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Genetics

Activation regions in a yeast transposon have homology to mating type control sequences and to mammalian enhancers

(*Ty1*/DNA sequence/deletion mutations/gene expression)

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ABSTRACT **The DNA sequence of the *Ty1* activating region from the *CYC7-H2* mutant of *Saccharomyces cerevisiae* is presented. Analysis of the data revealed the presence of four simian virus 40-type enhancer core sequences. Two of the *Ty1* enhancer cores are contiguous with sequences also homologous to the diploid control site at *MATα*. We postulate that these two *Ty1* regions of ≈30 base pairs are regulatory blocks, and we have analyzed deletions to ascertain whether they are necessary for effects of *Ty1* on adjacent gene expression. We found that activation is lost when a restriction fragment encompassing both postulated regulatory blocks is deleted. Deletion of restriction fragments that remove only one of the two regulatory blocks has little or no effect on *Ty1* activating ability in haploid yeast cells or on repression of this function in diploid yeast cells. Because the most significant internal homologies in the restriction fragments analyzed are the putative regulatory blocks, these observations suggest that enhancer-like sequences are involved in cell-type control of *Ty1* effects on gene expression.**

The *CYC7-H2* mutation in *Saccharomyces cerevisiae* causes overproduction of the respiratory protein, iso-2-cytochrome *c*. This regulatory mutation was caused by insertion of a 5.5-kilobase (kb) sequence in the 5′ noncoding region of the *CYC7* structural locus (1). The inserted sequence is a member of the *Ty1* family of dispersed repetitive elements found in yeast (2).

Ty1 insertion places *CYC7-H2* expression under control of yeast cell type, which can be **a**, α, or **a**/α (see ref. 3 for review). In either **a** or α haploid cells of yeast, 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. The **a** and α cell types are established by the presence of either **a** or α alleles, respectively, at the mating type locus (*MAT*). *MATα* codes for two regulatory proteins, *α1* and *α2*; *MAT***a** codes for one regulatory protein, ***a1***. The *MAT***a** and *MATα* cells are capable of mating with each other to give diploid cells with a heterozygous *MAT* constitution. The resulting **a**/α cell type is capable of sporulation but not mating. Establishment of this third cell type requires the expression of both the ***a1*** and *α2* gene products, which together form a negative regulator of haploid specific genes. It has been shown that transcription of several haploid specific genes including *MATα1*, *STE5*, *STE6*, and *HO* is repressed in **a**/α diploid cells (see ref. 4 and refs. therein). Also, a 14-base-pair (bp) sequence that is required for repression of *MATα1* transcription in diploid cells has been identified by deletion analysis of the *MATα* control region (5).

Cell-type dependent regulation of the *CYC7-H2* mutation is shared by certain other *Ty1* insertion mutations in yeast (see ref. 6 for review). Among these are the *CYP3-4*, *cargA*$^{+}$*O*^{h}, and *ADH2*^{c} mutations in which *Ty1* insertions were shown to be within a region between 125 and 600 bp upstream from the various coding sequences. In all of these insertion mutants, the *Ty* element is oriented so that its direction of transcription is opposite that of the affected gene. Also, it has been found that the steady-state mRNA corresponding to each of these genes is increased or made constitutively, but size and 5′ map position of the transcript are not affected. These observations show that the *Ty1* element is not providing a new transcription initiation site but is regulating transcription from various upstream positions. This effect is similar in certain respects to the action of enhancer sequences, such as that of the simian virus 40 (SV40) 72-bp repeat (see ref. 7 for review). However, unlike the SV40 enhancer, the orientation of *Ty* sequences inserted at the 5′ end of the affected genes may be important. A similar orientation effect is associated with activation of the *INT1* and *INT2* loci by mouse mammary tumor virus (8, 9). Finally, cell-type dependence of *Ty1* activating ability is analogous to the host and tissue specificity associated with activity of viral and cellular enhancers (10–15).

In a previous report, it was shown that 2 kb of *Ty1* sequences proximal to the *CYC7* coding region are sufficient for the activation and cell-type regulation characteristic of *CYC7-H2* (16). In this report, the DNA sequence of the *Ty1* activating region is presented. Further deletion analysis is used to test subfragments encompassing enhancer-like sequences for activating ability.

MATERIALS AND METHODS

Plasmid Constructions. pYe(CEN3)-30 (17), pAB25 (18), pAB45 (16), pNC3, and pNC16 plasmid DNAs used in constructions for these studies were prepared according to the procedure of Norgard (19). All constructions were amplified *in vivo* by $CaCl_2$ transformation of *Escherichia coli* strain HB101 (20). Plasmid DNA preparations for analysis of constructions and for yeast transformation were done according to the procedure of Holmes and Quigley (21).

Minichromosome plasmids designated pNC5 and pNC3 (Fig. 1) contain fragments from the *CYC7*$^{+}$ and the *CYC7-H2* genes, respectively. Three restriction fragment deletions corresponding to the *Pvu* II/*Hpa* I (Δ**a**), *Hpa* I/*Sph* I (Δ**b**), and *Pvu* II/*Sph* I (Δ**c**) fragments of *Ty1* were constructed (Fig. 2). Deletions were accomplished by treating the *CYC7-H2* minichromosome plasmid with corresponding enzymes (New England Biolabs) and rejoining plasmid ends with T4 DNA ligase (New England Biolabs). Deletions with an *Sph* I

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

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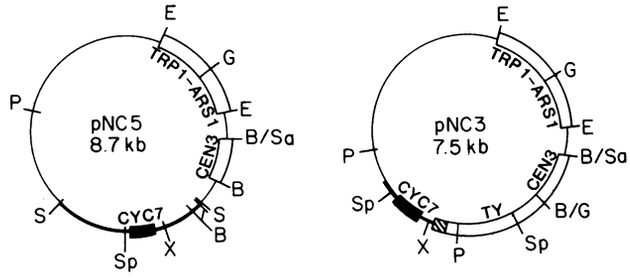

FIG. 1. *CYC7*$^+$ and *CYC7-H2* plasmids. The minichromosome plasmid designated pNC5 was constructed by attaching *Sal* I linkers (New England Biolabs) to the 2.2-kb *CYC7*$^+$ *Eco*RI fragment from pAB25 and inserting the fragment into the *Sal* I site of pYe(CEN3)-30. The minichromosome plasmid designated pNC3 was constructed by inserting the 4.4-kb *CYC7-H2 Bgl* II/*Bam*HI fragment from pAB45 into the *Bam*HI site of pYe(CEN3)-30. The resulting plasmid was treated with *Bam*HI endonuclease and nuclease BAL 31 to delete pBR322 sequences that were duplicated by insertion of the *CYC7-H2* fragment into the vector. One deletion end point in pNC3 occurs at pBR322 sequence position 1604 and the other occurs 146 bp from the *Sph* I site in the *CYC7* sequence (M. Teague, personal communication). Yeast *TRP1*, *ARS1*, *CEN3*, and *Ty* sequences are indicated by open boxes with corresponding labels. *Delta* sequences are indicated by the hatched box. *CYC7* coding region is indicated by solid box with flanking regions indicated by thick lines. Thin lines represent pBR322 sequences. Restriction sites shown are as follows: *Bam*HI, B; *Bgl* II, G; *Eco*RI, E; *Pvu* II, P; *Sal* I, S; *Sau*3A, Sa; *Sph* I, Sp; and *Xho* I, X.

end required linearization of the plasmid by partial cleavage and generation of blunt ends by using the 3′ exonuclease activity of T4 DNA polymerase (New England Biolabs). Restriction enzyme analysis confirmed deletion of fragments **a**, **b**, and **c** in the plasmids designated pNC21, pNC22, and pNC23, respectively.

Yeast Genetic Procedures and Yeast Strains. Media and conventional yeast genetic procedures for crosses, strain constructions, and scoring nutritional markers are described by Sherman *et al.* (22). Yeast strains were grown under derepressed conditions for determination of cytochrome *c* content. Media and procedures for these determinations are as described (16). A LiCl procedure (23) was used to

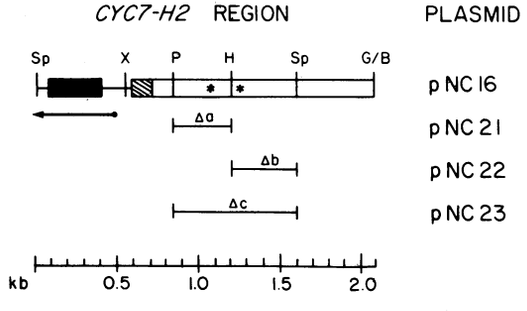

FIG. 2. Structure and expression of *CYC7-H2* deletion plasmids. The *CYC7*-Ty region from pNC16 is shown in expanded form. Sites for *Sph* I (Sp), *Xho* I (X), *Pvu* II (P), and *Hpa* I (H) endonucleases are shown. The *Bgl* II/*Bam*HI (G/B) junction between *CYC7-H2* sequences and vector sequences is indicated. pNC16 is a derivative of pNC3 (see Fig. 1), in which the *Pvu* II site in pBR322 was converted to a *Sal* I site by attachment of linkers. This modification makes the *Ty1 Pvu* II site in pNC16 unique. *CYC7* coding sequences are represented by solid box, *delta* sequences are represented by hatched box, and *epsilon* sequences are represented by open box. Arrow indicates the polarity (5′ to 3′) and limits of the *CYC7* transcription unit. Asterisks represent sequences identified by homology to the SV40 enhancer and to the *MATα* diploid control sequences. Restriction fragment deletions corresponding to the pNC21, pNC22, and pNC23 plasmids are indicated by bars below the diagram of the *CYC7*-Ty fragment.

Table 1. Yeast strains

Strain	Genotype
E378-1A	*MATa cyc1 CYC7-H2 cry1 his1 lys2*
E724-7A	*MATa cyc1 cyc7-67 gal2 can1 trp1-289 ura3-52*
E730-4A	*MATα cyc1 cyc7-67 can1 leu2 his5 trp1-289*
GM105-15A	*MATa cyc1 CYC7-H3 his1 trp2*
KZ8-5C	*MATa cyc1 CYC7*$^+$ *his4 ura1*

transform yeast strain E724-7A with the minichromosome plasmids described in these studies. The *trp1* mutation in this strain permits selection of the pYe(CEN3)-30 based plasmids by requiring growth on synthetic medium lacking tryptophan.

Complete genotypes for yeast strains used in these studies are given in Table 1. All strains are completely iso-1-cytochrome *c* deficient because of mutations at the iso-1-cytochrome *c* structural gene *CYC1*. Strains E724-7A and E730-4A are also deficient in iso-2-cytochrome *c* because of the *cyc7-67* allele, which is a 0.4-kb deletion encompassing the *CYC7* coding region (T. Cardillo and K. Zaret, personal communication). Strain GM105-15A carries the *CYC7-H3* allele, which causes a 20-fold overproduction of iso-2-cytochrome *c* (18). Unlike the *CYC7-H2* allele, the expression of *CYC7-H3* is not regulated by the constitution of the mating-type locus (24).

DNA Sequencing Strategy and Data Analysis. The DNA sequence of cloned fragments was determined by the chain-termination method using M13 plus strand template DNA with the universal sequencing primer purchased from Bethesda Research Laboratories. Shotgun cloning and sequencing procedures as described by Deininger (25) were used with the exceptions that buffer gradient gels and ^{35}S label were used (26). DNA sequence determinations from 32 independent M13 clones contributed data from both strands for positions 1–1484 of the reported sequence and data from only one strand for positions 1485–1556.

DNA sequence data were compiled by use of the Staden computer program (27) on a VAX-11/780 computer. Homology search programs written by S. Weaver and C. Hutchison (unpublished) were used to identify regions of the *Ty1* from *CYC7-H2* that show homology to the SV40 enhancer region and to the *MATα* diploid control sequence. The computer algorithm of White *et al.* (28) was used to find internal homologies in *Ty1*. These homology search programs were run on a TRS-80 model II computer.

RESULTS

DNA Sequence of the *Ty1* Activating Region. We have shown that *CYC7-H2* expression and regulation are maintained for the portion of the gene carried on the minichromosome plasmid pNC3 (Fig. 1). These results further localize the activating region of *Ty1* to a fragment delimited by the *Bgl* II site in *Ty1*, which is at the vector junction in pNC3. The DNA sequence of the 1.56-kb *Xho* I/*Bgl* II *Ty1* fragment (Fig. 2) was determined and is presented in Fig. 3. The DNA strand shown has the same sense as the *Ty1* transcription unit, which is opposite that of *CYC7*. Junctions between *CYC7*, *delta*, and *epsilon* regions are indicated above the sequence.

The DNA sequence revealed an unusual structure for the proximal *delta* of *CYC7-H2 Ty1*. This *delta* consists of an inverted repeat of sequences homologous to the 3′ domain of normal *deltas* and lacks all sequences normally comprising the 5′ two-thirds of conventional elements. We conclude that two-thirds of a normal *delta* element is not required for activation and cell-type regulation associated with *Ty*-mediated gene expression. This result is significant because it suggests that *epsilon* sequences may be important determinants of *Ty1* effects on adjacent genes.

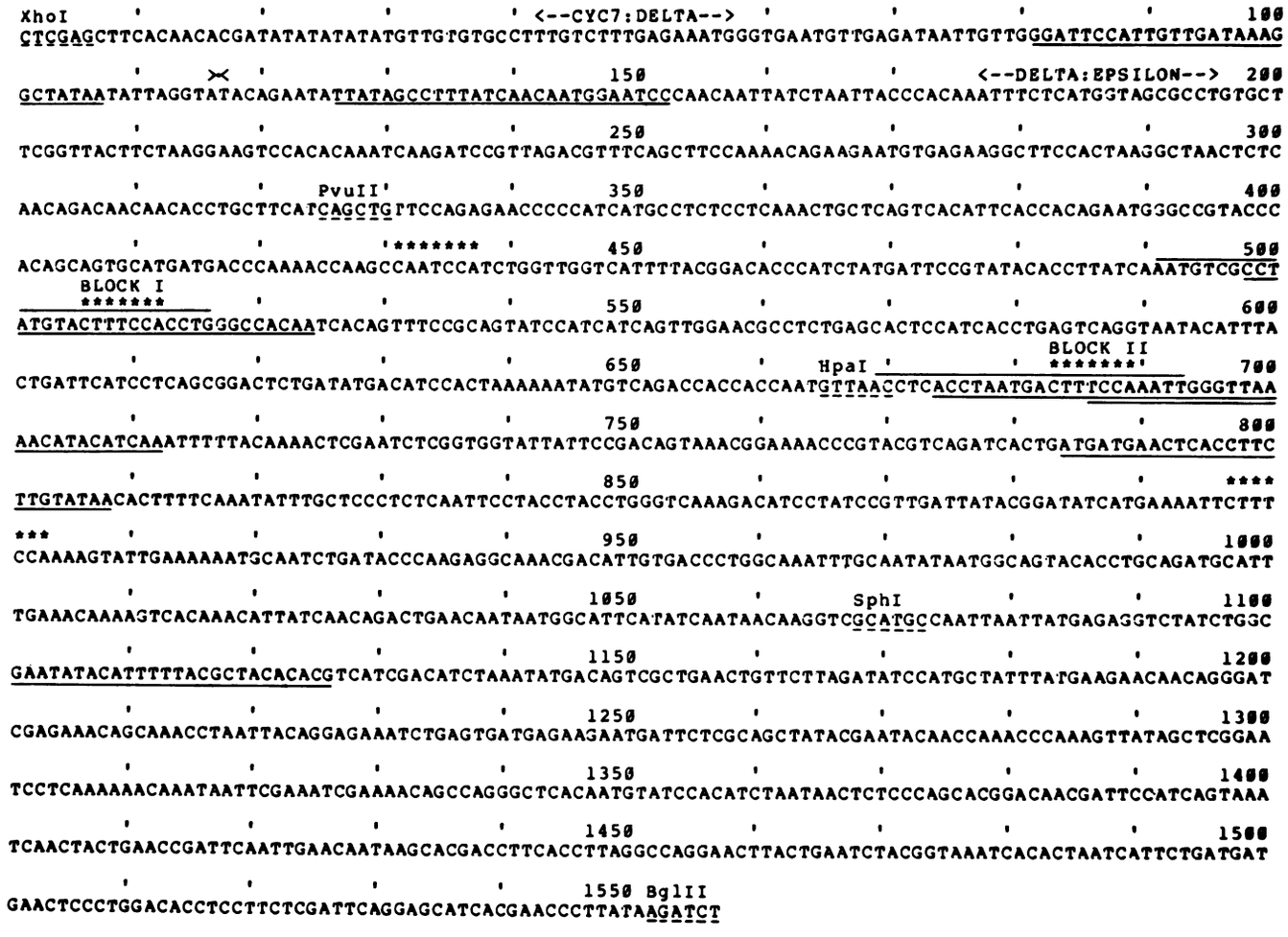

FIG. 3. DNA sequence of the *CYC7-H2* proximal *Ty1* region. The DNA strand shown has the same sense as the *Ty1* transcription unit, which is opposite that of *CYC7*. *CYC7:delta* (29) and *delta:epsilon* junctions are marked above the sequence. >·< marks the center of inverted *delta* sequences. Overlined sequences are homologous to the complement of nucleotides 1–26 of the SV40 72-bp repeat. Underlined sequences at positions 81–107 and 686–712 are homologous to *MATα* nucleotides 1643–1669, and underlined sequences at positions 126–152, 498–524, 674–700, 784–808, and 1100–1126 are homologous to the complement of the *MATα* sequence. Perfect enhancer core sequences are marked by asterisks. Landmark restriction sites are *Xho* I at position 1, *Pvu* II at position 325, *Hpa* I at position 665, *Sph* I at position 1068, and *Bgl* II at position 1552.

Possible Regulatory Sequences in *Ty1* Identified by Homology to Known Enhancer and Cell-Type Control Sequences. The ability of *Ty1* to regulate transcription at a distance stimulated us to search within its activating region for homology to known enhancer sequences. Analysis of SV40 deletion mutants indicated that 13–26 nucleotides at the 5′ end of the 72-bp repeat unit are required for viability of the virus and, presumably, enhancer function (30). Base-pair mutations in SV40 indicated enhancer activity correlates with a crucial core element located within these 13 nucleotides (31). Comparison with activating regions of three other known enhancers revealed that each element harbored a related core with the consensus being TGGLLLG (L is either A or T) (31). Four perfect matches, all to the complement of this enhancer core, occur in the 1556-bp activating region of *Ty1*. These are indicated by asterisks in Fig. 3. The *Ty1* sequence was searched for homology to 26 nucleotides from the 5′ end of the SV40 72-bp repeat sequence and to its complement. Using the criteria for a 15-bp match length with a 3-bp mismatch tolerance, two matches were found to the 26-base SV40 complement strand and none to the other strand. These two regions are overlined on the *Ty1* sequence in Fig. 3 and are designated block I and block II. An alignment of *Ty1* blocks I and II with the 26-bp SV40 sequence is shown in Fig. 4.

MATα1 and other haploid-specific genes are subject to repression by the **a1–α2** proteins present in *MAT***a**/*MATα* diploid cells. Siliciano and Tatchell (5) constructed a 14-bp deletion, designated R14, which encompasses nucleotides 1643–1657 of the *MATα* regulatory region. Unlike normal *MATα*/*MAT***a** diploid cells, *matαR14*/*MAT***a** diploid cells

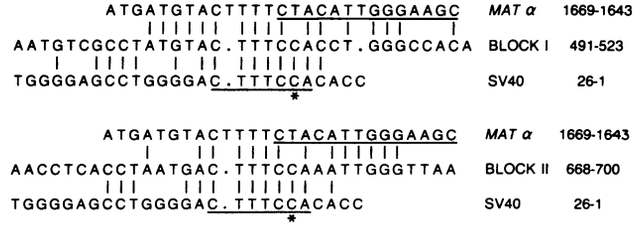

FIG. 4. Alignment of *Ty1* blocks I and II with SV40 and *MATα* regulatory sequences. Sequence blocks I and II of *Ty1* refer to regions indicated by both underlines and overlines in Fig. 3. The underlined region in *MATα* corresponds to the 14-bp deletion, R14, of Siliciano and Tatchell (5) that relieves repression of the *MATα1* transcript in diploid cells. Underlined region in SV40 sequence corresponds to the enhancer core region defined by Weiher *et al.* (31). The SV40 position marked by an asterisk indicates the C·G base pair that may be essential for enhancer function.

continue to synthesize $\alpha 1$ mRNA. This deletion is sufficient to relieve cell-type control, although the entire sequence required for **a**1–$\alpha 2$ repression may extend farther rightward from position 1657. Because *CYC7-H2* expression is also subject to **a**1–$\alpha 2$ repression, we reasoned that a homologous target sequence might be present in the *Ty1* controlling region. A search of *Ty1* for homology to *MAT*α nucleotides 1643–1669 and to its complement was undertaken. When the specified match length was 15 bases with a mismatch tolerance of 4, two matches to the *MAT*α sequence and five matches to the complement sequence were found in *Ty1*. These *MAT*α sequence homologies are underlined in Fig. 3. Two of the *Ty1* regions with matches to the *MAT*α diploid control site overlap sequences identified by homology to the SV40 enhancer. Alignments for these two *Ty1* regions with the *MAT*α sequence are shown in Fig. 4. From the alignments, it is apparent that the *MAT*α sequence also shares some homology to the SV40 enhancer sequence. The finding that *Ty1* sequences 491–524, designated block I, and 668–712, designated block II, are identified by homology to two different regulatory sequences strongly suggests these as possible regulatory sites in *Ty1*.

Deletion Mutant Analysis. Three deletions involving fragments **a**, **b**, and **c** as indicated in Fig. 2 were constructed to test whether the regions with homology to both enhancer and **a**1–$\alpha 2$ control sequences are necessary for effects of *Ty1* on adjacent gene expression. Deletion of fragment **a** (pNC21) removes block I but leaves block II, while deletion of fragment **b** (pNC22) has the reciprocal effect. Deletion of fragment **c** (pNC23) removes both blocks.

The cytochrome *c*-deficient yeast strain E724-7A was transformed with the *CYC7*$^+$ plasmid, with the *CYC7-H2* parent plasmid, and with the three *Ty1* deletion plasmids. Transformed strains were selected on the basis of the vector *TRP1* marker and then were tested for iso-2-cytochrome *c* production, plasmid copy number, and plasmid stability in yeast. Total DNA was prepared from representative transformed strains and Southern blot experiments were performed. The intensities of bands on autoradiograms corresponding to plasmid and genomic sequences hybridizing to a *CYC7* probe indicated that each of the above plasmids was present at 1–2 copies per cell (data not shown). To compare mitotic stability of the various plasmid constructions, representative transformed strains were grown for ≈6.5 generations under nonselective conditions in complete complex medium. The ratio of colony forming units on medium lacking tryptophan to that on complete medium gave a value of 0.50–0.55 for each of the transformed strains tested. Therefore, the values for comparison of iso-2-cytochrome *c* production reflect relative gene expression of the plasmid alleles. Results of these analyses are summarized in Table 2. The control plasmids, pNC5 and pNC16, produce amounts of iso-2-cytochrome *c* characteristic of *CYC7*$^+$ and *CYC7-H2* strains, respectively. Deletion of either fragment **a** or **b** from the *Ty1* activating region causes little if any deviation from *CYC7-H2* amounts of iso-2-cytochrome *c* produced. However, deletion of fragment **c**, which removes both homology blocks, abolishes overproduction of iso-2-cytochrome *c* and restores wild-type amounts. These results show that the fragment encompassing both homology blocks is necessary for *Ty1* activation of adjacent *CYC7* expression. Other deletion evidence indicates sequences more proximal to the *CYC7* coding region are also required for this effect.

The effect of **a**/α cell type on expression of the *CYC7-H2*-derived plasmids was also tested. *MAT*α tester strain, E730-4A, was crossed to *MAT***a** transformed strains as well as to control strains harboring the *CYC7*$^+$, *CYC7-H3*, or *CYC7-H2* allele. *MAT***a**/*MAT*α diploid strains were isolated by prototroph selection and amounts of iso-2-cytochrome *c* were determined. Results are shown in Table 2. Values

Table 2. Cell-type expression of *CYC7* plasmid and chromosomal alleles

Strain	Plasmid	*CYC7* allele*	Relative amount of cytochrome *c*†	
			*MAT***a**‡	*MAT***a**/*MAT*α§
E724-7A	pNC5	*CYC7*$^+$	2	1
E724-7A	pNC16	*CYC7-H2*	23	4
E724-7A	pNC21	*CYC7-H2* Δa	17	2
E724-7A	pNC22	*CYC7-H2* Δb	23	4
E724-7A	pNC23	*CYC7-H2* Δc	2	1
KZ8-5C	—	*CYC7*$^+$	1	1
E378-1A	—	*CYC7-H2*	20	1
GM105-15A	—	*CYC7-H3*	20	10

*In plasmid-containing strains, the *CYC7* allele refers to the fragment from the *CYC7*$^+$, *CYC7-H2*, or *CYC7-H2* derivative gene that is carried by the respective plasmids.

†Low temperature spectroscopic examination of intact cells was used to estimate the relative amount of iso-2-cytochrome *c*. One unit corresponds to the normal *CYC7*$^+$ amount.

‡Values represent the average from determinations on 10–20 independent isolates from transformation with the specified plasmid.

§*MAT***a** haploid strains specified in column one were crossed to *MAT*α tester strain E730-4A. Resulting *MAT***a**/*MAT*α diploid strains were isolated by prototroph selection. In the case of plasmid-containing strains, crosses were made with three representative isolates from transformation with the specified plasmid. Values represent the average from determinations on the three resulting diploid strains.

should be compared to the *cyc7-67*/*CYC7-H3* reference strain, which shows a decrease in amount of iso-2-cytochrome *c* expected from the decrease in gene dosage without associated **a**1–$\alpha 2$ repression (24). The amount of iso-2-cytochrome *c* produced by the *cyc7-67*/*CYC7-H2* strain indicates a 10-fold repression of *CYC7-H2* expression is caused by **a**1–$\alpha 2$. There is a decrease by a factor of 3–4 in iso-2-cytochrome *c* in the diploid strain with the pNC16 control plasmid and a comparable decrease for the **Δa** and **Δb** constructions carried by pNC21 and pNC22, respectively. The **a**1–$\alpha 2$ repression of *CYC7-H2* derivatives on the minichromosome plasmids is apparent but is less than one-half that shown by the *CYC7-H2* allele in its chromosomal position. Nevertheless, it appears that deletion of either fragment **a** or **b** does not relieve **a**1–$\alpha 2$ repression relative to the control plasmid.

Internal Homologies. Results of the deletion analysis with *CYC7-H2* minichromosome plasmid suggest that equivalent regulatory information is located within fragments **a** and **b**. A diagonal–traverse homology search algorithm (28) was used for locating similarities within the 1556-bp sequence. When 21 matches in a 33-base length is specified, two homologies between fragment **a** and fragment **b** are found. One internal homology is between positions 495 and 528 (block I) in fragment **a** and between positions 672 and 705 (block II) in fragment **b**. The second match is between positions 504 and 537 in fragment **a**, which includes part of block I, and positions 838–871 in fragment **b**. Under different search conditions, for example, when 17 matches in a 24-base length is specified, the only homology identified between fragments **a** and **b** involves positions 495–518 in block I and positions 672–696 in block II.

DISCUSSION

We have determined the DNA sequence from the activating region of *Ty1*. The composition of the sequence together with results from restriction fragment deletions in this region led us to believe that enhancer elements within the *epsilon* domain of *Ty1* are responsible for activation of *CYC7* expres-

sion in the *CYC7-H2* mutation. A computer search for homology to 26 bp of the SV40 enhancer region identified two enhancer-like sequences in *Ty1*. These two regions are contiguous with sequences that are also found by computer search to be homologous to the **a**/α control site from *MAT*α. Analysis of deletion derivatives in transformed yeast strains has shown that overproduction of iso-2-cytochrome *c* is lost when a restriction fragment encompassing the two putative regulatory blocks is deleted. Deletion of restriction fragments that remove only one of the two blocks has little or no effect on amounts of iso-2-cytochrome *c*. The latter finding indicates that the two deletion fragments contain equivalent regulatory information. *Ty1* sequences, involving blocks I and II, were also identified by computer search for internal homologies that could provide such equivalent regulatory information. Although it is difficult to identify short regulatory sequences by computer search, the *Ty1* regions designated block I and block II appear to be significant. First, they are identified by homology to two different regulatory sequences, the SV40 enhancer region and the *MAT*α diploid control region. Second, they comprise the most significant homology between two restriction fragments, which appear to contain equivalent regulatory information.

The finding that the *Ty1* activator sequences are repeated is not unusual. Activator sequences associated with a number of viral genomes are often present as short tandem repeats of 50–100 nucleotides. Similarly, the enhancer region of the immunoglobulin heavy chain gene contains repeating elements related to the viral enhancer core sequence (13). It is not surprising to find that only one of the two *Ty1* activating sequences is required for function. Only one of the two 72-bp repeat sequences of the SV40 enhancer region is required for its activating function (7). However, sequence duplication may play an important role in enhancer function under some conditions. For example, one mechanism by which polyoma can productively infect embryonal carcinoma cells is by mutation that causes tandem repetition of sequences with enhancer activity (32). It is intriguing to find that mammalian enhancer core sequences are present in yeast. The possibility that these sequences have similar function in such diverse organisms suggests a highly conserved role for these elements in eukaryotic gene expression.

One plausible model to account for cell-type modulation of *Ty1* effects on adjacent genes is as follows. In **a** or α haploid cells, *Ty1* enhancer sequences, either themselves or in association with enhancer-specific proteins, establish a configuration that is conducive for efficient utilization of normal initiation signals by RNA polymerase. In **a**/α diploid cells, the **a***1*–α*2* repressor binds to the target site on *Ty1*. Because this target region overlaps the enhancer core sequence, the interaction antagonizes the configuration required for activation of transcription. Affinity of the repressor for its site may be determined by the DNA sequence alone or by a DNA–protein complex. Recently, it has been observed that the **a***1* and α*2* proteins of yeast are homologous to the putative DNA binding domain of the homeotic proteins of *Drosophila* and frog (33). It is possible that the mechanism for **a***1*–α*2* regulation of haploid specific genes, including Ty-controlled genes such as *CYC7-H2*, represents a general one for determination of cell type in eukaryotic organisms.

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