

Involvement of SRE element of Ty1 transposon in TEC1-dependent transcriptional activation

Isabelle Laloux¹, Eric Jacobs³ and Evelyne Dubois^{1,2*}

¹Laboratoire de Microbiologie, Université Libre de Bruxelles, ²Institut de Recherches du CERIA, 1 avenue E.Gryson, B-1070 Brussels, Belgium and ³Transgene S.A., 11 rue de Molsheim, F-67082 Strasbourg, France

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ABSTRACT

Some Ty1 transposable element insertion mutations of *Saccharomyces cerevisiae* activate transcription of adjacent genes in a cell-type dependent manner. This activation requires at least *STE12* and *TEC1* gene products. The binding site for the *STE12* protein is located in the sterile responsive element (SRE), which is just downstream the 5' LTR of Ty1 and contains one copy of the pheromone response element (PRE). This report defines the sequences in Ty1 required for TEC1-dependent activation using a *TDH3::lacZ* reporter gene in which the UAS was replaced by different portions of a Ty1 or Ty2 element. The Ty1 SRE seems to be sufficient to ensure the TEC1 and *STE12*-mediated activation whereas Ty2 SRE can activate the expression of the adjacent genes in the absence of both proteins. Adjacent to the PRE element, there is a region (PAE) with extensive sequence divergence in Ty1 and Ty2 SREs. Swapping experiments between Ty1 and Ty2 sequences show that Ty1 PAE is required for the activation of adjacent gene expression in a TEC1 and *STE12*-dependent manner. The use of a *LexA::TEC1* construct indicates that the chimeric protein has no activation ability suggesting that TEC1 could act in conjunction with another factor.

INTRODUCTION

Ty1 and Ty2 are two retrotransposon families of *Saccharomyces cerevisiae*. Each of these elements has a unique epsilon (ϵ) region of about 5.5 kb surrounded by two long terminal repeats (LTR). Functional Ty1 and Ty2 elements encode Gag (TYA) and Pol (TYB) proteins similar to those of retroviruses and are able to transpose through an RNA intermediate which is encapsidated into intracellular virus-like particles, giving rise to new chromosomal transposon copies. Most of the laboratory strains contain 20–40 copies of such transposons dispersed in their genomes. Ty1 and Ty2 share well-conserved LTR and ϵ domains. However, in addition to local base substitutions, they contain two relatively large sequences of overall heterogeneity within TyA

and TyB (1). These elements have the capacity to alter expression of cellular genes. Indeed, many mutations in *S. cerevisiae* are known to result from the insertion of a Ty element adjacent to a structural gene. Such transposons, when inserted in the 5' non coding region of a gene can strikingly alter its expression by decreasing or activating its transcription efficiency. Inhibition of expression of the adjacent gene has been exemplified by several mutations at the *HIS4* and *LYS2* loci (2, 3) whereas a number of Ty insertions at the *CYC7*, *ADH2*, *CAR1*, *CAR2* and *DUR2,1* loci giving rise to increased expression of the adjacent gene have been reported (4–8).

In the cases of Ty insertions activating gene expression, the transposon and the adjacent gene are always divergently transcribed. Due to the proximity of the Ty insert, the adjacent gene is then subject to a transcriptional control by the mating type locus (*MAT*) (6, 9). These Ty-mediated mutations were named ROAM (Regulated Overproducing Alleles responding to Mating type) (6, 9). The adjacent gene activation observed in haploid cells is reduced in *MAT α /MAT α* diploid cells and in haploid mutants affected at *STE7*, *STE11* or *STE12* locus. The products of these 3 genes are required for expression of haploid specific genes (10).

Mutational analysis of the *CYC7-H2* Ty1 defined at least two regulatory elements, which are responsible for the observed pattern of cell type specific gene expression (11–14). A sequence within the unique ϵ region approximately 500–700 bp downstream from the Ty1 transcription start site, called region D, includes the element designated block II which has a homology to the SV40 enhancer core and to the $\alpha 1$ - $\alpha 2$ repressor site (13). A single copy of this domain acts autonomously as a mating type regulator but has very little enhancer activity. Reiterating this site has an additive effect on transcription (13). This region is recognized by a constitutively produced protein factor (15). Recently Errede, showed that MCM1 is the protein that binds to Ty1 at this sequence (16). SPT13 has been proposed to limit the enhancer function of the block II domain in haploids, by controlling the MCM1 activity (17). Another important region named A or TAS1 has been located in the ϵ region just downstream the 5' LTR (13, 14). This element seems to play

*To whom correspondence should be addressed at: Institut de Recherches du CERIA, 1 avenue E.Gryson, B-1070 Brussels, Belgium

a major role in activation of adjacent gene transcription in wild-type haploid cells and is involved in the mating type control of Ty1 and adjacent gene transcription. This region contains a 50 base pair element (SRE) which is the target of a protein complex formed between *STE12* gene product and an unknown 72 kD factor (18, 11). SRE contains a pheromone response element (PRE), a *cis*-acting function also found in several chromosomal genes and required for pheromone induced transcription (19).

Analysis of Ty917, a member of Ty2 class revealed the existence of multiple regulatory sequences responsible for activation of gene expression (20, 21). Liao *et al.* (20) have identified a putative UAS in the 5' LTR of the Ty2 element. There are also two enhancers within the translated portion of the element which modulate the transcription. The upstream enhancer includes a region similar to the Ty1 SRE (22).

Ty transcription and the effect of Ty insertions on expression of adjacent genes can also be affected by the products of a series of genes which are functionally unrelated to the MAT control. These genes are designated *SPT*, *TYE* and *TEC* (previously named *ROC*). Mutants for *SPT* genes were obtained by phenotypic reversion of *his4* and *lys2* mutations caused by Ty insertion in front of the transcription start site (23). However it is not known whether *SPT* gene products other than SPT13 (see above) are involved in Ty-mediated gene activation. Most of *spt* mutations cause a variety of other phenotypes including defects in sporulation, DNA repair and growth. These pleiotropic effects suggest that *SPT* genes are required for different aspects of normal gene expression in *S.cerevisiae*.

Additional genes involved in Ty-mediated gene activation were identified by recessive *tye* mutations which suppress the overexpression of the glucose repressible *ADH2* gene (24). The behaviour of *tye* mutant strains suggests that *TYE* gene products influence expression of many genes and not specifically Ty and Ty-mediated transcription (25).

The *tec1* mutation was isolated by suppression of overproduction of the *DURI*₂ gene product (5). *TEC1* is a *trans*-acting factor required for full Ty1 and Ty1-mediated gene activation. However, deletion of the *TEC1* gene had little effect (if any) on Ty2 transcript levels. Unlike most of the proteins involved in Ty and adjacent gene expression, *TEC1* gene product is not a regulator of mating or sporulation process and has no known cellular function (26). *TEC1* encodes a 486 aa protein which has a domain with homology to the TEF1 DNA-binding motif (27), which recognizes the SV40 enhancer sequence (28). Because the block II sequence is similar to the SV40 core enhancer sequence, an attractive prediction was that *TEC1* should be a block II DNA binding protein (27).

By analysis of deletions, fusions and *in vitro* created mutations, we show in this report that the SRE element but not the block II element is responsible for *TEC1*-dependent activation of Ty-mediated gene expression. We attempt to determine more precisely the sequences in the SRE required for the activation in a *TEC1* and *STE12*-dependent manner.

MATERIALS AND METHODS

Strains, media and genetic procedures

Saccharomyces cerevisiae isogenic haploid strains 01921c (*MAT α dur^{O^h-1} ura3*), 01921cTD3F4 (*MAT α tec1::ura3 dur^{O^h-1} ura3*) and diploid strain 01921c2N (*MAT α /MAT α dur^{O^h-1}/dur^{O^h-1} ura3/ura3*) used in this study have been described previously (26). Haploid strain 02501c (*MAT α tec1::ura3 dur^{O^h-1} ura3 leu2*)

was obtained by mating haploid strains 02463d (*MAT α ura3 leu2*) and 01921cTD3F4, sporulation and tetrad analysis. Isogenic *ste12::LEU2* derivatives of 02463d and 02501c were constructed by replacement of the *STE12* gene after transformation with a *SacI*–*SphI* restriction fragment containing the *ste12::LEU2* allele. This fragment was purified from plasmid pSI14-12 (kindly provided by S.Fields) in which an *XbaI*–*XbaI* fragment encompassing the 3' end of the *STE12* gene had been replaced with the *LEU2* gene. Gene replacements were confirmed by Southern blot analysis.

Yeast transformation was performed by the LiCl procedure (29). Yeast spheroplasts were transformed with DNA fragments by the method of Hinnen *et al.* (30). Yeast cultures were grown at 30°C in minimal medium containing 25 μ g/ml uracil or 50 μ g/ml leucine. Standard yeast genetic procedures for mating, sporulation, tetrad analysis and scoring of nutritional markers were carried out as described previously (31). *E.coli* XL1-blue [*recA1*, *endA1*, *gyrA96*, *thi-1*, *hsdR17*, *supE44*, *relA1*, *lac* (*F proAB lacIqZ Δ M15 Tn10(tet^r)*)] Transformations were performed by the calcium chloride procedure (32). Transformants were grown in LB medium containing 100 μ g/ml ampicillin.

DNA isolation and techniques

Small scale plasmid DNA preparations from *E.coli* were accomplished by the method of Birnboim and Doly (33) and with the Qiagen kit from Diagen. Restriction digestion and enzyme modifications of DNA were performed as recommended by the suppliers following standard methods (34). Oligonucleotides were synthesized with a BioResearch Cyclone DNA synthesizer.

Oligonucleotide directed *in vitro* DNA mutagenesis

Site-specific mutagenesis of the PAE region was achieved by using the oligonucleotide-directed *in vitro* mutagenesis system supplied by Amersham. This system is based on the method of Eckstein and his coworkers (35). Oligonucleotide directed *in vitro* DNA mutageneses were performed on single stranded DNA purified from the *E.coli* XL1-blue strain transformed with recombinant pBluescript phagemids. The mutagenised inserts were directly sequenced by the dideoxy method of Sanger *et al.* (36) on double strand DNA denatured with alkali (37) as recommended in the United States Biochemical Sequenase kit. Primers used for sequencing were commercial or synthesized in the laboratory.

Oligonucleotide directed *in vitro* mutageneses of the PAE segment were performed with the plasmids pILB52H and pILB53H containing the *PvuII*–*Sau3A* fragments from Ty1 and Ty2 respectively. Synthesized oligonucleotide PAE2-6 shown in Fig. 5 was used to *in vitro* mutagenize plasmid pILB52H. Plasmid pILB53H was mutagenized with oligonucleotide PAE1-6.

Construction of vectors pIL5 and pIL6 carrying the reporter gene

The plasmids in Figs 1, 2 and 4 are derived from the pIL5 vector. pIL5 is a 10.85 kb vector containing the *TDH3* (glyceraldehyde-3-phosphate-dehydrogenase) 5' sequence (UAS-less) fused to *lacZ* (26). This plasmid was constructed by assembly of three fragments: the 5.5 kb *SmaI*–*HindIII* fragment from YCp50 containing *CEN4* and *ARS1*, the *EcoRI*–*SnaBI* fragment from YE356R (38) containing the *lacZ* and *URA3* genes and the 176 bp *HindIII*–*EcoRI* fragment containing the 3' part of the *TDH3* promoter (including TATA region) without UAS (previously described in 26). In this construction, the *lacZ* gene is connected

to the *TDH3* (*Bam*HI) ATG codon (ATG GATCC) via the *Bam*HI–*Eco*RI portion of the pUC9 polylinker so as to provide the correct reading frame for translation (26). The pIL6 vector (11.3 kb) is the same as pIL5, except that the *TDH3* UAS sequences extending upstream from position –164 with respect to the ATG codon (39) are present, giving a fully functional *TDH3* promoter: the 715 bp *Eco*RI–*Hind*III fragment containing the complete promoter was ligated to the *Eco*RI (position 10,818) and *Hind*III (position 10,850) sites of pIL5.

Subcloning of Ty1 and Ty2 sequences into pUC18 and pBS-SK⁺

Plasmid pIL301 (4.9 kb) was constructed by inserting a 2.2 kb *Sal*I–*Bam*HI fragment from Ty1-pIL16 (26) into the *Sal*I and *Bam*HI sites of the pUC18 polylinker. This plasmid contains Ty1-pIL16 sequences from the 5' LTR to the *Sal*I site (position –2312 on Fig. 1). Plasmid pIL301ΔP (3.8 kb) was constructed by *Pst*I digestion of pIL301 followed by ligation of the vector. pIL301 was double-digested with *Bgl*III (position –1840 on Fig. 1) and *Sph*I (in pUC18), treated with T4 DNA polymerase and ligated to create plasmid pIL301ΔBgS (4.4 kb). This plasmid was double-digested with *Hpa*I and *Pst*I, treated with T4 polymerase and ligated to construct pIL301ΔBgSBII (4.1 kb). Plasmid pILBS1 contains the 0.104 kb *Sau*3A–*Hpa*I fragment from Ty1-pIL16 (26) inserted into the *Bam*HI and *Eco*RI sites of the pBS-SK⁺ multiple cloning site. pILBS2 and pILBS3 were constructed by insertion of the 96 bp *Pvu*II–*Sau*3A fragments respectively from Ty1-pIL16 (see Fig. 1) and Ty2-pJEF1510 (kindly provided by J.D. Boeke) into the *Eco*RV and *Bam*HI sites of pBS-SK⁺. The *Sac*I polylinker site of pILBS1, 2, and 3 was converted into an *Hind*III site after T4 DNA polymerase treatment and ligation of the extremities with a *Hind*III linker to create respectively pILBS1H, 2H and 3H plasmids.

Insertion of Ty1 and Ty2 NA portions upstream from the (UAS-less) *TDH3::lacZ* reporter gene into pIL5

Plasmids pIL51A, pIL13, pIL11 and pIL14 depicted in Fig. 1 were constructed by ligation of the *Hind*III–*Bam*HI fragments respectively from pIL301, pIL301ΔBgS, pIL301ΔP and pIL301ΔBgSBII with the pIL5 vector digested with *Hind*III and *Bam*HI. Vector pIL5 was linearized with *Hind*III, treated with T4 DNA polymerase and restricted with *Bam*HI before independent ligations with the *Hpa*I–*Bam*HI, *Pvu*II–*Bam*HI, and *Bst*XI–*Bam*HI from respectively, pIL301ΔP, pIL301ΔP and pIL301 to create pIL10, pIL12 and pIL20 plasmids. Fragments *Hind*III–*Hpa*I and *Pvu*II–*Bam*HI from pIL301 were ligated together with the pIL5 vector digested with *Hind*III and *Bam*HI to create pIL8. Fragments *Hind*III–*Pvu*II from pIL301 and *Xho*I (blunt-ended by T4 DNA polymerase)–*Bam*HI from Ty1-IL15 (26) were ligated together to the *Hind*III and *Bam*HI ends of pIL5 to construct pIL9. Plasmid pIL7 was constructed by the association of the *Hind*III–*Xho*I Ty2 (3.8 kb) segment from Ty2-pJEF1510 with the *Xho*I–*Bam*HI fragment from Ty1-IL15 into the pIL5 vector digested with *Hind*III and *Bam*HI. Fragment *Pvu*II–*Bam*HI from pIL7 was ligated to the *Hind*III (blunt-ended with T4 DNA polymerase) and *Bam*HI ends of pIL5 to give pIL19. Plasmids pIL32, pIL30, pIL35, pIL37, pIL41 and pIL42 depicted in Figures 2 and 3 were constructed by insertion of the *Hind*III–*Hind*III fragments, respectively, from pILBS1H, pILBS2H-PAE2-6 and pILBS3H-PAE1-6 into the *Hind*III linearized pIL5 vector. Integrity, orientation and copy number of the inserted sequences were checked by sequencing the insert

from position –114 inside the promoter using a synthetic oligonucleotide as primer (5'-CAATCAATACCTACCGT-CTTTATATACTTATTAG-3').

Construction of the *LexA::TEC1* fusion

Construction of in-frame *LexA::TEC1* gene fusion between the 5'-terminal part of the *E. coli LexA* ORF (encoding the DNA binding domain) and *TEC1* coding sequence at the level of the 9th codon was achieved in two steps. Firstly, the *TEC1 Sac*I–*Hind*III (1.9 kb) restriction fragment from pILDN478 (26) was blunt-ended with T4 DNA polymerase and inserted at the *Xho*I site of pQH51, blunt-ended with Klenow enzyme (40). In-frame fusion of the insert was checked by sequencing the junctions between the *TEC1* and the *LexA* sequences. Secondly, the *Bam*HI–*Bam*HI segment containing *ADH1* promoter–*LexA::TEC1* gene fusion was removed from this plasmid and inserted into the *Bam*HI site of the multicopy YEp13 plasmid to construct the pIL *LexA::TEC1* plasmid (pIL50).

β-Galactosidase assays

β-Galactosidase assays were performed with cellular extracts prepared using the French press. Plasmid bearing yeast transformants were grown to exponential phase on minimal medium. After centrifugation, the pellet from a 100 ml culture was resuspended in 5 ml Z buffer (41). After French press lysis, cells were centrifuged at low speed to eliminate cellular debris and assayed according to Miller (41). β-Galactosidase specific activity is expressed in nmole *O*-nitrophenyl-β-D-galactopyranoside hydrolyzed/min × mg protein. For each plasmid, two independent transformants were analysed and at least three assays per transformant were performed. Proteins were determined by the Folin method.

RESULTS

Ty1 sequences homologous to SV40 core enhancer are dispensable for *TEC1*-dependent transcription activation

To identify the sequences required for *TEC1*-dependent transcription activation we used the Ty1 element isolated from Ty-pIL16, described previously (26). This ROAM mutation consists of a Ty1 element inserted upstream the TATA box of the *TDH3* promoter, at nt –178 from the *TDH3* ATG codon. *LacZ* coding sequence was fused downstream of the *TDH3* ATG codon, and Ty1-*TDH3::lacZ* sequences were transferred into the low-copy number plasmid YCp50 (see Materials and Methods and Figure 1). Deletions inside the Ty1 element have been constructed as described in Materials and Methods (see Figure 1). All these plasmids were introduced in *TEC1,ura3* (01921c) haploid and diploid strains and in a *tec1::ura3* (01921c TD3F4) mutant strain.

The level of β-galactosidase produced in the absence of Ty1 element (pIL5) is very low (see Figure 1). The first 2150 nucleotides of Ty1 (pIL51A) are sufficient to ensure activation of the adjacent gene. This activation is reduced in a *tec1* mutant strain and in a wild-type diploid *MATa/MATα* strain (see Figure 1). All the deletions which do not remove the 97 bp *Pvu*II–*Sau*3A DNA fragment containing the SRE region defined by Errede and collaborators as the target of the STE12 protein (11) do not significantly affect the expression of the *TDH3::lacZ* reporter gene and the response to *tec1* mutation and mating type control (see Figure 1). Indeed, the activation of β-galactosidase synthesis is reduced in a *tec1::ura3* mutant strain and in a wild-

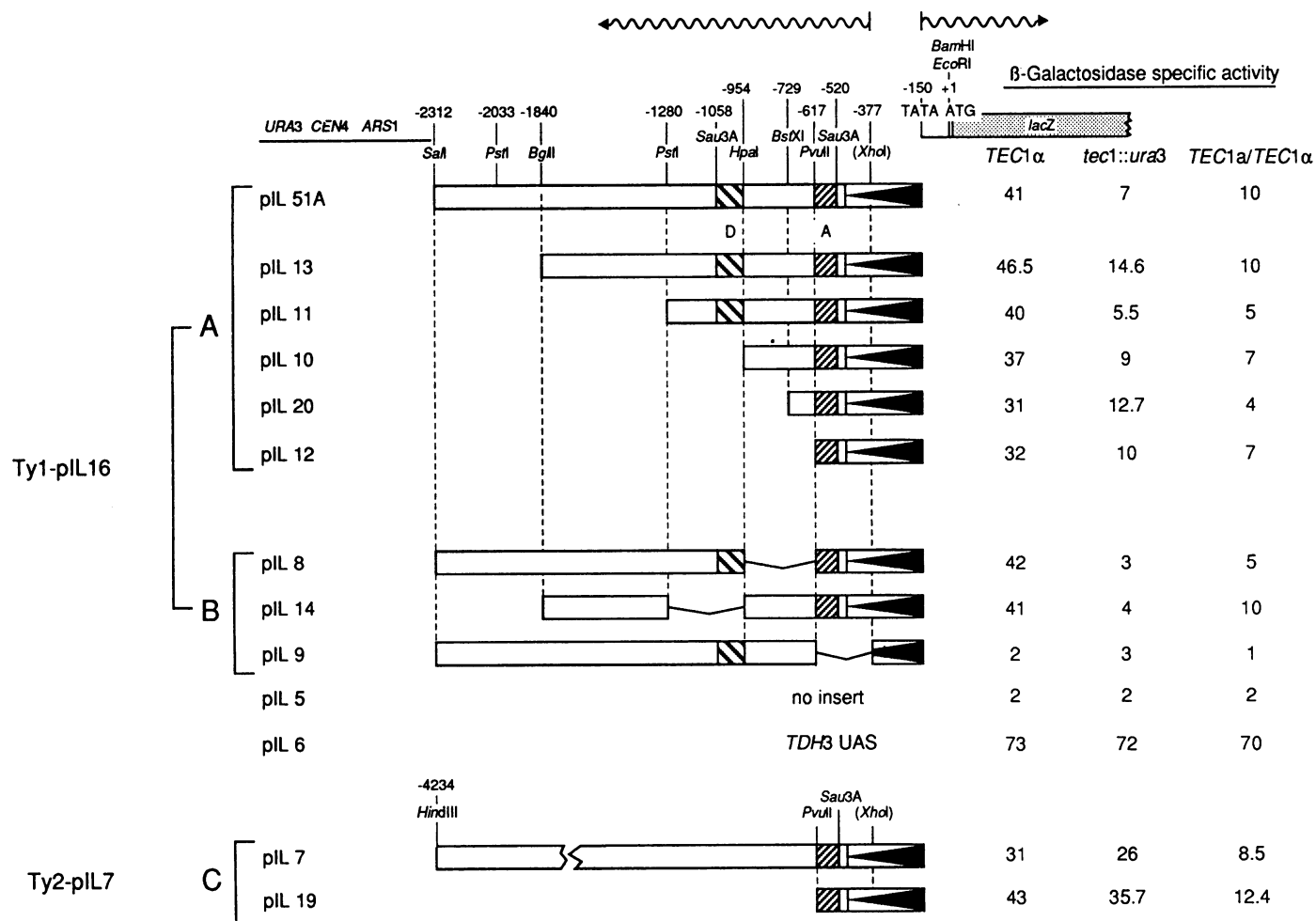


Figure 1. Effects of 3' and internal deletions inside Ty1 element inserted upstream the *TDH3::lacZ*. Panel A: Plasmids containing 3' deletions in Ty1. Panel B: Plasmids containing internal deletions in Ty1. Panel C: Plasmids containing 3' deletions in Ty2. The construction of the different plasmids is described in Materials and Methods. The waved arrows represent Ty and *lacZ* mRNAs. The black triangles represent the 5' LTR sequence of Ty. The hatched boxes represent the A region containing the SRE element (thin bars) and the D region containing the block II element homologous to SV40 enhancer (thick bars). The broken lines represent the extent of the internal deletions. The numbers indicate the positions of the restriction sites in Ty sequence. β-Galactosidase specific activities in various transformant strains grown on minimal medium are presented on the right-hand side. β-Galactosidase specific activities are expressed in nmoles *O*-nitrophenyl-β-D-galactopyranoside hydrolysed/min × mg protein.

Table 1. Activation ability of the LexA-TEC1 protein

| | | β-Galactosidase specific activity |
|--------|--|-----------------------------------|
| pIL50 | <i>LexA::TEC1</i> + reporter plasmid | 7 |
| pAII96 | <i>LexA::ARGRII</i> + reporter plasmid | 2,250 |
| p1840 | reporter plasmid bearing the <i>GAL1::lacZ</i> fusion gene | 4 |

The strain 02463d (*leu2, ura3*) was cotransformed with the reporter plasmid p1840 and plasmids carrying the *LexA::TEC1* and *LexA::ARGRII* fusion constructs. The *LexA::ARGRII* plasmid was used as positive control. The same strain was transformed with p1840 only as negative control.

The *LexA::TEC1* construction is described in Materials and Methods, the *LexA::ARGRII* construction is described in (40). β-Galactosidase specific activity is expressed in nanomoles of *O*-nitrophenyl-β-D-galactopyranoside hydrolyzed per minute per milligram of protein.

type diploid strain. In contrast the deletion of the *PvuII-XhoI* region (pIL9 in Table 1) leads to the loss of activation of the adjacent gene.

So, it is obvious that the sequence homologous to the SV40 core enhancer (region D) is not necessary to activate the expression of the adjacent gene in a *TEC1*-dependent manner and to ensure the mating type regulation. In a wild-type diploid

strain the decrease of β-galactosidase synthesis is likely to be the result of the reduction of *STE12* expression.

The SRE element is sufficient for *TEC1*-dependent transcription activation

To study the effects of region D and SRE elements on gene expression, we have inserted the 97 bp *PvuII-Sau3A* DNA

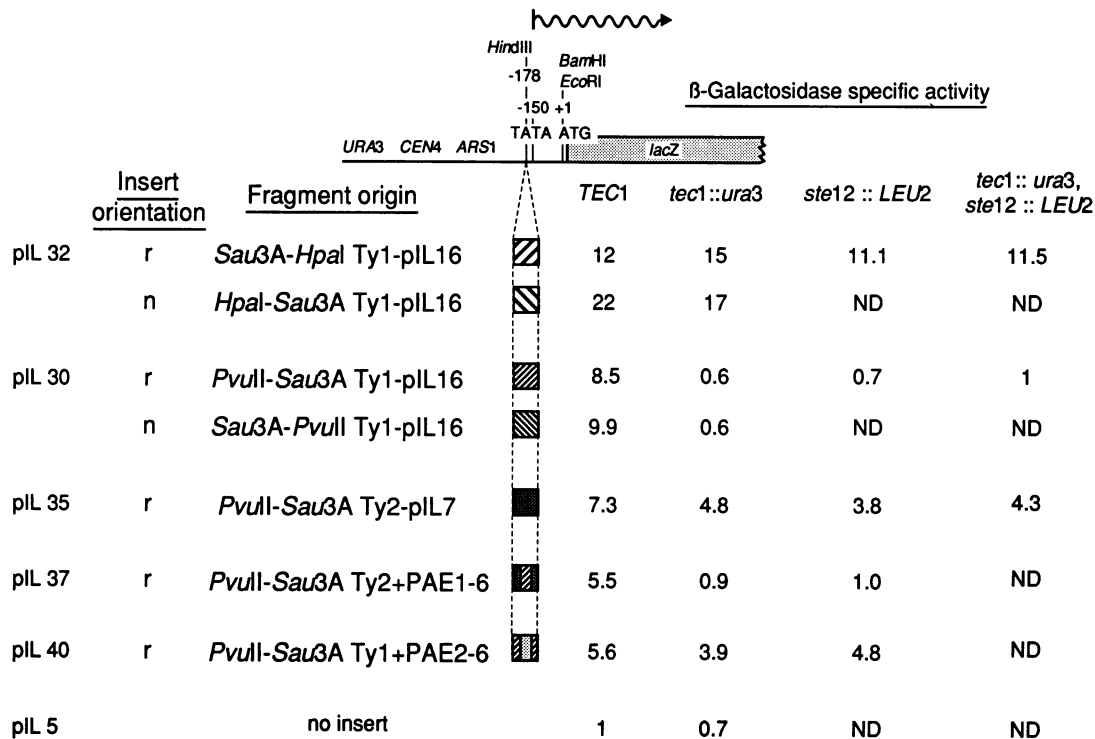


Figure 2. Effects of insertion of different portions of Ty1 and Ty2 in a UAS-less *TDH3* promoter fused to *E. coli lacZ* gene on expression of this reporter gene. The boxes represent the fragments listed on the left-hand side which are inserted in the unique *HindIII* restriction site of the plasmid pIL5 (see Materials and Methods). The 'r' orientation corresponds to the orientation of Ty in a ROAM mutation while the non-ROAM orientation is designated 'n'. β-Galactosidase specific activities in various transformant strains grown on minimal medium are presented on the right-hand side. Amounts of β-galactosidase are the average of at least 3 determinations on 2 independent transformation isolates for each plasmid. β-Galactosidase specific activity is expressed in nmoles *O*-nitrophenyl-β-D-galactopyranoside hydrolysed/min × mg protein. ND means not determined.

fragment containing the SRE element (pIL30) and the 104 bp *Sau3A-HpaI* DNA fragment containing the region D (pIL32) in the *HindIII* restriction site located at 28 bp upstream from the TATA box in the UAS-less *TDH3::lacZ* gene (see Figure 2 and Materials and Methods). The fragments were inserted in both orientations and we have also inserted the *PvuII-Sau3A* fragment from a Ty2 element (pIL35).

These plasmids were introduced in a *leu2 ura3* strain (02463d), in a *leu2, ura3, tec1::ura3* strain (02501c) and in both strains disrupted for *STE12* gene. The disruption of *STE12* by insertion of *LEU2* gene is described in Materials and Methods.

The three regions (Ty1 SRE, Ty2 SRE and Ty1 region D) inserted in the UAS-less *TDH3* promoter are able to activate the expression of the *lacZ* reporter gene (see Figure 2). The difference in β-galactosidase levels induced with the first 2000 nucleotides (Figure 1) and only with the SRE or the region D (Figure 2) is mainly due to a difference in the genetic backgrounds of the strains. Indeed, the plasmid pIL30, when introduced in the strain 01921c (*ura3*), leads to an activation of 22 units β-galactosidase instead of 8 in the non-isogenic strain 02463d (*ura3 leu2*).

This activation by SRE element and region D is orientation independent as for classical enhancers (see Figure 2). Only the Ty1 SRE activates transcription in a TEC1-dependent manner. We observe a reduction of *lacZ* expression to its basal level in the *tec1::ura3* mutant, and also in the *ste12::LEU2* mutant. There is no cumulative effect in the double mutant *tec1 ste12* but it is difficult to conclude whether the two act independently or not

because the β-galactosidase level is the same as in a strain transformed with the control promoter without insert (pIL5).

Ty2 SRE and Ty1 region D seem to have approximately the same activation capacity as Ty1 SRE. However this activation does not require the TEC1 gene product. The absence of *ste12* mutation effect on activation by region D is expected according to Errede's results. There is only a slight reduction of activation by Ty2 SRE in the *ste12* mutant although the two SRE sequences contain the same PRE element which seems to be the target of the STE12 protein. However there are nucleotide changes in a region adjacent to PRE which is also required for complex formation between STE12 and Ty1 SRE (see below).

Identification of nucleotides involved in TEC1-dependent transcription activation

We had previously shown that *tec1::URA3* disruption does not lead to a significant reduction of the total Ty2 transcript level (26). Moreover Ty2 SRE element activates expression of the reporter gene in a TEC1-independent manner (see Figs 1 and 2). So TEC1 does not seem to be required for Ty2 and its adjacent gene expression.

We have compared the nucleotide sequences of the *Sau3A-PvuII* DNA fragment containing the Ty1 SRE with the same fragment containing the Ty2 SRE (see Figure 3). The PRE sequence (TGAAACG) which is the target of STE12 in *STE2* promoter, is present in Ty1 and Ty2 SRE elements. In the case of *STE2*, PRTF is present in the complex formed between STE12 and *STE2* UAS (19) while in the case of the Ty1 regulatory

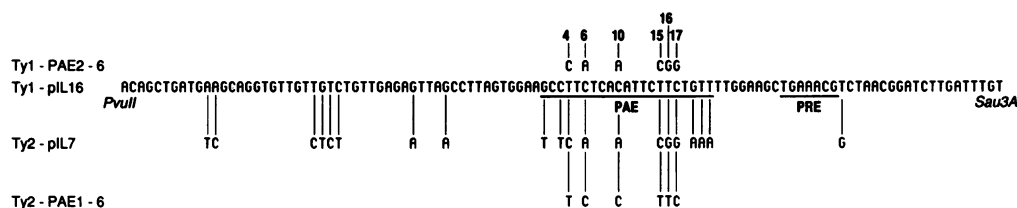


Figure 3. Comparison of nucleotide sequences of *PvuII*–*Sau3A* DNA fragments from Ty1 and Ty2. PRE means pheromone response element. PAE means PRE adjacent element in which most of the nucleotide changes between Ty1 and Ty2 are located. Ty2 PAE1-6 contains the *PvuII*–*Sau3A* fragment from Ty2 in which 6 nucleotides are replaced by the corresponding ones of Ty1. Ty1 PAE2-6 contains the *PvuII*–*Sau3A* fragment from Ty1 in which 6 nucleotides are replaced by the corresponding ones of Ty2. The numbers indicate the positions of nucleotides in PAE which are changed by *in vitro* mutagenesis.

element, the factor proposed to interact with STE12 has not been identified (11). However sequences in addition to the PRE are essential for complex formation (18). Most of the nucleotide changes between Ty1 and Ty2 SRE are precisely located in this region adjacent to the PRE sequence that we named PAE for PRE (P) adjacent (A) element (E). In this sequence (GCCTTCTCACATTCTTCTGTT) there are 11 mismatches on 21 nucleotides (see Figure 3).

We performed two *in vitro* mutageneses using synthetic oligonucleotides (see Materials and Methods) to replace in that region some nucleotides of Ty1 by those of Ty2 in Ty1 SRE element and the nucleotides of Ty2 by the corresponding nucleotides of Ty1 in a Ty2 SRE element (see Figure 3). The chosen nucleotides seemed to be involved in the formation of the STE12-dependent protein complex with Ty1 SRE (18). The 6 nucleotides (C₄ → T, A₆ → C, A₁₀ → C, C₁₅ → T, C₁₆ → T, G₁₇ → C) of Ty1 introduced in the Ty2 background (pIL37 plasmid) were sufficient to allow a TEC1-dependent activation (see Figure 2). In contrast, the substitutions of 6 nucleotides (T₄ → C, C₆ → A, C₁₀ → A, T₁₅ → C, T₁₆ → C, C₁₇ → G) in Ty1 by the corresponding nucleotides of Ty2 (pIL40) strongly reduced the effect of a *tec1* mutation (see Figure 2). Moreover in this last construction (pIL40) the response to *ste12* mutation was also reduced. It is worth noting that although the different nucleotide substitutions modify the response to TEC1 and STE12, they do not affect significantly the level of activation (see Figure 2 and Discussion).

Analysis of the TEC1 ability to activate transcription

To determine if TEC1 protein is able to activate transcription, we constructed a gene encoding the DNA binding region of the bacterial *LexA* repressor fused to the *TEC1* gene (see Materials and Methods). This technique was used to define transcription activating regions in several regulatory proteins (42, 43). This gene which encodes a protein called LexA–TEC1 was carried on a *LEU2-2μ* yeast expression plasmid. A *leu2*, *ura3* strain (02463d) and a *leu2*, *ura3*, *tec1::ura3* (02501a) strain were transformed with this plasmid and with another plasmid that contained a target gene, a *GAL1::lacZ* fusion construct that carried an upstream *LexA* operator (p1840). Expression of the target *GAL1::lacZ* gene was measured by assaying the amounts of β-galactosidase activity produced by cultures of the strains containing these two plasmids. According to the β-galactosidase assays, the LexA–TEC1 protein is not able to stimulate transcription of a *GAL1::lacZ* gene whose upstream activation site had been replaced with a single *LexA* operator (see Table 1). However, the chimeric LexA–TEC1 protein is functional

| Origin of SRE | Sequences | <i>TEC1</i> and <i>STE12</i> - dependent activation |
|---------------|---------------------------------|---|
| Ty1 | GCCTTCTCACATTCTTCTGTT | + |
| Ty1-PAE2-6 | <u>GCCTTATCAA</u> ATTCCGGTGT | — |
| Ty2 | TCTCTATCAAATTCGGTAAA | — |
| Ty2-PAE1-6 | TCTTTC <u>TCACATTCTTCT</u> TAAA | + |

Figure 4. Nucleotides of PAE involved in TEC1 and STE12-dependent transcriptional activation. + Means that activation of adjacent gene expression requires TEC1 and STE12 integrity. — Means that activation of adjacent gene expression is independent on TEC1 and STE12. The modified nucleotides are underlined.

in the cell since it can complement a *tec1::ura3* mutation. *Tec1* mutation suppressed overproduction of the *DUR2,1* gene product resulting of Ty1 insertion in front of the *DUR2,1* gene (*durO^h* mutation) (5). The plasmid coding for LexA–TEC1 protein introduced in the *durO^h tec1::ura3* mutant strain restores the overexpression of the *DUR2,1* gene.

DISCUSSION

Ty insertion mutations activate adjacent gene expression under control of yeast cell type. Moreover, this activation requires the production of STE12 and TEC1 proteins. Two regions of Ty1 have been identified as important for the STE and a/α regulation. According to Errede and her collaborators, region A includes a STE12-dependent activator of reporter gene expression (11) and region D functions as an enhancer in the context of other Ty1 sequences (13). Region D as well as region A cause repression of gene expression in diploid cells. The target of TEC1 protein has not been identified, but the fact that TEC1 shows homology with TEF1 which is a protein binding to sequences in the SV40 enhancer core, suggested that block II in region D might be the target of TEC1 (27).

In this report we have defined the sequences required for TEC1 control of transcription activation using a UAS-less *TDH3::lacZ* reporter gene expression system in which we have inserted different portions of the Ty1 and Ty2 elements. All insertion and deletion experiments allowed us to show that the SRE element is sufficient for the TEC1-dependent activation. The SV40 a/α diploid control element showed no TEC1-dependent activation.

Most of our data are in agreement with the results presented by Errede *et al.* (13) although we observe an activation of the

adjacent gene with only one copy of the block II. In contrast to Kingsman's group results (14), the SRE region and the block II can activate transcription in an orientation independent manner in our reporter system.

We have also shown that Ty2 SRE does not require TEC1 gene product to stimulate expression of the adjacent genes. Most of the nucleotides changes between Ty1 SRE and Ty2 SRE are located in a region identified by Errede as essential for complex formation between STE12, an unknown factor and Ty1 regulatory element (18, 11). The replacement of six of these nucleotides in Ty2 SRE by the corresponding nucleotides of Ty1 confers the ability to activate in a TEC1-dependent manner but there is no increase of activation. On the contrary, Ty1 SRE in which 6 nucleotides are replaced by the Ty2 nucleotides activates independently of TEC1 and even in the absence of STE12 protein. The base substitutions in Ty1 SRE do not affect significantly the level of activation. In Figure 4 we summarize the different PAE sequences with their abilities to control the TEC1 and STE12-dependent transcription activation. These results show the involvement of the PAE region in the activation of the adjacent gene expression. The Ty1 PAE region is required for the STE12 and TEC1 control of gene transcription whereas Ty2 PAE region could activate in the absence of TEC1 and STE12 proteins. The swapping experiment presented simply shifts from one situation to another. PAE in Ty1 could be a positive regulatory site requiring TEC1 and acting together with the PRE STE12-binding site. PAE in Ty2 would also be a positive regulatory element but functionally independent of TEC1 and the PRE STE12-binding site. In this model, TEC1 would directly or indirectly participate in *trans* in the positive regulatory role of Ty1 PAE. According to Baur *et al.* (18), an unknown factor would bind cooperatively with STE12 to the SRE region and the footprint analysis of the protein/SRE complex showed that the PRE and an adjacent sequence element are protected. PAE might represent the binding site for such an accessory factor and it is not excluded that this factor might be TEC1. A comparative study of gel retardation assays performed with Ty1 and Ty2 SRE and the different substituted elements could test the ability of TEC1 protein to bind PAE in the presence or the absence of STE12.

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REFERENCES

1. Warmington, J.P., Warring, R.B., Newlov, C.S. Indye, K.J. and Oliver, S.G. (1985) *Nucleic Acids Res.*, **13**, 6679–6693.
2. Simchen, G., Winston, F., Styles, C.A. and Fink, G.R. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2431–2434.
3. Eibel, H. and Philippsen, P. (1984) *Nature*, **307**, 386–388.
4. Degols, G., Jauniaux, J.C. and Wiame, J.M. (1987) *Eur. J. Biochem.*, **165**, 289–296.
5. Dubois, E., Jacobs, E. and Jauniaux, J.C. (1982) *EMBO. J.*, **1**, 1133–1139.
6. Errede, B., Cardillo, T.S., Sherman, F., Dubois, E., Deschamps, J. and Wiame, J.M. (1980) *Cell*, **25**, 427–436.
7. Lemoine, Y., Dubois, E. and Wiame, J.M. (1978) *Mol. Gen. Genet.*, **166**, 251–258.
8. Williamson, V.M., Young, E.T. and Ciriacy, M. (1981) *Cell*, **23**, 605–614.
9. Errede, B., Cardillo, T.S., Wever, G. and Sherman, F. (1981) *Cold Spring Harbor Symp. Quant. Biol.*, **45**, 593–902.
10. Fields, S., Chaleff, T.D. and Sprague, G.F.Jr. (1988) *Mol. Cell. Biol.*, **8**, 551–556.
11. Company, M., Adler, C. and Errede, B. (1987) *Mol. Cell. Biol.*, **8**, 2545–2554.
12. Company, M. and Errede, B. (1987) *EMBO.J.*, **1**, 1133–1140.
13. Errede, B., Company, M. and Hutchinson III, A. (1987) *Mol. Cell. Biol.*, **7**, 258–265.
14. Rathjen, P.D., Kingsman, A.J. and Kingsman, S.M. (1987) *Nucleic Acids Res.*, **15**, 7309–7324.
15. Company, M. and Errede, B. (1988) *Mol. Cell. Biol.*, **8**, 5299–5309.
16. Errede, B. (1993) *Mol. Cell. Biol.*, **13**, 57–62.
17. Yu, G. and Fassler, J.S. (1993) *Mol. Cell. Biol.*, **13**, 67–71.
18. Baur, H., Jachning, J. and Errede, B. (1991) *Yeast Genetics and Molecular Biology Meeting San Francisco*.
19. Errede, B. and Ammerer, G. (1989) *Genes and Dev.*, **3**, 1349–1361.
20. Liao, X., Clare, J.J., and Farabaugh, P.J. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 8520–8524.
21. Roeder, G.S., Rose, A.B. and Pearlman, R.E. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 5428–5432.
22. Farabaugh, P., Liao, X.B., Belcourt, M., Zhao, H., Kapakos, J. and Clare, J. (1989) *Mol. Cell. Biol.*, **9**, 4825–4834.
23. Winston, F., Chaleff, D.T., Valent, B. and Fink, G.R. (1984) *Gene*, **107**, 179–197.
24. Ciriacy, M. and Williamson, V.M. (1981) *Mol. Gen. Genet.*, **182**, 159–163.
25. Ciriacy, M., Freidel, K. and LTMhning, C. (1991) *Current Genetics*, **20**, 441–448.
26. Laloux, I., Dubois, E., Dewerchin, M. and Jacobs, E. (1990) *Mol. Cell. Biol.*, **10**, 3541–3550.
27. Burglin, T.R. (1991) *Cell*, **66**, 11–12.
28. Xiao, J.H., Davidson, I., Matthes, H., Garnier, J.M. and Chambon, P. (1991) *Cell*, **65**, 551–568.
29. Ito, H., Fukura, Y., Murata, K. and Kimura, A. (1983) *J. Bacteriol.*, **153**, 163–168.
30. Hinnen, A., Hicks, J.B. and Fink, G.R. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1929–1933.
31. Sherman, F., Fink, G.R. and Hicks, J.B. (1986) *Methods in Yeast Genetics*. Cold Spring Harbor University Press, Cold Spring Harbor, NY.
32. Cohen, S.N., Chiang, A.C.Y. and Hiu, L. (1972) *Proc. Natl. Acad. Sci. USA*, **69**, 2110–2114.
33. Birnboim, H.C. and Doly, J. (1979) *Nucleic Acids Res.*, **7**, 1513–1523.
34. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor University Press, Cold Spring Harbor, NY.
35. Sayers, J.R., Schmidt, W. and Eckstein, F. (1988) *Nucleic Acids Res.*, **16**, 791–802.
36. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **77**, 5463–5467.
37. Chen, E.J. and Seeburg, P.H.. (1985) *DNA*, **4**, 165–170.
38. Myers, A.M., Tzagoloff, A., Kinnuz, D.H. and Lusty, C.J. (1986) *Gene*, **45**, 299–310.
39. Bitter, G.A. and Egan, K.M. (1984) *Gene*, **32**, 263–274.
40. Qiu, H.F., Dubois, E. and Messenguy, F. (1991) *Mol. Cell. Biol.*, **11**, 2169–2179.
41. Miller, J. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor University Press, Cold Spring Harbor, NY.
42. Brent, R. and Ptashne, M. (1985) *Cell*, **43**, 729–736.
43. Hope, I.A. and Struhl, K. (1986) *Cell*, **46**, 885–894.