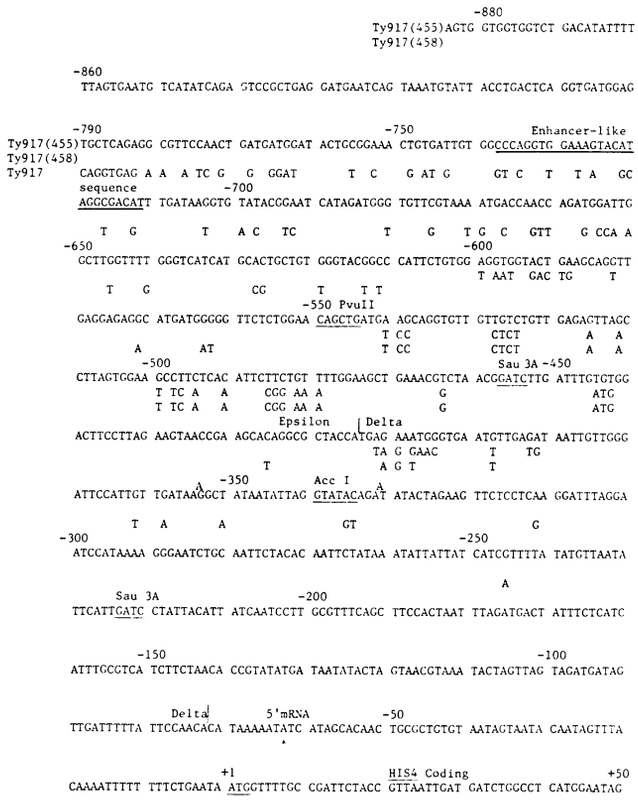

FIG. 5. DNA sequence of *Ty917*, *Ty917(455)*, and *Ty917(458)*. The sequence of *Ty917(455)* is shown in its entirety. The sequences of *Ty917(458)* and *Ty917* are shown only where they differ from this sequence. The sequences of *Ty917(455)* and *Ty917(458)* start ≈10 bp from the *Hpa* I site in the ε region (see Fig. 3*b*) and end 50 bp inside the *HIS4* coding region. The sequence of *Ty917* extends only to the *Cla* I site (as in Fig. 4) and thus is 90 bp shorter than the other *Ty* sequences. The two A residues written above the line at positions −331 and −354 reflect the fact that the δ sequences differ in length by 2 bp. The enhancer sequence shown in Fig. 6*c* is indicated. See Fig. 4 legend for symbols and numbering system.

phenotype, we carried out DNA sequence analysis of two additional *Ty917(URA3)* gene conversion derivatives that confer a His^+ phenotype. The strategy used to sequence *Ty917(455)* and *Ty917(458)* is indicated in Fig. 3*b*. In Fig. 5, these sequences are compared with that of *Ty917*.

DISCUSSION

In this paper, we examine a series of *Ty* elements that, when inserted at the same position and in the same orientation in the *HIS4* regulatory region, have different effects on *HIS4* expression. The elements studied were *Ty917(URA3)*, which renders the cell His^-, and *Ty917(467)* and *Ty917(480)*, which cause weak His^+ and strong His^+ phenotypes, respectively. Full-length hybrid *Ty* elements were constructed that carry some DNA sequences from *Ty917(URA3)* and the remaining sequences from either *Ty917(467)* or *Ty917(480)*. Analysis of strains carrying these hybrid elements indicates that the sequence differences responsible for the variations in *HIS4* expression are confined to the rightmost 730 bp of the element. A single base pair change within the ε region converts *Ty917(URA3)* to an element conferring a weak His^+ phenotype. When this base pair change is combined with certain changes in the δ sequence, the resultant His^+ phenotype is much stronger.

The experiments described here define DNA sequences that are required, but not necessarily sufficient, for *Ty* control of gene expression. It is possible that there are DNA sequences to the left of the 730-bp segment defined here that are also required for controlling gene expression. These sequences could be common to all three *Ty* elements examined. Errede *et al.* (26) have studied the *CYC7-H2* mutation, a *Ty* insertion leading to overproduction of iso-2-cytochrome *c*. Analysis of deletion mutants and subclones of the *Ty* element indicates that all the sequences necessary for overexpression are contained in the rightmost 1.7 kb of the *Ty* element.

Studies of diploid (**a**/α and α/α) strains carrying hybrid *Ty* elements indicate that all the hybrids constructed *in vitro* confer the *MAT* control effected by the parental *Ty917(467)* and *Ty917(480)* elements. These results are consistent with those obtained by Errede *et al.* (26). The sequences responsible for enhancing gene expression have not yet been separated from those responsible for *MAT* control.

The experiments described above indicate that sequences present in the ε region of some *Ty* elements can lead to *MAT*-controlled transcription of the adjacent *HIS4* gene. Other experiments have indicated that *HIS4* transcription initiates at the wild-type *HIS4* transcription start site (13, 27). The ability to activate transcription from a nearby promoter is a characteristic property of the "enhancer" sequences found in simian virus 40 (SV40) and several eukaryotic genes (28). We therefore searched *Ty917* and its derivatives for sequences homologous to the consensus "enhancer core" (29). *Ty917* does contain a core sequence; this sequence is flanked on both sides by sequences homologous to the SV40 enhancer (Fig. 6). The sequence difference that distinguishes *Ty917(467)* from *Ty917* lies within this region of homology.

If this putative enhancer sequence is important in controlling *HIS4* expression, then a similar sequence should be found in all those *Ty917* derivatives that lead to His^+ phenotypes. To examine this possibility, we analyzed two more *Ty917* derivatives, *Ty917(455)* and *Ty917(458)*, which confer His^+ phenotypes (9). These *Ty* elements are members of the *Ty1* class of elements, unlike *Ty917*, which is a member of the *Ty2* class. Members of the *Ty1* and *Ty2* classes of elements differ from each other by two large blocks of sequence heterology (2, 12, 14, 30). We determined the DNA sequence of the rightmost 808 bp of *Ty917(455)* and *Ty917(458)* and we searched these sequences for an enhancer core. As indicated in Fig. 6*c*, *Ty917(455)* and *Ty917(458)* share perfect homology with the SV40 enhancer for the 8 bp that constitute the enhancer core. The center of the enhancer sequence is located 660 bp from the extreme right end of *Ty917(455)* and *Ty917(458)*, ≈70 bp to the left of the enhancer-like sequence in *Ty917(467)* and *Ty917(480)*. The existence of

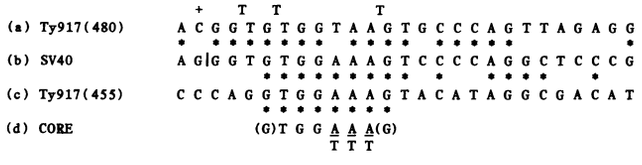

FIG. 6. Comparison of *Ty* and SV40 enhancer sequences. The *Ty917(480)* sequence [same as *Ty917(467)*] (*a*) extends from −685 (left) to −656 (right) (see Fig. 4). The nucleotide marked by + indicates the single base pair difference between these elements and *Ty917*. The three T residues shown above the *Ty917(480)* sequence have been displaced to maintain homologous register with the SV40 sequence. The SV40 sequence is shown in *b*; vertical line represents the boundary between SV40 late leader sequences (on the left) and the 72-bp repeat (on the right) (29). The *Ty917(455)* sequence shown in *c* extends from −738 (left) to −712 (right) (see Fig. 5). The enhancer core shown in *d* is a consensus sequence found in several enhancer elements and thought to be essential for enhancer function. A/T, either A or T residues can be found at these locations. Parentheses around the terminal G residues indicate that these are found in several but not all enhancer elements. Asterisks mark homologous nucleotides.

a sequence homologous to the enhancer core in *Ty* elements very different in sequence is consistent with the hypothesis that this sequence is important in enhancing *HIS4* expression. Note that the sequence in the *Ty* that is homologous to the SV40 enhancer is not the only region in the *Ty* that is important in controlling gene expression. The sequence differences that distinguish *Ty917(467)*, which confers a weak His^+ phenotype, from *Ty917(480)*, which confers a strong His^+ phenotype, lie within the δ sequence $\approx$280 bp away from region homologous to the SV40 enhancer.

Experiments from several laboratories indicate that *Ty* transcription and the transcription of *Ty*-adjacent genes are coordinately regulated. Thus, the transcription of *Ty* elements and the adjacent genes is repressed in strains heterozygous, $\mathbf{a}/\alpha$, at *MAT* and in strains that carry mutations at the *STE7*, *ROC1*, or *ROC2* loci (31). These observations suggest that sequences that control the transcription of adjacent genes may also control transcription of the *Ty* element itself. We therefore propose that the region spanning the ε/δ junction acts not only as an enhancer of transcription of *Ty*-adjacent genes but also as an enhancer of *Ty* transcription. [Note that the base change that distinguishes *Ty917(467)* from *Ty917* and 10 of the 11 changes that distinguish *Ty917(480)* from *Ty917* lie 3′ to the *Ty* transcription initiation site.] If the enhancer sequence regulates both *Ty* transcription and the transcription of the adjacent gene, then there should be a correlation between an element's transcriptional ability and its ability to enhance transcription of an adjacent gene.

The control of gene expression by *Ty* elements may be mechanistically related to the regulation of gene expression by integrated retroviruses in mammalian cells. DNA segments containing the long terminal repeats of several different retroviruses have been shown to act as enhancers of transcription (32–35). In both avian leukosis virus (35) and Rous sarcoma virus (34), there is reason to believe that the region necessary for enhancer activity consists of sequences from the long terminal repeat as well as sequences from the adjoining internal region of the element.

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