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TEC1, a Gene Involved in the Activation of Ty1 and Ty1-Mediated Gene Expression in *Saccharomyces cerevisiae*: Cloning and Molecular Analysis

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Ty and Ty-mediated gene expression observed in haploid cells of *Saccharomyces cerevisiae* depends on several determinants, some of which are required for the expression of haploid-specific genes. We report here the cloning and molecular analysis of *TEC1*. *TEC1* encodes a 486-amino-acid protein that is a *trans*-acting factor required for full Ty1 expression and Ty1-mediated gene activation. However, mutation or deletion of the *TEC1* gene had little effect on total Ty2 transcript levels. Our analysis provides clear evidence that *TEC1* is not involved in mating or sporulation processes. Unlike most of the proteins involved in Ty and adjacent gene expression, the product of *TEC1* has no known cellular function. Although there was no mating-type effect on *TEC1* expression, our results indicate that the *TEC1* and the a/α diploid controls on Ty1 expression are probably not cumulative.

Ty elements are retrotransposons of *Saccharomyces cerevisiae* similar to proviral forms of vertebrate retroviruses in structure, transcriptional properties, and gene organization (8, 20, 40, 45; for a review, see reference 4). They transpose through an RNA intermediate, encode a reverse transcriptase, and produce viruslike particles (5, 26). Most laboratory strains contain about 20 to 40 copies of Ty elements dispersed throughout the genome. They are subdivided into at least three subfamilies. Ty1 and Ty2 share well-conserved DNA domains. However, in addition to local base substitutions leading to restriction site divergences, they contain two large sequences of overall heterogeneity (11, 55). Ty3 is largely unrelated to Ty1 and Ty2 (27).

A large number of genes influence the transcript levels of Ty1 and Ty2 elements. Mutations in several of these genes give rise to deficiencies that are apparently unrelated to Ty expression, such as mating or sporulation defects (*ste7*, *ste11*, *ste12*, *spt3*, *spt7*, *spt8*, *tye1*, and *tye2*) and lethality of the cells (*spt6* and *spt14*) (see reference 59 for a review).

Similar to retroviruses, Ty1 and Ty2 elements exhibit enhancerlike properties leading to activation of yeast genes when inserted in their 5′ nontranscribed regions (ROAM mutations) (4). Activation of adjacent gene expression has been observed at several loci (*CYC7*, *CAR1*, *CAR2*, *DUR2,1*, *ADH2*$^+$, etc.) (21, 22, 58). In each case, transcription of the Ty element is divergent from that of the adjacent gene. It has been shown in several cases that the transcription start points are not modified in the presence of the retrotransposon (17, 57). Such insertions of Ty elements in the 5′ nontranscribed region place the adjacent gene under the control of a set of gene products (10, 21). These genes include those that affect mating (*MAT* locus, a number of *STE* genes, and the *TYE* genes) and another gene without known cellular function, the *TEC1* gene (previously named *ROC1* [19]; since the name *ROC1* has been previously attributed to another gene, we have renamed this locus *TEC1*, for transposon enhancement control). In contrast, a similar effect of *SPT* gene products on adjacent gene overexpression has not been reported.

In both Ty1 and Ty2 elements, computer analysis identified at least two enhancerlike domains homologous to the simian virus 40 enhancer core. These domains overlap with sequences of homology to the **a**1/α2 repression site (23, 46). Furthermore, Ty expression and adjacent gene activation are dependent on the *MAT* locus. The overexpression observed in mating-proficient cells (**a** and α) is reduced in *MAT***a**/*MAT*α diploid cells. Moreover, the products of the *STE7*, *STE11*, and *STE12* genes defined by mutations preventing mating in haploid cells may encode proteins involved in full Ty expression (21, 22).

By mutational analysis of the *CYC7-H2* gene, gel retardation assays, and footprinting, Errede and co-workers demonstrated that two regions of Ty1 (regions A and D) are sufficient to mimic regulation of adjacent gene expression characteristic of the entire Ty1 element (13, 24). Region A (overlapping with the junction between the δ element and ε sequence) seems to be the target of a *STE7*- and *STE12*-dependent activation (13). Region D (including the sequence with overlapping homology to the simian virus 40 enhancer and to the **a**1/α2 control site) is able, when tandemly repeated, to activate the expression of a reporter gene (14). This activation is controlled by the *MAT* locus. Region D is the binding site for another yet undefined factor whose synthesis is not cell type dependent. Binding of the factor to this region is necessary for the activation observed in vivo. Identification of genes encoding such factors is of particular interest in order to study this mechanism of activation. Previously, some mutations have led to the identification of *trans*-acting factors controlling Ty activation without affecting mating or sporulation processes. The product of one of these genes may be such a DNA-binding protein.

Insertion of Ty1 elements in front of the *DUR2,1* gene has been shown to induce overproduction of ureoamidolyase (*dur*O^h mutations) (21). The *tec1* mutation leads to a strong

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TABLE 1. *S. cerevisiae* strains

Strain	Genotype
Σ1278b	*MATα*
M1b	*MATα durO^h-1 cargB$^+$O^h-1*
02298b	*MATα tec1 durO^h-1 cargB$^+$O^h-1 ura3*
01921c	*MATα durO^h-1, ura3*
01921c2N	*MATa/MATα durO^h-1/durO^h-1 ura3/ura3*
01921cTD3F4[a]	*MATα tec1::ura3 durO^h-1 ura3*
01921cTD3F42N	*MATa/MATα tec1::ura3/tec1::ura3 durO^h-1/durO^h-1 ura3/ura3*
JWG2-1D	*MATα trp1 gal1 ura3*
JWG2-1DTD2F5[a]	*MATα tec1::ura3 trp1, gal1 ura3*
CL2Na	*MATa/MATα durO^h-1/durO^h-1 his3/HIS3 leu2/LEU2 ura3/ura3*
CL1a	*MATα durO^h-1*

[a] Spontaneous Ura$^-$ revertants obtained by selection of 5′-fluoroorotic acid-resistant clones on FOA medium (6).

reduction of ureoamidolyase synthesis (19). The same effect is observed on overproduction of arginase and ornithine transaminase, respectively, when *CAR1* and *CAR2* gene expression is enhanced by a Ty1 insertion. In addition, *tec1* mutation does not lead to mating or sporulation deficiencies. In this report, we present the sequence and molecular characterization of the cloned *TEC1* gene. The effect of the *TEC1* deletion on Ty expression and adjacent gene activation is examined.

MATERIALS AND METHODS

Strains and genetic procedures. The *S. cerevisiae* strains used are listed in Table 1. Diploid strains were constructed by crossing two isogenic strains of opposite mating types obtained by mating-type switching; the mating types were switched by using a 2μm plasmid carrying the *HO* gene received from A. Klar (Cold Spring Harbor Laboratory). Standard yeast genetic procedures for mating, sporulation, tetrad analysis, and scoring of nutritional markers were carried out as described previously (51). Yeast strains were grown on Difco media: YEP (1% yeast extract, 2% peptone)-2% glucose, YNB (without amino acids)-2% glucose, or YNB (without amino acids and ammonium sulfate)-2% glucose supplemented with 0.1% urea or ornithine. Auxotrophs were grown with 0.01% of the required amino acids. Yeast transformation was performed by the lithium acetate method (29). Yeast spheroplasts were transformed with DNA fragments as described elsewhere (28). *Escherichia coli* MM294 (r_K^- m_K^+ endoI BI$^-$ *lac*$^+$; nonlysogenic) transformations were performed by the calcium chloride procedure (12), and transformants were grown in LB medium containing ampicillin (100 μg/ml) (42).

DNA isolation and techniques. Plasmid DNA was prepared on cesium chloride-ethidium bromide equilibrium gradients as described by Maniatis et al. (39). Small-scale DNA preparation from *E. coli* was accomplished by the method of Birnboim and Doly (2). Genomic DNA was isolated from yeast cells by the method of Davis et al. (16).

Restriction digestions and other enzyme reactions were performed as recommended by the suppliers. DNA restriction fragments were separated by electrophoresis through 0.8% agarose gels in Tris hydrochloride-acetate buffer (pH 8). Electroelution, ligation, enzymatic treatment, and Southern blot hybridization analysis of DNA fragments were performed as described elsewhere (39). All probes were radiolabeled with [α-^{32}P]dCTP by nick translation (44) or by the multiprime DNA labeling method (25). M13 phages containing yeast DNA fragments (strand-specific probes) were ^{32}P labeled by using the hybridization probe primer supplied by Pharmacia.

Plasmids used to make *TEC1* strand-specific DNA probes were constructed by inserting the 2.3-kilobase (kb) *Eco*RI-*Xho*I DNA fragment containing the *TEC1* gene in both orientations into the multiple cloning region of M13mp18 and M13mp19 vectors (41).

Yeast chromosomes were separated by pulsed-field gel electrophoresis (54). The electrophoresis system was the LKB Pulsaphor. The pulses conditions were as follows: 200 s at 150 V for 72 h, 50 s at 300 V for 24 h, 100 s at 250 V for 24 h, 200 s at 150 V for 24 h, and 400 s at 150 V for 8 h. Gels were treated with 0.25 M HCl for 20 min at room temperature and then soaked in 0.2 M NaOH–0.6 M NaCl for 40 min and in 0.3 M Tris hydrochloride (pH 7)–10× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 40 min. The gels were then analyzed by Southern blot hybridization (39) with ^{32}P-labeled DNA probes.

Subcloning plasmids. The 6.0-kb *Bgl*II-*Cla*I and the 4.2-kb *Bgl*II-*Xho*I fragments containing the *TEC1* gene were subcloned from pBC43-2 into the *Bgl*II-*Cla*I and *Bgl*II-*Sal*I restriction sites, respectively, of the low-copy-number vector YCp50 (31). Plasmid pILC5-6 was constructed by subcloning the 5.7-kb *Bgl*II-*Sal*I fragment from pBC43-2 (Fig. 1) into the pUC19 multiple cloning region of the pFL44 vector (*Bam*HI and *Sal*I sites). pFL44 (2 μm origin, *URA3* derivative of pUC19) is a multicopy plasmid received from F. Lacroute.

pILC5-6ΔE is a pILC5-6 derivative in which the remaining *Eco*RI site of the pUC19 multiple cloning region was destroyed by T4 polymerase treatment. pILC1-6, pILC2-2, and pILC8-1 were obtained by inserting *Bgl*II-*Xho*I, *Pst*I-*Xho*I, and *Eco*RI-*Xho*I restriction fragments into the polycloning region of the pFL44 vector.

BAL 31 deletions within the *TEC1* gene. Plasmids pILDN and pILDC (Fig. 1) were made by creating extensive deletions from the *Eco*RI and *Xho*I sites, respectively, in pILC5-6ΔE with BAL 31 exonuclease. pILC5-6ΔE was first linearized by cutting with *Xho*I and treated with BAL 31 exonuclease (New England BioLabs, Inc.) (see reference 35 for calibration of BAL 31 nuclease for extensive DNA digestion). At 30-s intervals, samples were phenol extracted. The DNA samples were precipitated and digested with *Sal*I to remove the *Xho*I-*Sal*I sequence, treated with T4 polymerase to fill in the DNA ends, and ligated. After ligation, the deletion endpoints are flanked by the *Hin*dIII unique site of the pFL44 polycloning region. Each ligation was used to transform *E. coli* MM294. The extent of the *TEC1* 3′-terminal deletions was estimated by the sizes of the *Eco*RI-*Hin*dIII resulting fragments. To create 5′-terminal deletions of the *TEC1* region, pILC5-6ΔE was first digested with *Eco*RI and treated with BAL 31 nuclease. The DNA was then cut at the *Kpn*I site present in the pFL44 polycloning region to remove the sequences extending upstream from the *Eco*RI site, treated with T4 polymerase, and ligated. After ligation, the deletion endpoints are flanked by the *Sac*I unique site of the pFL44 polycloning region. After transformation into *E. coli* MM294, the extent of the 5′-terminal deletions was determined by the sizes of the resulting *Sac*I-*Sal*I fragments.

***TEC1-lacZ* fusions.** The *TEC1-lacZ* in-frame fusions were constructed by subcloning the 0.42- and 0.8-kb *Eco*RI-*Sph*I fragments from pILDC9 and pILDC138 (C-terminal deletions; Fig. 1 and 2) into the *Eco*RI and *Sph*I sites of the high-copy-number plasmid YEp356 (43) to yield pILDC9-Z

FIG. 1. Physical localization of the *TEC1* locus on chromosome II, restriction map, and deletion analysis of the cloned DNAs containing the wild-type *TEC1* gene. (A) Representation of the physical localization of *TEC1* on the right arm of chromosome II. Thin line represents chromosomal DNA. Some restriction sites are shown. Open boxes with arrowheads represent the side-by-side *TEC1* and *MIS1* genes, with arrows showing the orientations of their respective ORFs (see Results). Arrowheads indicate localization of the *TEC1* gene with regard to the *GAL* and *PHO3,5* clusters (see Results). (B) Plasmid pBC43-2, one of the Ura$^+$ clones that complemented the *tec1* mutation. Boxes correspond to yeast DNA sequences; lines represent vector DNA. pILC plasmids are derivative subclones bearing fragments (open boxes) inserted in the pUC19 multicloning region of the multicopy plasmid pFL44. Plasmids pILDN and pILDC carry deletion derivatives (BAL 31 exonuclease treatment) of the 5.7-kb *Bgl*II-*Sal*I region of pILC5-6 (see Materials and Methods). Strains 02298b (*tec1 ura3 dur*O^h*-1*, *cargB*$^+$*O*^{h}*-1*) and 01921cTD3F4 (*tec1*::*ura3 ura3 dur*O^h*-1*) were transformed with these plasmids. Complementation of *tec1* and *tec1*::*ura3* mutations was scored by the ability to grow on minimal medium containing urea or ornithine as the sole nitrogen source compared with the ability of *TEC1* strains Σ1278b and M1b (Table 1). Symbols: +, complementation. −, no complementation. (C) The 2.3-kb *Eco*RI-*Xho*I fragment containing the *TEC1* gene was replaced by a 1.6-kb *Eco*RI-*Sal*I fragment containing the *URA3* gene. The *Xmn*I-*Xmn*I fragment (*tec1*::*URA3*) was used for performing gene deletion. The *TEC1* ORF is shown as an open box; the hatched box indicates the minimal complementing fragment (when present on this multicopy plasmid). Restriction sites: A, *Sac*I; B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; S, *Sal*I; Sa, *Sau*3A; Sp, *Sph*I; Xh, *Xho*I; X, *Xmn*I. Sites in parentheses have been destroyed.

and pILDC138-Z. YEp356 is a yeast-*E. coli* shuttle vector suitable for fusing yeast promoter and coding sequences to the *lacZ* gene of *E. coli*.

Ty1-*lacZ*/*TDH3-galK* integrative plasmids. pILTI plasmids were generated as follows. Transposition of five independent Ty1 elements (Ty1-IL12, -IL15, -IL16, -IL17, and -IL18) occurred in the 5′ nontranscribed region of a silent *TDH3-galK* gene fusion on a yeast-*E. coli* high-copy-number shuttle vector. In this construct, the *galK* gene fragment is devoid of its own ATG codon and is connected to the glyceraldehyde-3-phosphate dehydrogenase (*TDH3*)-ATG codon via the *Bam*HI-*Eco*RI portion of the pUC9 polylinker so as to provide the correct reading frame for translation from the *TDH3* ATG codon (ATG GATCC). The *Eco*RI site is as in the YRpR1 construct described previously (48).

The *TDH3* sequences extending upstream from position −164 with respect to the ATG codon (3) were removed, giving rise to a silent gene (N. Harford, personal communication). Transcription-terminating signals are provided by *ARG3* sequences. The insertion points of the five transposition events were mapped by DNA sequencing at positions −139 (Ty-IL12 and -IL16), −144 (Ty-IL18 and -IL17), and −160 (TyIL15) in the nucleotide sequence of the *TDH3* (*GPD*) promoter (data not shown). Ty1 insertion in the 5′ nontranscribed region of this *TDH3-galK* gene resulted in an activation of gene expression. A 5-kb *Bgl*II-*Sal*I fragment was subcloned from each of these plasmids into the *Bam*HI and *Sal*I sites of the pUC19 multiple cloning region in the integrative plasmid YIp356R (43). These constructions create in-frame fusions between the *TYB* open reading frame (ORF) of each Ty1 element (at the *Bgl*II site position 1707) and the *E. coli lacZ* gene. These pILTI12 to pILTI18 vectors linearized by *Sac*II digestion were integrated in the genome at the *ARG3* locus (see Results).

Construction of the *tec1*::*ura3* deletion alleles. Figure 1B shows the *Xmn*I fragment used for constructing the *tec1*::*URA3* deletions. This fragment was used to transform *ura3* yeast strains. Total yeast DNA of Ura$^+$ transformants was digested with *Eco*RI and *Xho*I. DNA fragments were then separated by agarose gel electrophoresis and transferred to nitrocellulose. The Southern blots were then hybridized with the 2.3-kb *Eco*RI-*Xho*I and *Eco*RI-*Eco*RI fragments from pBC43-2 (Fig. 1). To allow transformation of *tec1*::*URA3* strains with plasmids bearing the *URA3* selection marker, positive selection of spontaneous Ura$^-$ revertants (*tec1*::*ura3*) was performed by plating the strains onto FOA medium (6). The Ura$^-$ revertants transformed with a *URA3 TEC1* low-copy-number plasmid showed a wild-type phenotype and perfect complementation of the *tec1* defect.

DNA sequence analysis. Sequence analysis of the *TEC1* DNA was performed on both strands by the dideoxy-chain termination technique (49), using the BAL 31-generated deletion plasmids described above (Fig. 1 and 2). Double-stranded plasmid DNA was denatured and directly sequenced (9) with the sequencing primers provided by Pharmacia for pUC vectors or with synthetic oligonucleotides.

DNA sequences were analyzed with the GenBank, EMBL, and NBRF data bases and sequence analysis software package of the Genetics Computer Group (version 6.0) (18). The nucleotide sequence reported in this paper has been submitted to the GenBank/EMBL data bank (accession no. M32797).

RNA analysis. Total cellular RNA was isolated from cells grown in minimal liquid medium to an optical density at 600 nm of 0.1 (10^7 cells per ml) by glass bead lysis (51). Poly(A)$^+$-enriched RNA preparations were obtained from total yeast RNA by chromatography on poly(U)-Sepharose (Pharmacia). Total or poly(A)$^+$-enriched RNA (10 to 50 μg) was size fractionated on 1.5% agarose–6% formaldehyde–10 mM sodium phosphate buffer (pH 8.5) denaturing gels. Transfer to nitrocellulose and hybridization with ^{32}P-labeled DNA probes are described by Thomas (52).

The probe used to detect *TEC1* mRNA was the 2.3-kb *Eco*RI-*Xho*I fragment isolated from plasmid pILC8-1 as an *Eco*RI-*Hin*dIII fragment. The *Hin*dIII site is from the pUC19 polylinker of pFL44 (see above).

The Ty1-specific probe was the 0.420-kb *Bgl*II (nucleotide position 3302)-*Eco*RI (nucleotide position 3722) fragment

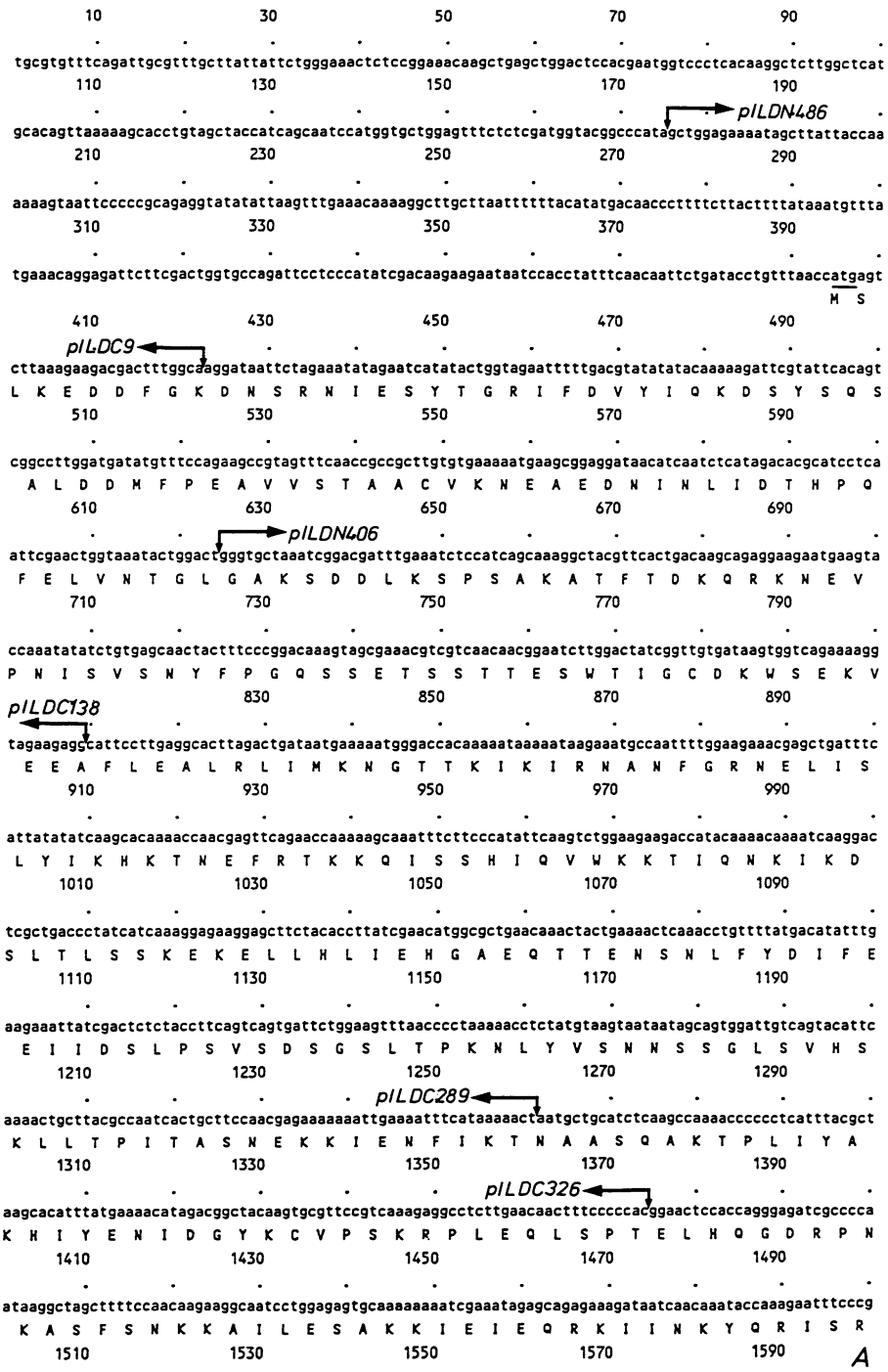

FIG. 2. DNA and deduced amino acid sequences of the *TEC1* gene. The entire nucleotide sequence of the 2.3-kb *Eco*RI-*Xho*I complementing region (Fig. 1) was determined on both strands. The sequence of the predicted 486-amino-acid polypeptide is presented beneath the nucleotide sequence, beginning at position 395 and extending to position 1855. Junctions of deletion derivatives of the *TEC1* gene depicted in Fig. 1 are indicated with arrowheads. The minimal complementing coding sequence is carried on plasmid pILDC289. The translational start codon is underlined, and a star indicates the translational stop codon.

of a Ty1 element isolated from plasmid YEp (*URA3*$^+$ *cargA*$^+$*O*^{h}-*1*) (30). The Ty2-specific probe was the 1.704-kb *Cla*I (nucleotide position 726)-*Cla*I (nucleotide position 2430) fragment of a Ty2 element received from J. D. Boeke. The probes used in this study as internal controls were the 0.930-kb *Eco*RI-*Sal*I fragment of the β-IPM dehydrogenase (LEU2) coding region isolated from the YEp13 vector (7) and the 2.75-kb *Eco*RI-*Eco*RI fragment revealing the mRNA coding for ribosomal protein rp73 of *S. cerevisiae* isolated from plasmid pA14, received from J. Warner. The *lacZ* probe was the 0.620-kb *Eco*RI-*Sal*I *lacZ* fragment of plasmid YEp357 (43). The *galK* probe was the 1.150-kb *Eco*RI-*Eco*RI fragment from plasmid YRpR1 (48). The *MFα1* probe was the 1.6-kb *Eco*RI-*Eco*RI fragment from plasmid p69A (33).

Assay of *TEC1-lacZ* fusion products. Yeast strains containing plasmid-borne *TEC1-lacZ* fusions were harvested in the

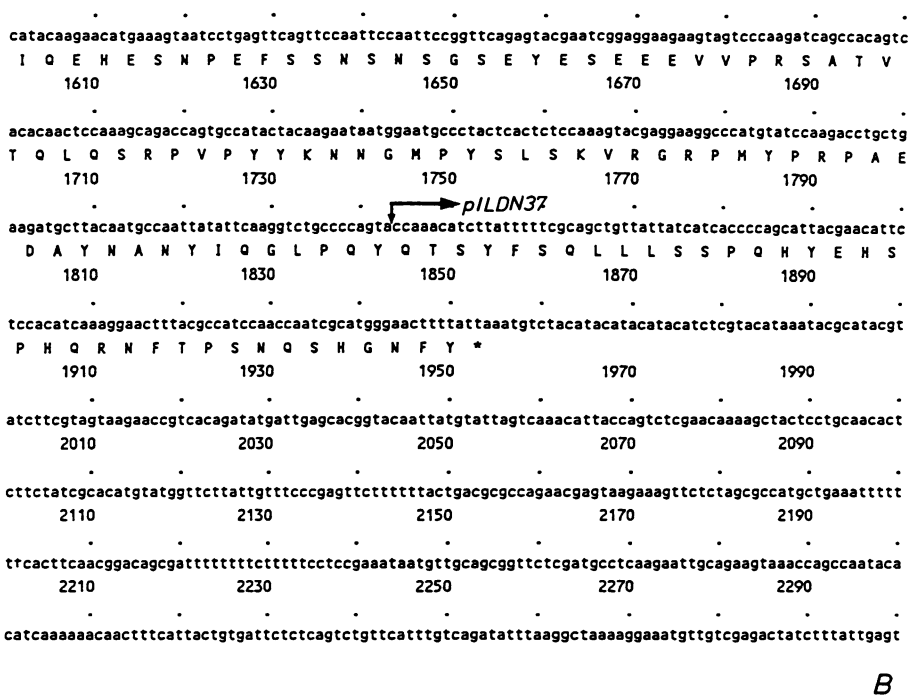

FIG. 2—*Continued.*

midlogarithmic phase. The cells were broken by vortexing in the presence of glass beads in Z buffer (42), and cellular debris was removed by centrifugation at 4000 × *g*. β-Galactosidase activity was assayed as described elsewhere (42) and expressed as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) cleaved per minute per milligram of protein. Samples containing 10 to 50 μg of protein were denatured with an equal volume of 0.125 M Tris hydrochloride (pH 6.8) containing 20% glycerol, 4% sodium dodecyl sulfate, 1.4 M β-mercaptoethanol, and 0.002% bromophenol blue by heating for 4 min at 100°C and subjected to electrophoresis on a 12.5% polyacrylamide-sodium dodecyl sulfate gel (34). Proteins were blotted onto nitrocellulose (53) and detected with purified monoclonal anti-β-galactosidase antibodies provided by Promega Biotec. The immunoreaction was developed by using the biotin-streptavidin peroxidase method (Amersham Corp.).

RESULTS

Isolation and characterization of the *TEC1* gene. Because of the insertion of Ty1 elements in the 5′ nontranscribed region of the *DUR2,1* and *CAR2* genes, the overproduction of ureoamidolyase and ornithine transaminase in a *durO*^{h}-*1* *cargB*$^+$O^h-*1* yeast strain is strongly reduced in the presence of the *tec1* mutation. This mutation gives rise to a growth deficiency on urea or ornithine as the sole nitrogen source (19). The *TEC1* gene was cloned by complementation of the *tec1* mutation in strain 02298b (*tec1* *durO*^{h}-*1* *cargB*$^+$*O*^{h}-*1*). This strain was transformed with an *S. cerevisiae* genomic bank constructed by M. Rose and containing partial *Sau*3A genomic fragments inserted in the unique *Bam*HI site of the low-copy-number plasmid YCp50. The transformants were selected for growth on urea or ornithine. Several independent transformants were obtained. Two different plasmids, named pBC7-2 and pBC43-2, were purified and found to complement the *tec1* mutation. The two plasmids were analyzed by restriction mapping and appeared to carry a common region of about 9-kb within their inserts.

Different DNA fragments from pBC43-2 were inserted in the YCp50 and pFL44 vectors (Fig. 1B). These plasmids were used to transform strain 02298b. Growth on urea or ornithine and restoration of enzyme overproduction (data not shown) were tested with the different transformants. A 2.3-kb *Eco*RI-*Xho*I fragment was shown to be sufficient for complementation of the *tec1* mutation (Fig. 1B, plasmid pILC8-1).

To define more precisely the boundaries of the *TEC1* complementation unit, we constructed unidirectional deletions in pILC5-6ΔE, generating deletions from each end of the 2.3-kb *Eco*RI-*Xho*I fragment (Fig. 1B).

Plasmids carrying selected deletions were introduced into strains 02298b (*tec1*) and 01921cTD3F4 (*tec1*::*ura3* Δ*tec1* [see below]). The transformant cells were tested for the ability to grow on urea or ornithine as the sole nitrogen source, which allowed us to localize a complementing activity in a region of about 1 kb (Fig. 1).

To confirm that the cloned DNA contains the *TEC1* gene, we examined the meiotic segregation of a chromosomally integrated plasmid relative to the *tec1* mutation. For this purpose, an *Eco*RI-*Eco*RI DNA fragment containing the entire *TEC1* gene (Fig. 1B) was subcloned into pBR322 carrying a *Hin*dIII-*Hin*dIII *URA3* fragment. Strain 02298b was then transformed with this integrative plasmid, and Ura$^+$ colonies were purified. One mitotically stable Ura$^+$ Tec$^+$ transformant, single-copy integration of which was verified by Southern blot analysis of total genomic DNA (not shown), was mated to the *durO*^{h}-*1* strain CL1a. The resultant diploids were sporulated and scored for Ura$^+$ and Tec$^+$ phenotypes. Among 45 tetrads analyzed, only two spores were Tec$^-$, indicating a tight linkage between the integrated plasmid carrying *TEC1* and the *tec1* mutation. The analysis

of the *tec1*::*URA3* mutant allowed us to confirm the allelism (see below).

Because the *tec1* mutation in strain 02298b could be leaky, we constructed a *TEC1*-deleted strain to ensure the complete loss of *TEC1* function. The Δ*tec1* null allele was constructed by the method of one-step replacement as described by Rothstein (47). The 2.3-kb *Eco*RI-*Xho*I fragment from pILC5-6 was replaced by a 1.6-kb *Eco*RI-*Sal*I *URA3* fragment (Fig. 1C). This plasmid was subsequently restricted with *Xmn*I to generate a DNA fragment with both ends having homology to the *TEC1* locus. This fragment was then used to transform the diploid strain CL2Na (a *ura3 durO*^{h}-*1*/α *ura3 durO*^{h}-*1*). Ura$^+$ transformants expected to be heterozygous at the *TEC1* locus were characterized by Southern blot analysis of the genomic DNA (not shown).

The transformants were sporulated and dissected. Tetrad analysis showed 2+:2− segregation for the *URA3* marker. All of the Ura$^+$ spores were shown to contain the *tec1*::*URA3* allele. Since these *tec1*::*URA3* spores were viable, the *TEC1* gene appears not to be essential for cellular growth.

Replacement deletion of the *TEC1* gene was achieved in several strains to obtain isogenic wild-type and *tec1*::*ura3* mutant strains (see Materials and Methods and Table 1). These strains were used in the subsequent experiments.

A *durO*^{h}-*1 tec1*::*URA3* strain was crossed with the *durO*^{h}-*1 tec1* mutant strain 02298b. The resulting diploids were unable to grow on urea as a nitrogen source, showing an absence of complementation. The segregation analyses confirmed the allelism of *tec1*::*URA3* and *tec1* (4 Tec$^-$:0 Tec$^+$).

The chromosomes of *TEC1* and *tec1*::*ura3* haploid strains were resolved by pulsed-field gel electrophoresis on agarose and transferred to a nitrocellulose membrane. Hybridizations of the chromosomal DNA with a *TEC1* DNA probe revealed that the *TEC1* gene is located on chromosome II, using a chromosome II-specific *DUR2,1* DNA probe as a control (data not shown). Southern blots of separated restriction fragments from a collection of chromosome II-specific cosmid clones (kindly provided by R. Stucka) were hybridized with the *Eco*RI-*Xho*I *TEC1* probe. This allowed us to assign the hybridization signals to one cosmid clone. To further analyze and to confirm the preliminary assignment, a more precise mapping of the *TEC1* locus was obtained by restriction analysis and subsequent hybridization of this cosmid clone, C1258 (received from R. Stucka). The clone derives from partially *Sau*3A-digested yeast DNA (strain C836) cloned into the unique *Bam*HI site of the vector pY3030. It contains a 30-kb DNA fragment encompassing the *PHO3,5* cluster, located to the right of the *GAL* cluster on the right arm of chromosome II. This allowed us to localize the *TEC1* gene at about 15 kb (5 centimorgans) from the *PHO3,5* locus (Fig. 1A).

Sequence analysis of the *TEC1* gene. The nucleotide sequence of the 2.3-kb *Eco*RI-*Xho*I DNA region containing the *TEC1* gene was determined on both strands. The DNA sequence shown in Fig. 2 was found to contain one large ORF of 487 codons, starting with an ATG codon at position 395 and extending to a TAA (stop) codon at position 1855. A second ATG codon is present at position 515. Two plasmids, pILDC9-Z and pILDC138-Z, in which 5′ sequences of the *TEC1* gene are fused to the *E. coli lacZ* gene, were constructed (Fig. 2). In both cases, *TEC1-lacZ* products were observed by Western blot (immunoblot) analysis (not shown), indicating that the ORF is translated into a protein. The observation of the shorter product demonstrates that the first AUG codon is used. The apparent molecular weights of these fusion products are in agreement with the size of the predicted protein (55 kilodaltons). The *TEC1* product exhibits no significant sequence similarity to any known protein in the NBRF, GenBank, and EMBL data bases. The polypeptide is very rich in serine residues (12.5%). The predicted *TEC1* amino acid sequence has a uniform distribution of acidic and basic residues and is rather hydrophilic. Computer analysis of the sequence and secondary-structure predictions failed to identify motifs such as zinc finger, leucine zipper, or α helix-turn-α helix found to be conserved in various transcriptional activators.

According to the sequence data, the smallest of the aforementioned 3′ deletions (pILDC289) that complements the *tec1* and *tec1*::*ura3* mutations removes all sequences downstream from position 1261 and encoding the 117 C-terminal amino acids of the protein (Fig. 1 and 2). These results imply that the C-terminal one-third of the *TEC1* product is dispensable for the activator role of *TEC1* at least when the truncated gene is present on a high-copy-number vector. Comparison of the *Eco*RI-*Xho*I sequenced fragment with DNA sequences present in GenBank and EMBL data bases revealed an overlapping homology of nucleotides 1515 to 2308 with nucleotides 1 to 794 (amino acid 12) of the *S. cerevisiae MIS1* gene (50). This finding indicates that the *TEC1* and *MIS1* genes are located side by side on chromosome II, the two ORFs being in the same orientation (Fig. 1A).

Analysis of *TEC1* transcription. Total RNA preparations were obtained from wild-type and *tec1* mutant strains. Poly(A)$^+$ RNA was subsequently purified on poly(U)-Sepharose membranes. Northern (RNA) hybridization analysis with the 2.3-kb *Eco*RI-*Xho*I DNA probe detected one polyadenylated transcript of about 1.5 kb (data not shown). This size is consistent with the length of the *TEC1* ORF. By hybridization with single-strand-specific probes, we confirmed that the sense of transcription is consistent with the observed ORF (data not shown).

Effect of *TEC1* deletion on Ty transcripts levels. To further characterize the *TEC1* effect on Ty expression, Ty1 and Ty2 mRNA levels were analyzed independently by Northern blotting in couples of isogenic *TEC1* and *tec1*::*ura3* or *tec1* strains, using specific DNA probes (Fig. 3). The absence of the *TEC1* product strongly reduced the total Ty1 transcript level (Fig. 3A). A decrease of at least 5- to 10-fold was observed in 02298b and 01921c genetic backgrounds, but a lower effect (3- to 5-fold) was observed in the JWG2-1D genetic background. In contrast with these results, there was little or no fluctuation in Ty2 mRNA levels between *TEC1* and *tec1*::*ura3* or *tec1* strains (Fig. 3B). Indeed, in strains with 02298b and JWG2-1D backgrounds, no significant effect was observed, and a two- to threefold effect was detected in strains with a 01921c background.

Coordinate effect of the *TEC1* gene product on Ty1 and adjacent gene expression. The effects of the *TEC1* deletion were analyzed by using five different Ty1 elements transposed in the 5′ nontranscribed region of a silent *TDH3-galK* gene fusion. To measure the expression level of each Ty1 element, Ty-*lacZ* fusions were used. In-frame *TYB-lacZ* fusions were constructed and inserted together with the *TDH3-galK* reporter gene on the YIP356R plasmid (Fig. 4A). After linearization, these five constructs were integrated at the *ARG3* locus of isogenic *TEC1* and *tec1*::*ura3* strains (01921c background). Single-copy integrations were obtained (data not shown), and the Ty1-*lacZ* and *TDH3-galK* mRNA levels were analyzed by Northern blotting (Fig. 4B). For each of these Ty1 elements, *TEC1* control of *TDH3-galK*

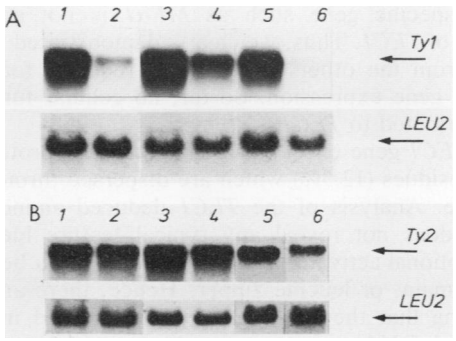

FIG. 3. Effect of *TEC1* function on Ty1 and Ty2 transcription. Total RNA was isolated from cells in early log phase. RNA (50 μg) was fractionated on a 1.5% agarose–6% formaldehyde gel and transferred to nitrocellulose. (A) RNA hybridized with the Ty1-specific ^{32}P-labeled 0.42-kb *Bgl*II-*Eco*RI DNA fragment; (B) RNA hybridized with the Ty2-specific ^{32}P-labeled 1.7-kb *Cla*I-*Cla*I DNA fragment (see Materials and Methods. The mRNA level of the *LEU2* transcript hybridized with the ^{32}P-labeled 0.93-kb *Eco*RI-*Sal*I fragment was used as an internal control. Lanes: 1, 02298b transformed with plasmid YCp50 containing the 5.7-kb *Bgl*II-*Sal*I fragment from pBC43-2 (Fig. 1); 2, 02298b (*tec1*); 3, JWG2-1D (*TEC1*); 4, JWG2-1DTD2F5 (*tec1*::*ura3*); 5, 01921c (*TEC1*); 6, 01921cTD3F4 (*tec1*::*ura3*).

reporter gene expression was observed, comparing *TEC1* and *tec1*::*ura3* strains. The Ty1-*lacZ* RNA levels were similarly altered by the *TEC1* deletion except for one element, named Ty-IL15 (Fig. 4B, lanes 3 and 4); in this particular case, the Ty1-*lacZ* transcript was undetectable. It is possible that a mutation occurred during the transposition process that altered the promoter region of this element. Interestingly, the Ty-IL15 element was the most efficient in activating reporter gene expression, and this element also exhibited the most pronounced *TEC1* effect.

Relationship between the *TEC1* expression and the mating and sporulation functions. We have shown previously that the *tec1* mutation does not lead to mating or sporulation defects (18). However, a leaky effect was not ruled out. Therefore, we studied whether a complete loss of *TEC1* function could alter the conjugation and sporulation processes. Our observations were that the *tec1*::*ura3* null mutant was mating proficient and that a *tec1*::*ura3* homozygous **a**/α diploid strain showed no decrease in sporulation efficiency.

In addition, by Northern blot analysis, we observed that the *MFα1* transcript level in the *tec1*::*ura3* strain was indistinguishable from that of the wild type (data not shown), confirming that the *TEC1* gene does not belong to the group of Ty expression determinants, such as *STE7*, *STE11*, and *STE12*, that are also involved in haploid-specific gene expression.

However, the possibility existed that mating type can control expression of the *TEC1* gene. By Northern blot analysis of the *TEC1* transcript and by measurement of the level of a *TEC1-lacZ* fusion protein in isogenic haploid and **a**/α diploid strains, no transcriptional or posttranscriptional *MAT* control of *TEC1* expression was observed (Table 2).

Interaction between the *MAT* and the *TEC1* controls on Ty1 transcript levels. The **a**/α *MAT* control represses expression of Ty and Ty-controlled genes. The analyses presented above demonstrated that *TEC1* function affects Ty1 expression but that *TEC1* expression itself is not influenced by the **a**/α control. Therefore, we wished to investigate the interaction between the *MAT* repression and *TEC1* activation of the Ty1 and Ty1-controlled genes. The **a**/α effect on Ty1 transcripts levels in the absence of the *TEC1* product was analyzed by Northern blotting. Ty1 transcript levels were similar in a *tec1*::*ura3* haploid and in a homozygous

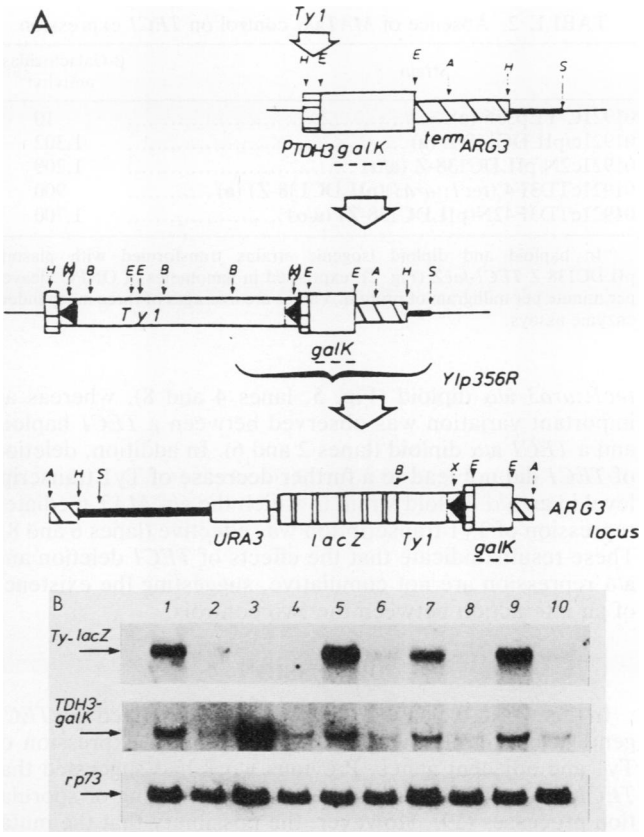

FIG. 4. *TEC1* activation of Ty1 and adjacent gene transcription. (A) Construction and single-copy integration in the yeast genome of the Ty1-*lacZ*/*TDH3-galK* constructs. Transposition of five independent Ty1 elements was obtained in the 5′ nontranscribed region of a silent *TDH3-galK* gene fusion (transcription-terminating signals were provided by *ARG3* sequences). In each case, *TDH3-galK* expression was activated by the Ty1 element. Five-kilobase *Bgl*II-*Sal*I DNA fragments carrying Ty1, *TDH3-galK*, and *ARG3* sequences were inserted in the integrative plasmid YIp356R, creating in-frame fusions between the *TYB* ORF of each Ty1 element and the *E. coli lacZ* gene. Integration of the *Sac*II-linearized plasmids was targeted at the *ARG3* locus of *TEC1* and *tec1*::*ura3* isogenic strains. Symbols: ▯, *TDH3* sequences; □, *galK* gene; ▨, *ARG3* sequences; ▬, pBR322 sequences; —, vector DNA; ◀, long terminal repeat sequences; ▭, epsilon region of Ty1; ▯▯, *lacZ* sequences. Restriction sites (▼): H, *Hin*dIII; E, *Eco*RI; A, *Sac*II; S, *Sal*I; X, *Xho*I; B, *Bgl*II. Parentheses indicate polymorphic sites. (B) Northern hybridization of Ty1-*lacZ* and *TDH3-galK* mRNA levels in isogenic *TEC1* and *tec1*::*ura3* strains. Total RNA was isolated from early-log-phase cells. RNAs (10 μg) were separated by electrophoresis on a 1.5% agarose–6% formaldehyde gel, transferred to nitrocellulose, and hybridized with the 1.150-kb *Eco*RI-*Eco*RI ^{32}P-labeled *galK* fragment, followed by hybridization with the 0.620-kb *Eco*RI-*Sal*I *lacZ* DNA fragment. The mRNA level of the *S. cerevisiae* rp73 gene revealed with the ^{32}P-labeled 2.75-kb *Eco*RI-*Eco*RI fragment was used as an internal control. Lanes: 1, 01921c (*TEC1*) (pILTI12); 2, 01921cTD3F4 (*tec1*::*ura3*) (pILTI12); 3, 01921c(pILTI15); 4, 01921cTD3F4(pILTI15); 5, 01921c(pILTI16); 6, 01921cTD3F4(pILTI16); 7, 01921c(pILTI17); 8, 01921cTD3F4 (pILTI17); 9, 01921c(pILTI18); 10, 01921cTD3F4(pILTI18).

TABLE 2. Absence of *MAT*a/α control on *TEC1* expression

Strain	β-Galactosidase activity[a]
01921C/YEp356 (a)	10
01921c/pILDC138-Z (a)	1,302
01921c2N/pILDC138-Z (a/α)	1,209
01921cTD3F4 (*tec1*::*ura3*)(pILDC138-Z) (a)	900
01921cTD3F42N(pILDC138-Z) (a/α)	1,700

[a] In haploid and diploid isogenic strains transformed with plasmid pILDC138-Z *TEC1-lacZ* (Fig. 2), expressed in nanomoles of ONPG cleaved per minute per milligram of protein. Values are averages of three independent enzyme assays.

tec1::*ura3* a/α diploid (Fig. 5, lanes 4 and 8), whereas an important variation was observed between a *TEC1* haploid and a *TEC1* a/α diploid (lanes 2 and 6). In addition, deletion of *TEC1* did not lead to a further decrease of Ty1 transcript level in an a/α diploid strain in which the a/α *MAT*-mediated repression of Ty1 transcription was effective (lanes 6 and 8). These results indicate that the effects of *TEC1* deletion and a/α repression are not cumulative, suggesting the existence of an interaction between the two controls.

DISCUSSION

In this work, we have isolated and characterized the *TEC1* gene, which is involved in the activation of expression of Ty1 and adjacent genes. Previous work had suggested that *TEC1* was not involved in the control of mating or sporulation processes (19). However, the possibility that the mutation was leaky was not ruled out. Construction of the null allele has allowed us to show that the gene is not essential for cellular growth and is required neither for the mating process nor for sporulation. In addition, the transcript level of a haploid-specific gene such as *MFα1* is not affected by deletion of *TEC1*. Thus, we clearly demonstrated that *TEC1* differs from the other determinants required for Ty1 and adjacent gene expression. So far, no cellular function has been attributed to *TEC1*.

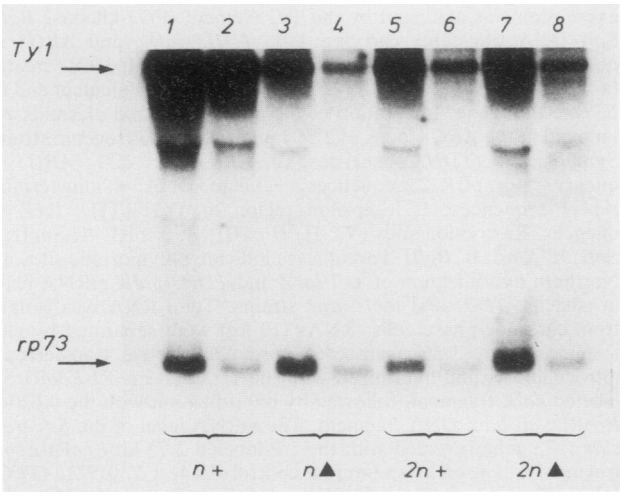

FIG. 5. Northern hybridization analysis of Ty1 mRNA levels in isogenic *TEC1* and *tec1*::*ura3* haploid or diploid strains. Total RNA was isolated from cells in early log phase. RNAs were fractionated in a 1.5% agarose–6% formaldehyde gel and transferred to nitrocellulose. Odd and even lanes contain, respectively, 40 and 8 μg of RNA. RNA was hybridized with the ^{32}P-labeled 0.42-kb *Bgl*II-*Eco*RI Ty1 DNA fragment. The rp23 mRNA hybridized with the ^{32}P-labeled 2.75-kb *Eco*RI-*Eco*RI fragment was used as an internal control. Lanes: 1 and 2, 01921c (*TEC1*); 3 and 4, 01921cTD3F4 (*tec1*::*ura3*); 5 and 6, 01921c2N (a/α); 7 and 8, 01921cTD3F42N (a/α *tec1*::*ura3*/*tec1*::*ura3*). n, Haploid; 2n, diploid; +, wild type; Δ, *tec1*::*ura3*.

The *TEC1* gene encodes a 486-amino-acid protein rich in serine residues (12.5%) which are dispersed throughout the sequence. Analysis of the *TEC1* deduced amino acid sequence does not reveal any typical feature identified in transcriptional activators, such as zinc finger, α helix-turn-α helix domain, or leucine zipper. Hence, there are no data suggesting that the *TEC1* gene product could interact directly with DNA sequences or with transcription factors.

TEC1 control on Ty and adjacent gene expression was investigated in isogenic *tec1*, *tec1*::*ura3*, and *TEC1* strains. We have observed that *TEC1* deletion strongly reduces Ty1 transcript levels. The amplitude of this effect is dependent on the genetic background. Such variation could be due to strain-to-strain differences in the Ty1 population or to differences in other Ty1 transcriptional controls.

The effect of *TEC1* deletion on the transcript levels of five individual Ty1 elements (measured as Ty1-*lacZ* transcription products) and coordinate effect on the transcript levels of adjacent genes (*TDH3-galK* gene fusion) were also investigated. These experiments revealed that the *TEC1* control was effective in each case. One of these elements appeared to be transcriptionally deficient, possibly as a result of a mutation that occurred during the transposition process. In this situation, the *TDH3-galK* transcript level was particularly efficiently increased. This finding is in agreement with results reported recently by Coney and Roeder (15) indicating that a transcriptionally deficient Ty element gives rise to an increased enhancement of adjacent gene expression. In addition, the *TEC1* control is extremely efficient in this particular case, suggesting an essential role of the *TEC1* gene in the increase of adjacent gene activation.

The effect of the *TEC1* deletion on overall Ty2 transcript levels was also measured. Surprisingly, the *TEC1* deletion significantly decreased Ty1 transcript levels but had little effect on Ty2 transcripts. A relatively weak effect on Ty2 was observed in only one of our strains. One explanation could be that only some Ty2 elements are controlled by *TEC1*. To complete this study, the expression of a series of individual Ty2 elements and the activation of the adjacent gene need to be examined in *TEC1* and Δ*tec1* strains.

From the analysis of the *tec1* and *tec1*::*ura3* mutants, we conclude that the *TEC1* protein participates in a positive control of Ty1 and Ty1-mediated gene expression. We do not know whether this control is modulated by external or cellular signals. Indeed, we have observed that *TEC1* expression is not controlled by the *MAT* locus either at the transcriptional or at the translational level. However, analysis of *MAT* and *TEC1* effects on Ty1 transcript levels revealed that these two controls are not cumulative. In the absence of *TEC1* gene product (*TEC1* deletion), Ty1 transcripts levels are relatively low and the *MAT* effect is abolished. Moreover, under the condition of a/α repression (in a/α diploids), *TEC1* control could no longer be observed. These results suggest the existence of an interaction between *TEC1* and *MAT* controls. We can speculate that *TEC1* encodes one of the elements required for the enhancement of Ty1 and adjacent gene expression whose action is modulated by factors that are responsible for *MAT* regulation. Gel retardation experiments involving Ty enhancer fragments should allow us to elucidate this relationship.

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