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TSF1 to *TSF6*, Required for Silencing the *Saccharomyces cerevisiae GAL* Genes, Are Global Regulatory Genes

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ABSTRACT

The *Saccharomyces cerevisiae GAL1* and *GAL10* genes are controlled in response to the availability of galactose and glucose by multiple activating and repressing proteins bound at adjacent or overlapping sites in UAS_G. Negative control elements in UAS_G, designated *GAL* operators $GALO_1$ to $GALO_6$, are required to silence basal level transcription of *GAL1* and *GAL10* when galactose is absent. We isolated and characterized recessive mutations in six nuclear genes, *TSF1* to *TSF6*, that impair silencing of *GAL1* and *GAL10* gene expression. Surprisingly, the results of several experiments suggest that the *TSF* genes encode global regulatory factors. *tsf1* to *tsf6* mutations derepressed expression from yeast *CYC-GAL* hybrid promoters (fused to *lacZ*) that harbor a variety of operator sequences, and caused pleiotropic defects in cell growth, mating, and sporulation. S1 mapping and Northern blot results for *tsf3* suggest that the molecular defect is at the transcriptional level. Mutant phenotypes were additive in certain combinations of *tsf* double mutants, implying that more than one silencing pathway is involved in *TSF1* to *TSF6* function. Most significantly, mutations in all six *TSF1* to *TSF6* genes activated expression from *GAL1* and *CYC1* promoters (fused to *lacZ*) lacking upstream activating sequences. Combined, the simplest interpretation of these results is that *TSF1* to *TSF6* encode factors that control the function of the basic RNA polymerase II transcriptional machinery.

REGULATION of transcription of eukaryotic genes is a very complex process. Transcriptional control of the *Saccharomyces cerevisiae GAL1* and *GAL10* genes, for example, relies on the coordinate interactions of multiple regulatory proteins that recognize activating and silencing elements in a 365-bp control region designated UAS_G, shown in Figure 1 (Guarente, Yocum and Gifford 1982; Struhl 1985; Johnston 1987; Finley *et al.* 1990; Flick and Johnston 1990, 1992; Nehlin, Carlberg and Ronne 1991). A gene-specific activator protein, GAL4, binds as a dimer molecule cooperatively to four adjacent sites within UAS_G (Giniger and Ptashne 1988; Carey *et al.* 1989). Six repression elements, designated *GAL* operators $GALO_1$ to $GALO_6$, reside adjacent to or overlap the GAL4 binding sites (West *et al.* 1987; Finley and West 1989; Finley *et al.* 1990; Nehlin, Carlberg and Ronne 1991; Flick and Johnston 1992). Finally, two general activating elements, GAE_1 and GAE_2 (GAL4/galactose-independent activating elements), are present at positions adjacent to or overlapping the GAL4 sites and *GAL* operators (Finley and West 1989; Finley *et al.* 1990). Regulatory proteins that recognize these various elements control the amount of *GAL1* and *GAL10* transcription over a range of four orders of magnitude (Yocum *et al.* 1984; West, Yocum and Ptashne 1984; Finley *et al.* 1990).

The regulatory model in Figure 1 suggests that the role of the *GAL* operators is to prevent general regulatory proteins bound at GAE_1 and GAE_2 from activating *GAL1* and *GAL10* transcription in the absence of galactose (*i.e.*, noninducing conditions) (West, Yocum and Ptashne 1984; Finley and West 1989; Finley *et al.* 1990; Finley 1990). Two general regulatory proteins that bind GAE_1 ($GALO_2$) are REB1 (*Y* factor/GRF2/QBP; Brandl and Struhl 1990; Chasman *et al.* 1990; Ju, Morrow and Warner 1990; Wang, Nicholson and Stillman 1990) and BUF (binding URS1 protein) (Luche, Smart and Cooper 1992; T.G. Cooper, personal communication; see also Luche, Sumrada and Cooper 1990; Hollingsworth, Goetsch and Byers 1990; Buckingham *et al.* 1990). Both proteins have been demonstrated to play positive as well as negative transcriptional regulatory roles in *S. cerevisiae* dependent on promoter context, coinciding with the positive and negative regulatory functions previously ascribed to GAE_1 and $GALO_2$, respectively (Finley and West 1989; Finley *et al.* 1990). A third general regulatory protein, GCR1 (Baker 1991), also may bind to GAE_1 since a recog-

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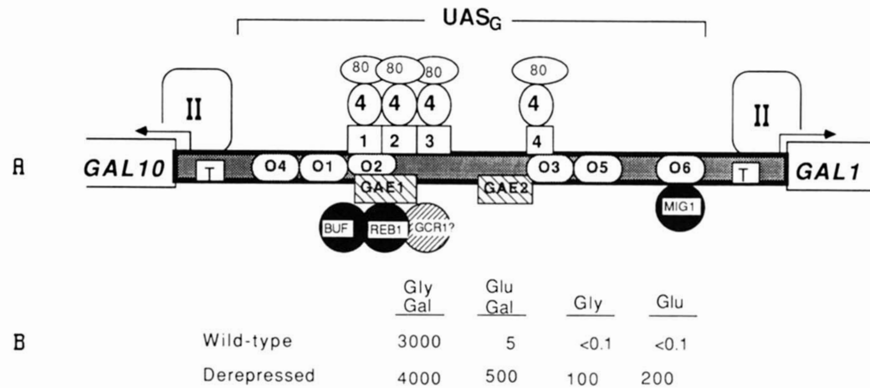

FIGURE 1.—Model of *GAL1* and *GAL10* promoter structure and function. (A) The intact *GAL1-GAL10* divergent promoter region is shown, denoting UAS$_G$ (nomenclature of GUARENTE, YOCUM and GIFFORD 1982), activating elements GAE$_1$, GAE$_2$ and the four *GAL4*-binding sites (rectangles), *GAL* operators O$_1$ to O$_6$ (ovals), and the regulatory proteins GAL4 ("4") and GAL80 ("80"; open ovals). T, TATA box. II, RNA polymerase II basal transcription apparatus. Regulatory proteins BUF (binding URS1 factor), REB1, GCR1 and MIG1 are described in the text. (B) Regulation of *GAL1* transcription: β-galactosidase activities from *GAL1-lacZ* fusions (on 2-μm plasmids) in a *GAL4*$^+$ yeast strain grown on Glu (2% glucose), Glu + Gal (2% glucose plus 2% galactose), Gly (3% glycerol plus 2% lactate), or Gal (2% galactose) medium. "Wild-type," normal *GAL1* promoter activity (plasmid pRY131) in a wild-type yeast strain; "Derepressed," abnormal *GAL1* promoter activity due to *cis*-acting (FINLEY *et al.* 1990) or *trans*-acting (this report) mutations.

nition sequence for this protein (5′-CTTCC-3′) is present adjacent to the REB1 site, overlapping GAL4 binding site 3 (Figure 1). The repressor protein MIG1, which recognizes and appears to be responsible for the activity of operator *GALO*$_6$ (NEHLIN, CARLBERG and RONNE 1991), together with DNA-binding proteins that recognize other *GAL* operators (identified in partially purified yeast crude extracts; FINLEY 1990), are likely to be responsible for inhibiting transcriptional activation by REB1, BUF, and other GAE$_1$- and GAE$_2$-binding proteins.

The repressing activity of the *GAL* operators is constitutive, and though sufficient when galactose is absent to block the relatively weak activating potential of the general regulatory proteins bound to GAE$_1$ and GAE$_2$, is insufficient when galactose is present to inhibit the strong activity of GAL4 dimers bound to the four *GAL4* sites. Interestingly, the *MAT*α2 operator, a well characterized, efficient yeast operator which represses *MAT***a**-specific genes in *MAT*α cells (JOHNSON and HERSKOWITZ 1985; KELEHER, PASSMORE and JOHNSON 1989), is likewise insufficient to block strong GAL4 activity when substituted for *GAL* operators in single or multiple copies in UAS$_G$ (R. W. WEST, JR., unpublished data). To prevent GAL4 from activating transcription of *GAL1* and *GAL10* when galactose is absent, a specific inhibitory protein, GAL80, is obligatory. Galactose or a metabolic derivative releases the GAL80 block on GAL4 activity, thereby allowing induction of *GAL1* and *GAL10* transcription (JOHNSTON 1987; OSHIMA 1991; LEUTHER and JOHNSTON 1992). Inhibition of GAL4 by GAL80 coupled with *GAL* operator-mediated silencing of GAE$_1$ and GAE$_2$ activity creates an extraordinarily effective genetic "off switch." Neither the mechanism of GAL4-GAL80 interaction, nor transcriptional silencing by the *GAL* operators, is well understood.

We wished to further characterize the mechanism of transcriptional silencing of *GAL1* and *GAL10* by the *GAL* operators. For this purpose, we employed a genetic selection scheme where hybrid yeast promoters that harbor specific subsets of *GAL* operators were used to identify mutants having lesions in genes required for *GAL* operator activity. Subsets of *GAL* operators were inserted between UAS$_C$, from the *CYC1* (iso-1-cytochrome *c*; GUARENTE *et al.* 1984) promoter, and a UAS-less *GAL1*, *GAL10* or *CYC1* promoter fused to the *Escherichia coli lacZ* gene. The *GAL* operators prevent activator proteins HAP1 to HAP4 that bind UAS$_C$ (FORSBURG and GUARENTE 1989) from fully inducing promoter activity, so yeast cells containing these hybrid promoters form white instead of blue colonies on X-gal indicator plates (WEST *et al.* 1987; FINLEY and WEST 1989; FINLEY *et al.* 1990). Mutants having lesions in genes required for silencing should be detectable by the appearance of blue colonies. In this fashion, we identified six nuclear genes, *TSF1* to *TSF6*, which appear to encode factors that are required to silence expression of *GAL1*- and *GAL10-lacZ* fusions when galactose is absent. Mutations in two of these genes, *TSF3* and *TSF6*, individually increased expression substantially of a wild-type *GAL1-lacZ* fusion in the absence of galactose or GAL4. Studies with various combinations of *tsf* double mutants showed that the effects of the mutations are additive, suggesting that more than one regulatory pathway is operative. Results are presented which suggest that rather than encoding *GAL* operator-binding proteins, however, *TSF1* to *TSF6* encode global regulatory factors. For mutations in at least one of the six genes, *TSF3*, the molecular defect appears to be solely or primarily transcriptional, and the combined

data suggest that the target of TSF3 function is likely to be the activity of the basic RNA polymerase II basal transcriptional machinery. This view is supported by the fact that *TSF3* was recently shown to be identical to *SIN4* (JIANG and STILLMAN 1992; CHEN *et al.* 1993), a negative transcriptional regulator of mating type function in *S. cerevisiae* (NASMYTH, STILLMAN and KIPLING 1987; STERNBERG *et al.* 1987; NASMYTH and SHORE 1987; HERSKOWITZ 1989).

(*TSF1* to *TSF6* were formerly designated *GAL20* to *GAL25*, respectively; CHEN *et al.* 1991.)

MATERIALS AND METHODS

Strains and plasmids: *S. cerevisiae* strains used in this study are listed in Table 1. YM335 (JOHNSTON and DAVIS 1984) and the congenic $GAL4^+$ strain YM256 were provided by MARK JOHNSTON. *E. coli* strain DH5α was used for routine cloning work. pLGΔ-312, a $URA3^+$ 2-μm plasmid containing a wild-type *CYC1-lacZ* fusion (GUARENTE *et al.* 1984), and pLG670Z (same as above but lacking UAS_C), were gifts of LENNY GUARENTE. Plasmids pRY131 (wild-type *GAL1-lacZ* fusion), pRY133 (wild-type *GAL10-lacZ* fusion), 121-593, pLR1Δ1, pLR1Δ23B (each containing the *GAL1-lacZ* fusion harboring a respective deletion of UAS_G), 121-632 (*GAL1-lacZ* fusion completely lacking UAS_G), 121-688 (*GAL1-lacZ* fusion lacking UAS_G and the *GAL1* TATA box), and 632-37a-1 (single copy of GAE_2 upstream of the TATA box in 121-632) were described previously (YOCUM *et al.* 1984; WEST, YOCUM and PTASHNE 1984; FINLEY *et al.* 1990). Plasmid 1Δ1-37a contains a single copy of GAE_2 (oligonucleotide 37a; FINLEY *et al.* 1990) upstream of the *GAL1-lacZ* fusion in pLR1Δ1, and was a gift of RUSSELL FINLEY. UAS_C-*GAL1*, UAS_C-*GAL10* and UAS_G-*CYC1* hybrid promoters (fused to *lacZ*) were described previously (WEST *et al.* 1987; FINLEY and WEST 1989; FINLEY *et al.* 1990). Plasmid pRY131-I was constructed from pRY131 by deleting the 2241-bp *Eco*RI fragment harboring the yeast 2m origin of replication, and was used to construct strains BWY19 and BWY20 (Table 1) by integrating it into the *URA3* locus of strains YM335 and BWY115, respectively. pBD6 (a gift of JIM YARGER) is a *GAL7-lacZ* fusion plasmid that harbors the *GAL7-GAL10* intergenic region and adjacent sequences (YARGER, GORMAN and POLAZZI 1985). *MATα* derivatives of YM335 and YM256, BWY3 and BWY6 (Table 1), respectively, were obtained following transformation of each strain with YCp-*HO*-12 (a gift of TOM FOX), as described previously (JENSEN, SPRAGUE and HERSKOWITZ 1983). A *PDC1-lacZ* fusion plasmid (pyruvate decarboxylase; BUTLER, DAWES and MCCONNELL 1990) was obtained from GERALDINE BUTLER and DAVID MCCONNELL. An *SSN20*-containing plasmid and an *ssn6* strain were obtained from JANET SCHULTZ and MARIAN CARLSON. A *GAL11/SPT13*-containing plasmid, and an *spt13* strain, were provided by JAN FASSLER. Plasmids containing the *SIN1*, *SIN2*, *SWI1*, *SWI2* (*SNF2*) and *SWI3* genes were provided by CRAIG PETERSON, WARREN KRUGER and IRA HERSKOWITZ. A *cyc9* (*tup1*) strain was obtained from FRED SHERMAN. A *SIN3*-containing plasmid, and *sdi4* and *sdi2* strains, were obtained from DAVID STILLMAN. *SSN6*- and *TUP1*-containing plasmids were obtained from BOB TRUMBLY. *SPT5*-, *SPT10*- and *SPT11/SPT12*-containing plasmids were provided by FRED WINSTON. A *SIT3*-containing plasmid was provided by ANNE SUTTON and KIM ARNDT.

Media and chemicals: *S. cerevisiae* cells were routinely grown in YEP (1% yeast extract, 2% peptone) medium containing 2% glucose (YEP-D). Selection media for yeast transformation or for assaying β-galactosidase activity were synthetic complete (SC) medium (0.67% yeast nitrogen base without amino acids) containing either 3% glycerol and 2% lactate (Gly medium), 2% galactose plus 3% glycerol and 2% lactate (Gal medium), or 2% glucose (Glu medium). Ethyl methanesulfonate (EMS) and *O*-nitrophenyl-β-D-galactopyranoside (ONPG) were obtained from Sigma. The chromogenic dye 5′-bromo-4′-chloro-3′-indolyl-β-D-galactoside (X-gal) was purchased from Boehringer-Mannheim.

Yeast transformation and β-galactosidase assays: Yeast were transformed using the spheroplast technique (SHERMAN, FINK and HICKS 1986). Transformants were selected on SC (lacking uracil) agar plates containing 2% glucose. Determination of β-galactosidase activity in individual transformants was performed as described previously (FINLEY *et al.* 1990) and values are given in units, according to MILLER (1972). Multiple identical constructs were tested simultaneously, individual samples were analyzed in duplicate in each experiment, and the results of multiple independent determinations (at least four) performed on different days were averaged. Error was less than 20%. Values lower than 0.02 unit were not determined with precision.

Mutant isolation: For mutant isolation, YM335 cells containing *CYC1*-UAS_G-110A, *CYC1*-UAS_G-150, UAS_C-*GAL1-1* and UAS_C-*GAL10-6* were used (WEST *et al.* 1987; FINLEY and WEST 1989; FINLEY *et al.* 1990; see Figure 2). YM335 cells harboring *CYC1*-UAS_G-110A or *CYC1*-UAS_G-150 and grown in Glu medium are repressed 30-fold and 100-fold, respectively, compared to the wild-type *CYC1* promoter, and thus produce white colonies on X-gal Glu plates (corresponding to 1.5–5 units of β-galactosidase activity). YM335 cells harboring plasmid UAS_C-*GAL1-1* or UAS_C-*GAL10-6* and grown in Gly medium are repressed 2300-fold and 450-fold, respectively, compared to the analogous hybrid promoters lacking *GAL* operators, UAS_C-*GAL1-8* and UAS_C-*GAL10-8* (WEST *et al.* 1987), and thus produce white colonies on X-gal Gly plates (0.6 and 0.4 unit of β-galactosidase activity, respectively; WEST *et al.* 1987). The respective strains containing plasmids were mutagenized with EMS to about 75% survival (LOWRY and ZITOMER 1984; SHERMAN, FINK and HICKS 1986), and plated directly onto Glu (*CYC1*-UAS_G-110A and *CYC1*-UAS_G-150) or Gly (UAS_C-*GAL1-1* and UAS_C-*GAL10-6*) minimal selective agar plates containing X-gal. Seventy blue colonies were picked after screening a total of approximately 100,000 colonies, streak purified at least twice on selective medium and tested on two or more separate occasions for production of β-galactosidase. Colonies whose production of β-galactosidase was reproducibly derepressed by a factor of at least 2-fold compared to wild-type were characterized further. Mutations were identified as acting in *trans* after segregating the original plasmids (strains were grown in the nonselective medium YEP-D for approximately 20 generations) followed by transforming the segregants with the respective unmutagenized plasmids. *tsf1* mutants were obtained using UAS_C-*GAL10-6*, *tsf2*, *tsf3*, and *tsf4* mutants using *CYC1*-UAS_G-110A, *tsf5* and *tsf6-1* using *CYC1*-UAS_G-150, and *tsf6-2* using UAS_C-*GAL1-1* (see Table 2).

Genetic analysis: Genetic procedures were performed as described by SHERMAN, FINK and HICKS (1986) and GUTHRIE and FINK (1991). For determining complementation, it was first necessary to obtain isogenic *MATα leu2* derivatives of each mutant strain. This was achieved by crossing each mutant strain with wild-type strain BWY6 (*MATα leu2::HIS3* $GAL4^+$; see Table 1), and dissecting tetrads from the respective heterozygous diploids. BWY6, in turn, was obtained from YM256 by transforming the latter with YCp-*HO*-12 to

TABLE 1

List of *S. cerevisiae* strains

Strain[a]	Genotype
Haploids	
YM335	*MAT***a** *ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
YM256	*MAT***a** *ura3-52 lys2-801 his3-200 ade2-101 met GAL4⁺*
BWY3	*MATα ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536 leu2::HIS3⁺*
BWY6	*MATα ura3-52 lys2-801 his3-200 ade2-101 met GAL4⁺ leu2::HIS3⁺*
BWY19	*MAT***a** *TSF3⁺ ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536* pRY131I::*URA3⁺*
BWY20	*MAT***a** *tsf3-10 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536* pRY131I::*URA3⁺*
BWY54	*MAT***a** *Δtsf3-2::LEU2 ura3-52 lys2-801 his3-200 ade2-101 met GAL4⁺ leu2::HIS3⁺*
BWY55	*MATα Δtsf3-2::LEU2 ura3-52 lys2-801 his3-200 ade2-101 met GAL4⁺ leu2::HIS3⁺*
BWY100	*MAT***a** *tsf2-1 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY101	*MAT***a** *tsf2-2 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY102	*MAT***a** *tsf4-1 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY104	*MAT***a** *tsf2-5 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY105	*MAT***a** *tsf2-6 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY106	*MAT***a** *tsf2-17 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY107	*MAT***a** *tsf2-18 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY108	*MAT***a** *tsf2-19 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY109	*MAT***a** *tsf2-21 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY110	*MAT***a** *tsf2-22 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY111	*MAT***a** *tsf2-23 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY113	*MAT***a** *tsf3-7 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY115	*MAT***a** *tsf3-10 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY116	*MAT***a** *tsf3-11 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY117	*MAT***a** *tsf3-12 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY118	*MAT***a** *tsf3-13 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY119	*MAT***a** *tsf3-14 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY120	*MAT***a** *tsf3-15 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY122	*MAT***a** *tsf3-24 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY124	*MAT***a** *tsf1-1 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY125	*MAT***a** *tsf1-2 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY126	*MAT***a** *tsf1-3 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY127	*MAT***a** *tsf1-4 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY128	*MAT***a** *tsf1-5 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY132	*MAT***a** *tsf5-1 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 met*
BWY133	*MAT***a** *tsf6-1 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY134	*MAT***a** *tsf6-2 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY135	*MAT***a** *tsf3-27 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536*
BWY136	*MAT***a** *tsf3-10 ura3-52 lys2-801 his3-200 ade2-101 met GAL4⁺*
BWY143	*MATα tsf3-27 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536 leu2::HIS3*
BWY155	*MATα tsf2-5 ura3-52 his3-200 ade2-101 GAL4⁺ leu2::HIS3⁺ met*
BWY158	*MATα tsf2-23 ura3-52 his3-200 ade2-101 GAL4⁺ leu2::HIS3⁺ met*
BWY160	*MATα tsf4-1 ura3-52 his3-200 ade2-101 Δgal4-536 leu2::HIS3⁺ met*
BWY161	*MAT***a** *tsf4-1 ura3-52 his3-200 ade2-101 GAL4⁺ leu2::HIS3⁺ met*
BWY162	*MAT***a** *tsf5-1 ura3-52 his3-200 ade2-101 GAL4⁺ met*
BWY163	*MATα tsf5-1 ura3-52 his3-200 ade2-101 GAL4⁺ leu2::HIS3⁺ met*
BWY164	*MATα tsf6-1 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536 leu2::HIS3*
BWY168	*MATα tsf2-1 ura3-52 his3-200 ade2-101 GAL4⁺ leu2::HIS3 met*
BWY171	*MATα tsf3-7 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536 leu2::HIS3*
BWY173	*MATα tsf3-14 ura3-52 lys2-801 his3-200 ade2-101 met Δgal4-536 leu2::HIS3*
BWY177	*MAT***a** *tsf3-27 ura3-52 lys2-801 his3-200 ade2-201 met GAL4⁺*
BWY178	*MAT***a** *tsf6-2 ura3-52 lys2-801 his3-200 ade2-201 met GAL4⁺*
BWY400	*MATα tsf4-1 Δtsf3-2::LEU2 ura3-52 lys2-801 his3-200 ade2-101 Δgal4-536 leu2::HIS3 met*
BWY402	*MATα tsf2-5 Δtsf3-2::LEU2 ura3-52 lys2-801 his3-200 ade2-101 GAL4⁺ leu2::HIS3 met*
BWY403	*MATα tsf5-1 Δtsf3-2::LEU2 ura3-52 lys2-801 his3-200 ade2-101 GAL4⁺ leu2::HIS3 met*
BWY515	*MATα tsf2-22 ura3-52 his3 ade leu2-3 Δgal4-536 met*
BWY521	*MATα tsf3-13 ura3-52 his3 ade leu2-3 Δgal4-536 lys2-801*
BWY525	*MATα tsf3-11 ura3-52 his3 ade leu2-3 Δgal4-536 lys2-801*
BWY527	*MATα tsf3-12 ura3-52 his3 ade leu2-3 lys2-801 met GAL4⁺*
BWY537	*MATα tsf1-1 ura3-52 leu2-3 GAL4⁺*
BWY538	*MATα tsf1-3 ura3-52 his3 ade leu2-3 GAL4⁺*
BWY540	*MATα tsf1-4 ura3-52 his3 ade leu2-3 lys2-801 Δgal4-536 met*
BWY543	*MATα tsf1-5 ura3-52 ade leu2-3 lys2-801 GAL4⁺*

TABLE 1

Continued

Strain[a]	Genotype
SJ1031-7b	*MAT***a** *tsf3-1j*[b] *ade2 ade6 leu2-3, 112 ura3-52 gal1 trp1 his7*
SJ1031-14c	*MATα tsf3-1j*[b] *ade2 ade6 leu2-3, 112 ura3-52 gal1 trp1*
SJ5899-8a	*MAT***a** *TSF3*$^+$ *ade6 ade2 leu2-3, 112 ura3-52 gal1 trp1 his7*
SJ5899-18d	*MATα TSF3*$^+$ *ade2 lys5 leu2-3, 112 ura3-52 trp1 his7*
DBY745	*MATα ura3-52 leu2-3, 112 his3-Δ1 ade1-101 GAL4*$^+$
Diploids	
BWY5001	YM335 × BWY3
BWY7001	YM335 × DBY745

YM and BWY strains are isogenic, except for BWY515 to 543, and BWY7001. All strains, except YM and DBY strains, originated with this study.

[a] Construction of strains BWY54, 55, 400, 402 and 403 was described in CHEN *et al.* (1993).

[b] *tsf3-1j* was isolated in an independent screen (S. JOHNSON and B. BYERS, unpublished data).

switch the mating type, and by inserting a 1.8-kb *Bam*HI fragment containing the *HIS3* gene into the *LEU2* locus. Thereafter, each original mutant was crossed in pairwise combinations with various other *tsf1* to *tsf6* mutants (see Table 2). Resulting diploids were examined for the characteristic mutant phenotype (derepression of the hybrid promoters, temperature sensitivity, flocculence and abnormal cell morphology). Complementation was manifest as a repressed phenotype (*e.g.*, white colony, non-clumpy, non-temperature sensitive) in a diploid strain derived by mating two derepressed (blue colonies) haploid strains (liquid assays of β-galactosidase activity were performed to corroborate X-gal plate results). Complementation was scored as "+," wild-type phenotype "−," mutant phenotype (Table 2). Genetic linkage was determined by performing tetrad analysis with sporulation products of representative diploids from each complementation set. Parental ditype (PD), non-parental ditype (NPD) and tetratype (T) asci were ascertained by the presence of either 4 out of 4 (PD), 2 out of 4 (NPD), or 3 out of 4 (T) spores (respectively) giving rise to blue colonies on X-gal plates. X-gal plate results were always confirmed by assays for β-galactosidase activity. Linkage was ascertained if the PD:NPD was $>1:<1$ (SHERMAN, FINK and HICKS 1986).

GAL4$^+$ and Δ*gal4* derivatives of *tsf1* to *tsf6* mutants were distinguished following outcrossing by the growth on SC agar plates containing galactose as sole carbon source. Plate results were confirmed by testing for the presence of a 815-bp fragment on a 1% agarose gel following polymerase chain reaction (PCR) amplification corresponding to the N terminus of the *GAL4* gene, using the protocol described in AUSUBEL *et al.* (1990). Two synthetic oligonucleotides of 18 bp (see below) derived from the *GAL4* sequence (LAUGHON and GESTELAND 1984) were used as primers for PCR amplification. Primer I corresponds to nucleotides 880–897 of the *GAL4* sense strand, overlapping the *Cla*I site (LAUGHON and GESTELAND 1984), primer II corresponds to nucleotides 1695–1678 of the *GAL4* nonsense strand, overlapping the *Sal*I site.

Primer I (sense strand): 5′ GTATCGATT-GACTCGGCA 3′

Primer II (nonsense strand): 5′ CTGCACATCGTCGA-CAGA 3′

Mating and sporulation efficiency: Quantitative mating assays were performed according to the method of WILLIAMS and TRUMBLY (1990). Cells (10^6) from a given *MAT***a** mutant strain or its isogenic *MATα* derivative were mixed with 10^7 cells from the wild-type strains BWY6 (*MATα*) or YM256 (*MAT***a**), respectively. Diploids were selected on minimal diploid selection plates (SC medium lacking histidine and leucine) and counted. Mating efficiency was expressed as the fraction of diploids obtained from mutants *vs.* wild-type strains. To measure sporulation efficiency, we obtained homozygous diploids for each representative mutant by crossing each original mutant with its corresponding isogenic *MATα*-derivative. Approximately 10^4 homozygous diploid cells subjected to sporulation conditions for 7 days were examined (500 per ocular field), and the number of asci present was calculated. Sporulation efficiency was expressed as the fraction of sporulated cells present in mutant *vs.* wild-type strains (see Table 5). Subsequent revival of sporulation products on rich medium plates showed that the number of colonies formed was proportionate to the fraction of tetrads observed by microscopy.

Methionine feeding indicator assay: The procedure to test for excretion of methionine (and/or a biosynthetic intermediate) from *tsf1* to *tsf6* strains was adapted from the method of KLINE (1972). Briefly, a 5-ml culture of YM335 (*met*) cells grown to a density of about 10^8 per ml was added to 250 ml of molten (42°) SC agar medium containing glucose and lacking methionine. The seeded medium was poured onto Petri plates and allowed to harden. Thereafter, inocula of fresh *tsf1* to *tsf6* cultures were patched onto the seeded agar plates. The plates were incubated for 3 days at 30° prior to scoring. Feeding was determined by the presence of a halo of growth surrounding a given patch of *tsf1* to *tsf6* inoculum.

Poly(A)$^+$ RNA isolation and Northern blot analysis: Total cellular RNA was prepared by the method of SHERMAN, FINK and HICKS (1986). Poly(A)$^+$ RNA was selected on an oligo(dT) column (Boehringer Mannheim), prepared and used in accordance with the manufacturer's instructions. Other procedures were performed as described in CHEN *et al.* (1993). ^{32}P-Radiolabeled DNA probes were prepared using a random primer labeling kit (Boehringer Mannheim, Inc.) according to the manufacturer's instructions. The *GAL1* probe was a 600-bp *Xho*I-*Bam*HI DNA fragment obtained from plasmid 121-330 (WEST, YOCUM and PTASHNE 1984). The *GAL7* and *GAL10* probe was a 1.6-kb *Bam*HI-*Sal*I fragment obtained from plasmid pBD6 (Yarger, Gorman and POLAZZI 1985). *ACT1* and *MATα2* gene probes were described previously (CHEN *et al.* 1993).

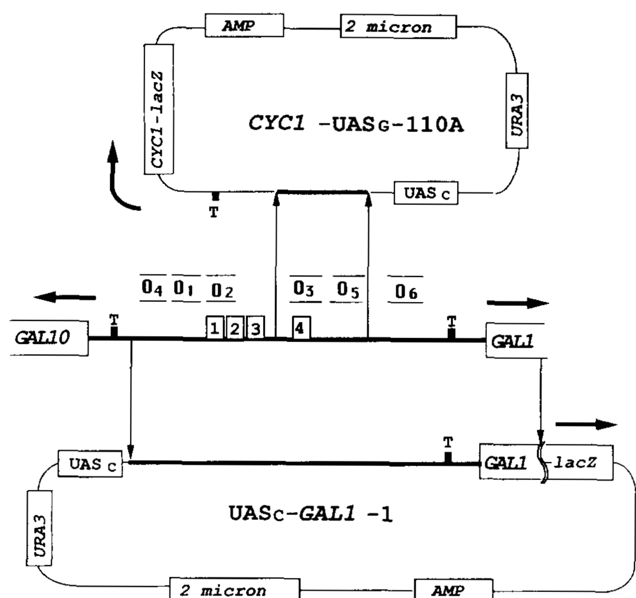

FIGURE 2.—Reporter plasmids that harbor *CYC-GAL* hybrid promoters. *CYC1*-UAS$_G$-110A is a *CYC1-lacZ* fusion that contains a 110-bp fragment of UAS$_G$, harboring operators *GALO*$_3$ and *GALO*$_5$, inserted into the unique *Xho*I site between UAS$_C$ and the *CYC1* TATA box (WEST *et al.* 1987; FINLEY and WEST 1989). UAS$_C$-*GAL1-1* is a *GAL1-lacZ* fusion that harbors the six operators *GAL* O$_1$ to *GALO*$_6$ between UAS$_C$ and the *GAL1* TATA box (WEST *et al.* 1987).

S1 mapping: S1 mapping analysis was performed as described previously (WEST, YOCUM and PTASHNE 1984; WEST *et al.* 1987). The single stranded probe extends from position 688 to 930 of the *GAL1* promoter (YOCUM *et al.* 1984; WEST *et al.* 1987), and was obtained as a 240-bp *Bam*HI-*Xho*I fragment from plasmid pLR1Δ24 (WEST, YOCUM and PTASHNE 1984).

RESULTS

Genetic screen for silencing mutants: Reporter plasmids that contain *CYC-GAL* hybrid promoters harboring specific subsets of the *GAL* operators *GALO*$_1$ to *GALO*$_6$ (WEST *et al.* 1987; FINLEY and WEST 1989; FINLEY *et al.* 1990; CHEN 1992) were employed to isolate mutations in *trans*-acting regulatory genes required for *GAL* operator activity. Figure 2 shows that restriction fragments containing various parts of UAS$_G$ that harbored combinations of operators that would repress promoter activity sufficiently (from 3- to 2500-fold, see MATERIALS AND METHODS), were placed between UAS$_C$ and the TATA box of a *CYC1* or *GAL1* (or *GAL10,* see MATERIALS AND METHODS) promoter fused to *lacZ*. *CYC1*-UAS$_G$-110A (Figure 2; WEST *et al.* 1987; FINLEY and WEST 1989) contains operators *GALO*$_3$ and *GALO*$_5$ inserted between UAS$_C$ and the TATA box of a *CYC1-lacZ* fusion. *CYC1*-UAS$_G$-150 (FINLEY *et al.* 1990) is similar, but contains operators *GALO*$_5$ and *GALO*$_6$ (refer to Figure 1). UAS$_C$-*GAL1-1* (Figure 2; WEST *et al.* 1987) harbors all six *GAL* operators between UAS$_C$ and the TATA box of *GAL1*-lacZ fusion. UAS$_C$-*GAL10-6* (WEST *et al.* 1987) contains operators *GALO*$_1$, *GALO*$_2$ and *GALO*$_4$ between UAS$_C$ and the TATA box of *GAL10-lacZ* fusion. YM335 cells containing *CYC1*-UAS$_G$-110A, *CYC1*-UAS$_G$-150, UAS$_C$-*GAL1-1*, or UAS$_C$-*GAL10-6* were treated with EMS (see MATERIALS AND METHODS), and approximately 70 blue (derepressed) colonies were isolated from over 100,000 colonies examined. Quantitative β-galactosidase activities indicated that expression from the hybrid promoters was derepressed by 2–140-fold (data not shown; see below). Thirty mutants were chosen for further characterization. Segregation analysis showed that the mutation in each of the 30 strains was unlinked to the plasmid and therefore was acting in *trans.* Heterozygous diploid strains derived by crossing each original mutant strain containing the respective reporter plasmid with wild-type strain BWY3 or DBY745 showed amounts of β-galactosidase only slightly higher than the homozygous diploid wild-type control strains BWY5001 and BWY7001, suggesting that each mutation was recessive. Tetrad analysis (6–10 asci tested per cross) following sporulation of representative diploids showed each mutation segregated 2:2, demonstrating that each resides in a single nuclear gene.

Six genes designated *TSF1* to *TSF6*: Pairwise crosses were made to determine the number of complementation groups represented by the 30 mutants. Subsequently, allelism was determined upon sporulating representative heterozygous diploids and performing tetrad analysis (5–12 asci dissected per cross). Table 2 shows that the 30 mutant alleles comprise 6 different genes, arbitrarily designated *TSF1* to *TSF6* (*T*ranscriptional *S*ilencing *F*actor). The reporter plasmids used to select mutations in each gene are shown in Table 2.

Several other findings are noteworthy. First, mutations in *TSF3*, *TSF4* and *TSF6* caused prominent growth defects which segregated 2:2 with derepression of the hybrid promoter (discussed below). Second, heterozygous diploids derived from crosses between *tsf2* and *tsf4* strains, and *tsf4-1* and *tsf5-1* strains, showed only partial complementation (Table 2), suggesting possible functional interactions between the *TSF2*, *TSF4*, and *TSF5* gene products (STEARNS and BOTSTEIN 1988; see DISCUSSION). Third, diploids derived by crossing *tsf1* to various *tsf2* to *tsf6* strains showed exceptionally poor sporulation (<0.1%) and poor spore viability, thereby precluding a complete allelism assignment, and indicating that in combination certain *tsf* mutations behave in a semidominant fashion.

***TSF1* to *TSF6* effects are not *GAL* operator-specific:** Mutations in each *TSF1* to *TSF6* gene had a general effect on expression of *CYC-GAL-lacZ* fusions on reporter plasmids. In Table 3 is shown the amount of β-galactosidase synthesized from the reporter vec-

TABLE 2

Thirty mutations comprising the six genes *TSF1* to *TSF6*

Mutant allele	Reporter plasmid	*tsf1-α*					*tsf2-α*			*tsf3-α*								*tsf4-α*	*tsf5-α*	*tsf6-α*	
		1	2	*3*	*4*	*5*	*1*	*5*	22	*1j*	*7*	*10*	*11*	*12*	*13*	*14*	27	*1*	*1*	*1*	2
tsf1-1	UAS$_C$-*GAL10*-6	−	−	−	−	−		+				+			+			+			
2		−	−	−	−	−						+			+						
3		−				−															
4		−	−	−	−	−															
5		−						+													
tsf2-1	*CYC1*-USA$_G$-110A						−	−	−		+	+	+	+	+	+		±[a]			
2							−	−	−		+	+	+	+	+	+		±[a]			
5		+					−	−	−		+	+	+	+	+	+		±[a]			
6							−	−	−		+	+	+	+	+	+		±[a]			
17							−	−	−		+	+	+	+	+	+		±[a]			
18							−	−	−		+	+	+	+	+	+		±[a]			
19							−	−	−		+	+	+	+	+	+		±[a]			
21							−	−	−		+	+	+	+	+	+		±[a]			
22							−	−	−		+	+	+	+	+	+		±[a]			
23		+					−	−	−		+	+	+	+	+	+		±[a]			
tsf3-1j[b]	*CYC1*-USA$_G$-110a	+	+					+				−		−	−	−					
7							+	+	+		−	−	−	−	−	−		+			
10		+	+				+	+	+	−	−	−	−	−	−	−		+			
11							+	+	+		−	−	−	−	−	−		+			
12							+	+	+		−	−	−	−	−	−		+			
13		+	+				+	+	+	−	−	−	−	−	−	−		+			
14							+	+	+		−	−	−	−	−	−	−	+			
15							+	+	+		−	−	−	−	−	−		+			
24							+	+	+		−	−	−	−	−	−		+			
27		+	+					+								−		+			
tsf4-1	*CYC1*-	+					±[a]	±[a]	±[a]		+	+	+	+	+	+		−			
-2	UAS$_G$-110A						±[a]	±[a]	±[a]		+	+	+	+	+	+		−			
tsf5-1	*CYC1*-UAS$_G$-150	+						+							+	+	+	±[a]	−	+	+
tsf6-1	*CYC1*-UAS$_G$-150	+						+				+			+	+	+	+	+	−	−
-2	UAS$_G$-*GAL1-1*	+						+				+			+	+	+	+	+	−	−

Thirty original mutants (*MAT***a**) containing the indicated reporter plasmids were crossed to the respective *MATα* derivatives and the resulting diploids tested for complementation (see MATERIALS AND METHODS). Complementation was scored as "+," wild-type phenotype, "−," mutant phenotype.

[a] Unliked noncomplementation (see RESULTS).

[b] *tsf3-1j* was isolated from an independent screen (S. JOHNSON and B. BYERS).

tors *CYC1*-UAS$_G$-110A, *CYC1*-UAS$_G$-150 and UAS$_C$-*GAL1-1* in *tsf1* to *tsf6* strains grown under inducing (glycerol and lactate, or "Gly" medium) *vs.* repressing (glucose, or "Glu" medium) conditions (with respect to the activity of UAS$_C$; see MATERIALS AND METHODS; GUARENTE *et al.* 1984). First, each *tsf1* to *tsf6* mutation derepressed expression of all three *CYC-GAL-lacZ* fusions rather than one containing a given subset of *GAL* operators. This reduces the likelihood that the mutations are in genes encoding operator-specific binding proteins. Second, hybrid promoter activity in the mutant strains was generally derepressed when UAS$_C$ was maximally active (Gly medium) as well as repressed (Glu medium), indicating that the *tsf1* to *tsf6* mutations are not involved in a specific repression pathway like catabolite repression. Third, none of the *tsf* mutations individually fully derepressed expression of the *CYC-GAL-lacZ* fusions relative to the wild-type *CYC1-lacZ* fusion, suggesting that more than one silencing mechanism is operative.

***tsf3* and *tsf6* mutations allow wild-type *GAL1-lacZ* expression in the absence of galactose and GAL4:** Transcription from the wild-type *GAL1* and *GAL10* promoters ordinarily cannot occur in the absence of galactose and GAL4 (see Introduction; Figure 1). We found upon examining expression of the wild-type *GAL1-lacZ* fusion that this requirement was relaxed in strains that harbor *tsf3* or *tsf6* mutations. *tsf1* to *tsf6* strains were transformed with multicopy plasmids that harbor the wild-type *GAL1* (pRY131) or *GAL10* promoter (pRY133) fused to *lacZ*, and expression was measured after growing the cells in the absence (Gly medium) or presence of galactose (galactose plus glycerol and lactate or "Gal" medium). Table 4 shows that

TABLE 3

Effects of *tsf1* to *tsf6* on expression from *CYC-GAL* hybrid promoters on reporter plasmids

Strain	Allele	Growth medium	Hybrid promoter: *CYC1*-UAS_G-110A (*CVYC1*; O_3,O_5)	*CYC1*-UAS_G-150 (*CYC1*; O_5,O_6)	UAS_G-*GAL1-1* (*GAL1*; O_1–O_6)
YM335	*TSF*$^+$	Gly	200 (1×)	150 (1×)	0.6 (1×)
		Glu	5 (1×)	1.5 (1×)	0.1 (1×)
BWY124	*tsf1-1*	Gly	375 (1.9×)	220 (1.5×)	15 (25×)
		Glu	9 (1.8×)	4.5 (3.0×)	0.4 (≥4.0×)
BWY104	*tsf2-5*	Gly	360 (1.8×)	150 (1.0×)	4.5 (7.5×)
		Glu	20 (4.0×)	5.0 (3.3×)	0.2 (≥2.0×)
BWY118	*tsf3-13*	Gly	345 (1.7×)	228 (1.5×)	72 (120×)
		Glu	50 (10×)	9.0 (6.0×)	14 (≥140×)
BWY102	*tsf4-1*	Gly	300 (1.5×)	160 (1.1×)	20 (33×)
		Glu	30 (6.0×)	7.0 (5.0×)	1.0 (≥10×)
BWY132	*tsf5-1*	Gly	340 (1.7×)	160 (1.0×)	16 (27×)
		Glu	7.0 (1.4×)	11 (7.0×)	1.8 (≥18×)
BWY133	*tsf6-1*	Gly	480 (2.4×)	600 (4.0×)	174 (290×)
		Glu	16 (3.2×)	38 (25×)	30 (≥300×)

Numbers represent units of β-galactosidase activity in the indicated strains (harboring the corresponding hybrid promoters) grown in Glu (repressed) or Gly (derepressed) medium. UAS_C, common to all promoters, is 4–25-fold less active in Glu *vs.* Gly medium (GUARENTE *et al.* 1984; WEST *et al.* 1987). Numbers in parentheses indicate fold derepression relative to the wild-type strain for the strains grown in the corresponding medium. Units of β-galactosidase activity derived from the corresponding wild-type (*CYC1*) or hybrid (UAS_G-*GAL1* or *CYC1*-UAS_G) promoters lacking *GAL* operators (WEST *et al.* 1987) were as follows: *CYC1*, 600 (Gly) and 150 (Glu); UAS_G-*GAL1-8*, 1380 (Gly) and 235 (Glu).

TABLE 4

Expression of wild-type *GAL1*- and *CYC1-lacZ* fusions in *tsf1* to *tsf6* strains

Strain	Allele: *TSF*	*GAL4*	Growth condition[a]	Promoter: *GAL1*	*CYC1*
YM256	*TSF*$^+$	*GAL4*	Induced	4400	565
			Noninduced	<0.1	165
BWY124	*tsf1-1*	Δ*gal4*	Induced	1.0	602
			Noninduced	0.8	164
BWY155	*tsf2-5*	*GAL4*	Induced	2600	670
			Noninduced	0.4	205
BWY136	*tsf3-10*	*GAL4*	Induced	3700	475
			Noninduced	35	178
BWY115	*tsf3-10*	Δ*gal4*	Induced	60	600
			Noninduced	35	150
BWY161	*tsf4-1*	*GAL4*	Induced	3300	805
			Noninduced	0.6	210
BWY162	*tsf5-1*	*GAL4*	Induced	2500	390
			Noninduced	0.2	290
BWY178	*tsf6-2*	*GAL4*	Induced	2490	853
			Noninduced	7.0	238

Numbers represent units of β-galactosidase activity produced in the indicated strains harboring the wild-type *GAL1* (pRY131) or *CYC1* (pLGΔ-312) promoter fused to *lacZ*.

[a] For *GAL1*, induced is Gal and noninduced is Gly. For *CYC1*, induced is Gly and noninduced is Glu.

in the absence of galactose, β-galactosidase activity derived from a wild-type *GAL1-lacZ* fusion rose well above background in *tsf3* and *tsf6* strains, and increased in other *tsf1* to *tsf6* strains as well. This was not an effect of increased reporter plasmid copy number, since proportionate derepression was observed in a *tsf3-10* strain that harbors an integrated copy of the wild-type *GAL1-lacZ* fusion [7.0 units in strain BWY20 (Table 1) *vs.* 35 units in BWY136, compared to <0.1 unit in BWY19, a *TSF*$^+$control strain harboring an integrated wild-type *GAL1-lacZ* fusion (Table 1); see MATERIALS AND METHODS]. Equivalent results were obtained in isogenic *tsf1* to *tsf6* strains that lacked *GAL4* (see for example BWY115, Table 4; data not shown). When the respective strains were grown under catabolite repressing conditions (Glu medium), expression in the absence of galactose and GAL4 was reduced by about 10-fold with respect to noninducing conditions (Gly medium). This may largely be due to increased repression by operator $GALO_6$, which is at least partly controlled by glucose (FINLEY *et al.* 1990; NEHLIN, CARLBERG and RONNE 1991; FLICK and JOHNSTON 1992). GAE_1 and GAE_2 activity, which likely contributes substantially to high basal level *GAL1* expression observed in *tsf* strains, can be repressed by $GALO_6$ alone (see below; FINLEY *et al.* 1990; R. FINLEY and R. WEST, unpublished data; CHEN 1992). For reasons that are unclear, the wild-type *GAL10* promoter was derepressed to a detectable level in only one *tsf* strain examined [BWY143 (*tsf3-27*, Table 1), data not shown], though allele *tsf3-1j* (SJ1031-7b, Table 1) was originally isolated using a *GAL10-lacZ* reporter plasmid.

Fully induced expression of a wild-type *GAL1-lacZ* fusion (presence of galactose or Gal medium; Table 4) or wild-type *GAL10*- or *GAL7-lacZ* fusions (data not shown) was marginally lower in *tsf2* to *tsf6* strains (*GAL4*$^+$) than in the isogenic *TSF*$^+$ control strain. Figure 3 shows that consistent results were obtained

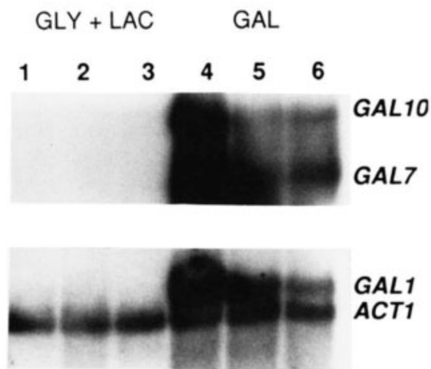

FIGURE 3.—Northern blot analysis of *GAL1*, *GAL7* and *GAL10* chromosomal transcripts in *tsf3* and *tsf6* strains. RNA was prepared for Northern blot analysis from strains YM256 (lanes 1 and 4), BWY136 (*tsf3-10*; lanes 2 and 5), and BWY 178 (*tsf6-2*; lanes 3 and 6). Strains were grown under noninducing (Gly medium, lanes 1–3) *vs.* inducing (Gal medium, lanes 4–6) conditions. Approximately 10 ug of poly(A)$^+$ RNA was loaded per lane, blotted, and probed with ^{32}P-radiolabeled DNA fragments corresponding to *GAL1* and *ACT1* (see MATERIALS AND METHODS). The membrane was stripped and re-probed with a ^{32}P-radiolabeled DNA fragment that harbors both *GAL7* and *GAL10* sequences.

by Northern blot analysis of *GAL1*, *GAL7* and *GAL10* chromosomal transcripts in *tsf3* and *tsf6* strains (*GAL4*$^+$) grown under fully inducing conditions. Notwithstanding, expression of *GAL1*, *GAL7* and *GAL10* was not impaired significantly enough to prevent growth on galactose as a sole carbon source (Table 5). *GAL1*, *GAL7* and *GAL10* chromosomal transcripts were not detectable in *tsf3* and *tsf6* strains grown under noninducing conditions (Fig. 3), since the amount of β-galactosidase activity derived from a wild-type *GAL1-lacZ* fusion under noninducing conditions was at least 100-fold lower than under inducing conditions (Table 4) and β-galactosidase activities were derived from strains harboring multicopy plasmids.

Expression of a wild-type *CYC1-lacZ* fusion was not appreciably altered in fully induced *tsf1* to *tsf6* strains (Gly medium), consistent with the fact that *tsf1* to *tsf6* strains grow at a normal rate in media containing glycerol as sole carbon source. Induced expression of at least two other genes, *PDC1* (encoding pyruvate decarboxylase; BUTLER, DAWES and MCCONNELL 1990) and *ACT1*, appeared to be unaffected by *tsf1* to *tsf6* mutations (CHEN 1992; J. MA and R. W. WEST, unpublished results).

tsf1* to *tsf6* cause pleiotropic growth defects:** *tsf1* to *tsf6* mutations did not significantly affect cellular growth rate at 30° in rich medium or in minimal medium containing any of the following sugars as sole carbon source(s): glucose, galactose, sucrose, raffinose, or glycerol plus lactate. Table 5 shows, however, that *tsf3*, *tsf4* and *tsf6* mutations caused both flocculence (dispersible by EDTA or dithiothreitol, reversible by $MgSO_4$ or $CaCl_2$) and temperature-sensitive lethality (36° was the nonpermissive temperature) which segregated 2:2 with derepressed promoter activity upon outcrossing. Additionally, *tsf3*, *tsf4* and *tsf6* mutations caused aberrant cell morphology (2–4-fold greater cell size and occasional schmooing; not shown). *tsf3* mutations furthermore significantly reduced the mating efficiency of both *MATa** and *MAT*α cell types, the severity of which was allele-dependent and ranged from 4- to 5-fold for *tsf3-14*, to 50–100-fold for *tsf3-7* and *tsf3-10* (Table 5). Consistent with this finding, the results of Northern blot analysis, shown in Figure 4, indicated that the silent mating type locus HMLα is expressed in strain BWY115 (MATa *tsf3-10*). Homozygous diploid *tsf3* strains further exhibited a significantly reduced sporulation efficiency, the severity of which was likewise allele-dependent (Table 5).

tsf1, *tsf2* and *tsf5* mutants were not as severely growth defective as *tsf3*, *tsf4* and *tsf6* mutants (Table 5). Surprisingly, however, while the parental strain YM335 requires methionine for growth, *tsf2*, *tsf4* and *tsf5* mutants reverted to Met$^+$ (Table 5) which cosegregated with transcriptional derepression upon outcrossing. Using a feeding-indicator plate assay (see MATERIALS AND METHODS), we observed that methionine and/or a metabolic precursor was excreted by *tsf2*, *tsf4* and *tsf5* strains, though not by YM335, *tsf3* or *tsf6* strains (data not shown). The respective *met* mutation in YM335 has not been characterized but was derived from a genetic screen to obtain mercury-resistant mutants of *S. cerevisiae* (SINGH and SHERMAN 1974; M. JOHNSTON, personal communication). One interesting possibility is that the defect lies in a regulatory gene required for transcription of *MET* structural genes (see, for example, THOMAS, JACQUEMIN and SURDIN-KERJAN 1992), and that *tsf2*, *tsf4* and *tsf5* mutations circumvent this requirement. Post-transcriptional roles, however, for Met$^+$ phenotypic reversion in *tsf2*, *tsf4* and *tsf5* mutants cannot be excluded.

***tsf1* to *tsf6* activate expression of UAS-less *GAL1*- and *CYC1-lacZ* fusions:** Unexpectedly, yeast *GAL1* and *CYC1* promoters (fused to *lacZ*) lacking their upstream activating elements were expressed at unusually high basal levels in *tsf1* to *tsf6* strains, a phenomenon reported previously for *ssn20* (*SPT6/CRE2*; NEIGEBORN, CELENZA and CARLSON 1987; DENIS and MALVAR 1990) and *spt10 S. cerevisiae* mutants (*CRE1*; DENIS and MALVAR 1990). Figure 5 shows that the amount of β-galactosidase activity derived from a UAS-less *GAL1-lacZ* fusion (plasmid 121-632 in Figure 6) ranged from 4 to 26 units in *tsf* strains grown in Glu medium, or 20–130-fold higher than in the isogenic wild-type (*TSF*$^+$) control strain. A UAS-less *CYC1-lacZ* fusion (plasmid pLG670Z, Figure 6) was expressed at levels ranging from 0.3 to 32 units, or 1.5–160-fold higher than in the wild-type strain. In *tsf3* strains, UAS-less promoter activity reached as high as 13% of the fully derepressed (*i.e.*, GAE-dependent; Figure 1) wild-type *GAL1* promoter activity,

TABLE 5

Effects of *tsf1* to *tsf6* on cellular growth, mating and sporulation

Allele	Temperature sensitivity	Flocculence	Gal	Met	Mating efficiency *MAT***a**	Mating efficiency *MAT*α	Sporulation efficiency
TSF⁺	−	−	+	−	1	1	1
tsf1-1	−	−	ND	−	0.8	ND	ND
tsf1-2	−	−	ND	−	0.8	ND	ND
tsf2-5	−	−	+	+	0.6	0.5	1
tsf2-23	−	−	+	+	1	0.7	0.6
tsf3-7	+	++	ND	−	0.02	0.01	0.01
tsf3-10	+[a]	++	+	−	0.02	0.01	1
tsf3-13	+[a]	++	ND	−	ND	ND	ND
tsf3-14	+	+	+	−	0.25	0.20	<0.0001
tsf4-1	+[a]	+	+	+	1	0.7	0.4
tsf5-1	−	−	+	+	0.5	0.6	0.2
tsf6-1	+[a]	++	ND	−	0.2	0.5	0.5
tsf6-2	+[a]	++	+	−	ND	ND	ND

Respective isogenic *MAT***a** and *MAT*α derivatives of *tsf1* to *tsf6* strains are from Table 1. The *TSF*⁺ control strain was YM256. Temperature sensitivity was tested on YEP-D medium at 36°. Flocculence was detected in liquid YEP-D medium at 30°. Gal (galactose utilization) phenotype was determined using SC medium containing galactose as sole carbon source. Met (methionine prototrophy/auxotrophy) phenotype was determined using SC medium lacking methionine. Mating and sporulation efficiency were determined as described in MATERIALS AND METHODS. ND, not determined.

[a] Leaky growth.

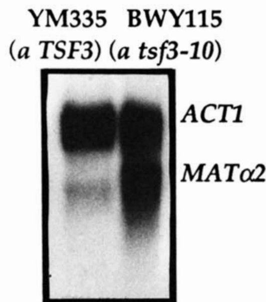

FIGURE 4.—*tsf3* derepresses the silent mating-type locus HMLα in a *MAT***a** strain. Approximately 15 μg of poly(A)⁺ RNA prepared from strains YM335 (*MAT***a** *TSF3*) and BWY115 (*MAT***a** *tsf3-10*) grown in Glu medium was fractionated on a denaturing 1% agarose gel, transferred to a nylon membrane following electrophoresis, and probed with a ³²P-radiolabeled 1.12-kb *Eag*I-*Nde*I fragment of *MAT*α*2*. A ³²P-radiolabeled 0.6-kb *Eco*RI-*Hin*dIII fragment of the yeast *ACT1* (actin) gene was used as an internal standard for RNA concentration. A faint transcript in YM335 migrating ahead of the *MAT*α*2* position likely corresponds to *MAT***a**, which cross-hybridizes with the *MAT*α probe (FASSLER and WINSTON 1989).

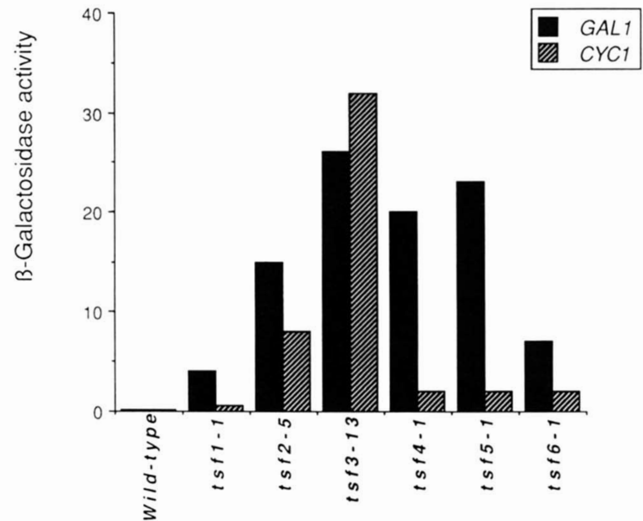

FIGURE 5.—*tsf1* to *tsf6* activate expression of UAS-less *GAL1*- or *CYC1-lacZ* fusions. Bars show levels of β-galactosidase activity in the respective strains harboring a UAS-less *GAL1* (plasmid 121-632; see MATERIALS AND METHODS) or UAS-less *CYC1* (plasmid pLG670Z) promoter fused to *lacZ* and grown in Glu medium. The corresponding amount of β-galactosidase activity for UAS-less *GAL1*- or *CYC1-lacZ* fusions in the wild-type strain (YM335) was 0.2 unit for both. Under fully derepressing (but noninducing; see Figure 1, Table 4 and text) conditions, wild-type (YM335) cells harboring the respective wild-type *GAL1-lacZ* (plasmid pRY131) or *CYC1-lacZ* (pLGΔ-312) fusions produced 200 and 150 units of β-galactosidase, respectively.

and 17% of the wild-type *CYC1* promoter activity (Figures 5 and 6). Comparable results were obtained with *tsf1* to *tsf6* strains that harbored a UAS-less *GAL10-lacZ* fusion, and comparable levels of expression were observed in the respective *tsf* strains grown in Gly medium (data not shown). It is noteworthy that *tsf3* was more effective than other *tsf1* to *tsf6* mutations in activating expression of the UAS-less *GAL1-lacZ* fusion and, surprisingly, considerably more effective in activating the UAS-less *CYC1-lacZ* fusion (Figure 5). This and other results in Figure 5 denote a certain degree of promoter specificity to *TSF1* to *TSF6* function. Combined with the fact that *tsf* mutations cause pleiotropic growth defects, these results suggest that *TSF1* to *TSF6* are global regulatory genes, and that their respective gene products act independently of specific upstream promoter elements.

***tsf3* appears to cause a specific defect in transcription:** Figure 6 shows that expression of the UAS-less

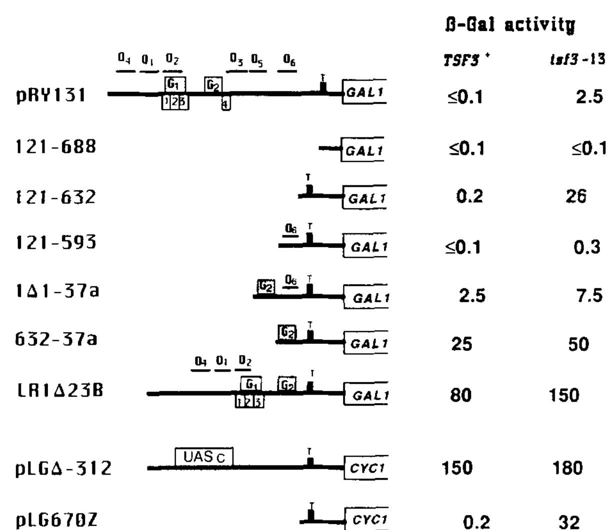

FIGURE 6.—*tsf3* removes a block on the function of upstream as well as downstream promoter elements. Presence or absence of *GAL* operators (horizontal bars), *GAL4* sites (open boxes 1–4), GAE_1 (stippled box G_1), GAE_2 (stippled box G_2), TATA box (T box), and UAS_C (large open box) in the respective promoter construct is denoted. Units of β-galactosidase activity from the respective *GAL1*- or *CYC1-lacZ* fusion in strains YM335 (*TSF3*+) and BWY118 (*tsf3-13*), grown in Glu medium, are shown at right. Expression of pRY131 in the *tsf3-13* strain grown in Glu medium is repressed 14-fold with respect to Gly medium (Table 4), due to catabolite repression acting via the *GAL* operators, in particular $GALO_6$ (FINLEY *et al.* 1990; NEHLIN, CARLBERG and RONNE 1991; FLICK and JOHNSTON 1992).

GAL1-lacZ fusion in strain BWY118 (*tsf3-13 Δgal4*) requires the normal *GAL1* TATA box (compare plasmids 121-688 *vs.* 121-632, Figure 6). Figure 7 shows S1 mapping results which demonstrate that the amount of *GAL1-lacZ* specific mRNA in strains BWY118 and BWY115 (*tsf3-10 Δgal4*) harboring plasmid LR1Δ23B, grown in the absence of galactose and GAL4, is substantially greater than in the isogenic *TSF*+ control strain YM335 (see also WEST, YOCUM and PTASHNE 1984). Figure 7 further shows that mRNA derived from a UAS-less *GAL1-lacZ* fusion (plasmid 121-632), though in lower abundance than that derived from LR1Δ23B (see Figure 6), is detectable in strains BWY118 and BWY115 but not in the isogenic *TSF*+ control strain YM335. The 5′ *GAL1* mRNA start sites for each construct appear to be unaltered in *tsf3* strains relative to *TSF*+ strains (Figure 7). These results in conjunction with those above suggest that *tsf3* causes a defect in transcriptional efficiency.

UAS-dependent *vs.* UAS-independent promoter activity in *tsf* strains: The amount of β-galactosidase activity derived from the wild-type *GAL1-lacZ* fusion or from *CYC-GAL-lacZ* fusions in *tsf1* to *tsf6* strains grown under noninducing or repressing conditions (Tables 3 and 4) is roughly equivalent to that derived from UAS-less *GAL1*- or *CYC-lacZ* fusions (Figure 5). In the former instances, however, expression likely derives from activation mediated by UAS-binding proteins (for example, GAE_1- and GAE_2-binding proteins for UAS_G, and HAP proteins for UAS_C) as well as from the removal of a constraint on the basal activity of the RNA polymerase II transcriptional machinery. Figure 6 shows, for example, that $GALO_6$ present upstream of the UAS-less *GAL1* promoter (plasmid 121-593) blocked derepression by *tsf3*. A similar result occurred when $GALO_3$ was placed upstream (data not shown). By contrast, GAE_1 and/or GAE_2 upstream of the UAS-less *GAL1* promoter (632-37a and LR1Δ23B) increased the amount of transcription above that derived from derepression by *tsf3* (Figure 6). Similar results were observed in other *tsf1* to *tsf6* strains (data not shown). These results are consistent with the notion that the products of the *TSF1* to *TSF6* genes act independently of specific upstream promoter elements, and that net *GAL1* promoter activity is commensurate to the number and strength of positive *vs.* negative control elements upstream (1Δ1-37a, Figure 6; see also FINLEY *et al.* 1990).

***TSF* genes may comprise more than one repression pathway:** Availability of the cloned *TSF3* gene (CHEN *et al.* 1993) made it possible to analyze expression in *tsf* double mutant strains, by constructing a *tsf3* null mutation in other *tsf1* to *tsf6* strains. Table 6 shows that the amount of expression in *Δtsf3tsf2*, *Δtsf3tsf4*, or *Δtsf3tsf5* double mutant strains from a wild-type *GAL1-lacZ* fusion (repressing conditions), a UAS-less *GAL1*- or *CYC1-lacZ* fusion, or the *CYC-GAL* hybrid promoter *CYC1*-UAS_G-110A was greater than the sum of the amount of expression in the two corresponding single mutant strains. The double mutants also were more growth defective, exhibiting increased flocculence, greater sensitivity to high temperature, and in some instances, reduced growth rate at 30° in glucose medium (*i.e.*, non-mitochondrial growth defect). These results are consistent with the fact that *tsf3* mutations caused different growth and/or regulatory defects than *tsf2*, *tsf4* or *tsf5* single mutations (Table 5). Interestingly, even in *tsf* double mutant strains expression of *CYC1*-UAS_G-110A remained beneath that derived from the wild-type *CYC1* promoter (Table 6), raising the possibility that an additional repression pathway(s) is involved.

DISCUSSION

Our studies suggest that a silencing mechanism(s) dependent on *TSF1* to *TSF6* represses basal level expression of the *GAL* structural genes (*i.e.*, noninducing conditions) and that this comprises an important pathway(s) in addition to GAL4/GAL80-mediated control for regulating *GAL* gene expression. Our results suggest that, rather than encoding *GAL* operator-specific binding proteins, *TSF1* to *TSF6* en-

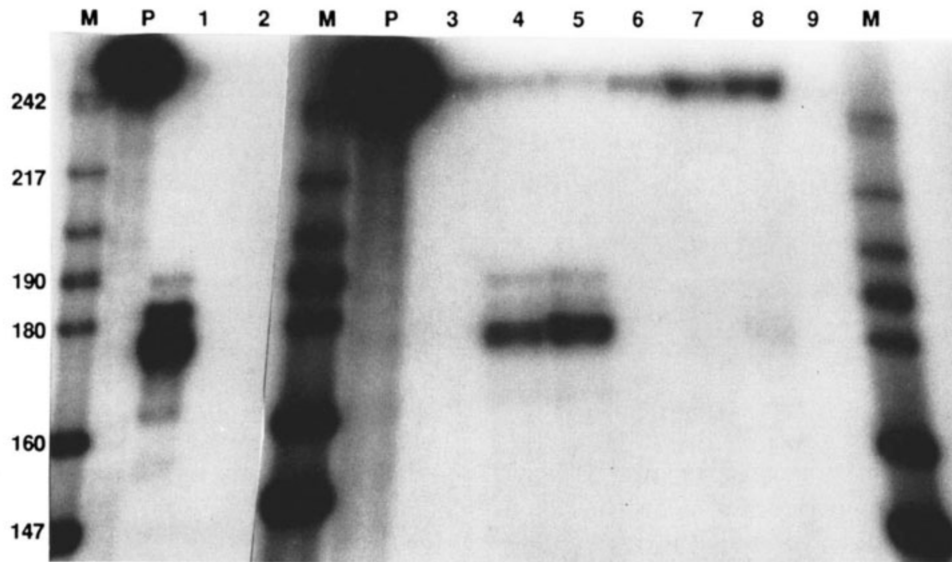

FIGURE 7.—*GAL1-lacZ* mRNA abundance and 5′ start sites in *tsf3 vs. TSF3*⁺ strains. S1 mapping of RNA made *in vivo* from wild-type or mutant (for example, UAS-less) *GAL1-lacZ* fusions in *TSF*⁺ *vs. tsf3* strains. Total cellular RNA was isolated from the respective strains harboring one of the plasmids shown in Figure 6, following growth in Gal medium (lane 1), Gly medium (lane 2) or Glu medium (lanes 3–9). The single-stranded *GAL1-lacZ* probe was derived from a 240-bp *XhoI-BamHI* fragment of pLR1Δ24. Lane 1 (25 μg RNA) pRY131 (wild-type *GAL1-lacZ* fusion plasmid)/YM256 (*TSF*⁺ *GAL4*⁺); lane 2 (50 μg RNA) pLR1Δ23B (mutant *GAL1-lacZ* fusion plasmid)/YM256; lane 3 (100 μg RNA) pLR1Δ23B/YM335 (*TSF*⁺ *Δgal4*); lane 4 (50 μg RNA) pLR1Δ23B/BWY115 (*tsf3-10 Δgal4*); lane 5 (50 μg RNA) pLR1Δ23B/BWY118 (*tsf3-13 Δgal4*); lane 6 (50 μg RNA) 121-632/YM335; lane 7 (50 μg RNA) 121-632/BWY115; lane 8 (50 μg RNA) 121-632/BWY118; lane 9 (50 μg RNA) 121-593/BWY118. Other lanes: (M) molecular size markers (*HpaII*-digested pBR327 DNA); (P) undigested probe. Further details are provided in Figure 6 and MATERIALS AND METHODS (see also WEST, YOCUM and PTASHNE 1984; WEST *et al.* 1987).

TABLE 6

***TSF1* to *TSF6* genes comprise more than one silencing pathway**

	Promoter			
		UAS-less		
Allele(s)	Wild-type *GAL1*	*GAL1*	*CYC1*	Hybrid *CYC1*-UAS$_G$-110A
TSF⁺	≤0.1	0.2	0.2	5.0 (150)
tsf2-5	0.1	15	8.0	20
Δtsf3-2	3.0	38	52	38
tsf4-1	≤0.1	20	2.0	30
tsf5-1	≤0.1	23	2.0	7.0
Δtsf3-2 tsf2-5	20	120	114	100
Δtsf3-2 tsf4-1	12	125	110	88
Δtsf3-2 tsf5-1	4.0	80	110	45

Numbers represent units of β-galactosidase activity measured from the respective strains harboring the indicated promoter (fused to *lacZ*), grown in Glu medium (for further details see Tables 2 and 3, and Figures 3 and 4). Strains used were YM256 (*TSF*⁺), BWY155 (*tsf2-5*), BWY54 (*Δtsf3-2*), BWY160 (*tsf4-1*), BWY163 (*tsf5-1*), BWY402 (*Δtsf3-2tsf2-5*), BWY400 (*Δtsf3-2tsf4-1*), and BWY403 (*Δtsf3 tsf5-1*). For *CYC1*-UAS$_G$-110A, the number in parentheses indicates units of β-galactosidase activity obtained from the respective wild-type *CYC1-lacZ* fusion (lacking *GAL* operators), provided for comparison.

code regulatory proteins that function in a more global manner. This perspective derives from the fact that one or more *tsf1* to *tsf6* mutations: (1) derepressed expression of a variety of *CYC-GAL-lacZ* fusions, (2) activated high basal level expression of UAS-less *GAL1-* and *CYC1-lacZ* fusions and (3) caused pleiotropic defects in cell growth and development. These results are consistent with our previous finding that *tsf3* partially suppresses repression in a *MAT***a** strain of *CYC-GAL* hybrid promoters that contain single or multiple copies of the *MATα2* operator (CHEN *et al.* 1993). At least in the case of *tsf3* mutations, S1 mapping and Northern blot analysis in conjunction with activation of UAS-less *GAL1-* and *CYC1-lacZ* fusions suggest that the molecular basis of the defect is transcriptional. Failure to obtain mutations in genes encoding *GAL* operator-specific binding proteins might be due to a failure to saturate our genetic screen with respect to the number of genes that can be identified (since for several of the *TSF* complementation groups only one or two alleles were identified, Table 2), or might instead suggest that such genes are either essential, repeated or functionally redundant, and therefore the corresponding mutations could not be detected by our genetic screen. Success in obtaining *tsf1* to *tsf6* mutations suggests that equally important silencing components act in conjunction with repressor proteins bound upstream to control *GAL1* and *GAL10* expression. We cannot rule out the possibility, of course, that one or more *TSF1* to *TSF6* genes encodes a "general" repressor protein having degenerate sequence specificity, capable of binding to a broad number of *S. cerevisiae* sequences including one or more *GAL* operators as well as downstream promoter sites.

A number of yeast transcriptional regulatory genes have been characterized in which mutations cause pleiotropic defects in cell growth and development, and which likewise appear to affect the expression of many genes. They include *SIT* genes (*HIS4* promoter; ARNDT, STYLES and FINK 1989), *SIN*, *SDI* and *SWI* genes (*HO* promoter; STERN, JENSEN and HERSKOWITZ 1984; BREEDEN and NASMYTH 1985), *SNF* and *SSN* genes (*SUC2* promoter; CARLSON 1987; SCHULTZ and CARLSON 1987; SCHULTZ, MARSHALL-CARLSON and CARLSON 1990), *CRE* genes (*ADH2* promoter; DENIS and MALVAR 1990), *UME* genes (*SPO* promoter; STRICH, SLATER and ESPOSITO 1989), and *SPT* genes (Ty element promoter; WINSTON *et al.* 1984; FASSLER and WINSTON 1988). Follow-up studies have revealed that such genes encode a variety of proteins having a general role in transcription, including histone H2A (*SPT11*; CLARK-ADAMS *et al.* 1988), histone H2B (*SPT12*; CLARK-ADAMS *et al.* 1988), histone H3 (*SIN2*; KRUGER and HERSKOWITZ 1991), an HMG-like protein (*SPT2/SIN1*; KRUGER and HERSKOWITZ 1991) and other proteins affecting the structure/function of chromatin (*SNF2/SWI2* and *SNF5*; HIRSCHHORN *et al.* 1992), transcription factor TFIID (*SPT15*; EISENMANN, DOLLARD and WINSTON 1989), the protein kinase SNF1 (CARLSON 1987), the protein phosphatase SIT4 (ARNDT, STYLES and FINK 1989; SUTTON, IMMANUEL and ARNDT 1991), the putative "mediator" or "potentiator" protein GAL11 (SUZUKI *et al.* 1988; FASSLER and WINSTON 1989; NISHIZAWA *et al.* 1990; HIMMELFARB *et al.* 1990) and the general repressor protein complex SSN6-TUP1 (TRUMBLY 1992; KELEHER *et al.* 1992). Results of complementation tests, in most cases involving transformation of *tsf* strains with cloned *S. cerevisiae* genes (see MATERIALS AND METHODS) and analysis of reversion to wild-type transcriptional (using UAS-less promoters and *CYC-GAL* hybrid promoters fused to *lacZ*) and cell growth phenotypes, suggest that none of the *TSF1* to *TSF6* genes is allelic to the following genes: *SIN1* (*SPT2*, ROEDER *et al.* 1985; KRUGER and HERSKOWITZ 1991), *SIN2* (*HHT1*; KRUGER and HERSKOWITZ 1991), *SIN3* (*SDI1/UME4/RPD1*; NASMYTH, STILLMAN and KIPLING 1987; STRICH, SLATER and ESPOSITO 1989; WANG and STILLMAN 1990; VIDAL *et al.* 1991), *SDI2* and *SDI4* (NASMYTH, STILLMAN and KIPLING 1987), *SWI1*, *SWI2* (*SNF2*) or *SWI3* (PETERSON and HERSKOWITZ 1992), *SSN6* (SCHULTZ and CARLSON 1987; SHULTZ, MARSHALL-CARLSON and CARLSON 1990), *SSN20* (*SPT6/CRE2*; NEIGHEBORN, CELENZA and CARLSON 1987; SWANSON, CARLSON and WINSTON 1990; DENIS and MALVAR 1990), *TUP1* (WILLIAMS and TRUMBLY 1990), *GAL11* (*SPT13*; SUZUKI *et al.* 1988; FASSLER and WINSTON 1989), *SIT3* (*GCR1*; ARNDT, STYLES and FINK 1989; BAKER 1991; DEVLIN *et al.* 1991), *SPT5* (SWANSON, MALONE and WINSTON 1991), *SPT10* (*CRE1*; FASSLER and WINSTON 1988; DENIS and MALVAR 1990), or *SPT11/12* (*HTA1/HTB1*; CLARK-ADAMS *et al.* 1988). Recently, *TSF3* was found to map to a new chromosomal location, and DNA cloning and sequencing revealed that *TSF3* and *SIN4*, a negative regulator of yeast *HO* (homothallism) gene transcription (NASMYTH, STILLMAN and KIPLING 1987; STERNBERG *et al.* 1987; NASMYTH and SHORE 1987; HERSKOWITZ 1989), are allelic (JIANG and STILLMAN 1992; CHEN *et al.* 1993). This indicates that the promoters for the *GAL* genes and mating-type genes share certain transcriptional silencing as well as activating (see PETERSON and HERSKOWITZ 1992) components and mechanisms.

Several results are compatible with the possibility that the *TSF1* to *TSF6* gene products comprise more than one silencing pathway. This is primarily suggested by the finding that the *tsf1* to *tsf6* mutations cause different categories of cell growth defects. For instance, *tsf2*, *tsf4* and *tsf5* each results in phenotypic suppression of the methionine auxotrophy in the genetic background used. Though the mechanism of suppression has not been worked out in this case, the simplest explanation is that these mutants either relieve transcriptional silencing at some uncharacterized *met* locus, or allow for overexpression of its gene product. This raises the possibility that of the six *TSF1* to *TSF6* genes, only these three are involved in this mechanism of transcriptional control. Consistent with the idea that *TSF2*, *TSF4* and *TSF5* are involved in a single pathway is the finding of unlinked non-complementation among *tsf2*, *tsf4* and *tsf5* mutations, which suggests direct protein-protein interaction among their gene products (STEARNS and BOTSTEIN 1988; SWANSON and WINSTON 1992). By contrast, only *tsf3* mutations caused severe defects in mating and sporulation, implying that *TSF3* is involved in a distinct pathway from the other *TSF* genes. Consistent with the above findings, double mutants between the *tsf3* deletion allele and *tsf2*, *tsf4*, or *tsf5* mutations exhibit additive effects on derepression of repressed promoters, lending further support to the possibility that more than one silencing mechanism has been identified by the *tsf1* to *tsf6* mutations. Multiple silencing mechanisms would ensure that expression of the *GAL* structural genes would remain at a very low basal level when galactose is not present.

It remains to be determined how the *TSF1* to *TSF6* gene products modulate gene expression. One possibility is that they directly mediate the interaction between upstream regulatory proteins and the basic transcription apparatus. According to this type of model, the respective proteins would function similarly to the yeast Gal11 protein, which is postulated to assist GAL4 in inducing transcription of the *GAL* structural genes (HIMMELFARB *et al.* 1990; NISHIZAWA

et al. 1990). While in this regard Gal11 has been viewed as a positive regulator, it also has been shown to act as a negative transcriptional regulator (FASSLER and WINSTON 1989), and a *gal11* mutation like *tsf* mutations activates constitutive expression from UAS-less *GAL1* or *CYC1* promoters and derepresses the uninduced level of transcription from the wild-type *GAL1* promoter, implying that the respective proteins share certain functional properties (CHEN *et al.* 1993). Several of our present findings, however, are difficult to reconcile by any simple mediator/potentiator model of *TSF* gene product function. Alternatively, the *TSF* genes may affect the structure or assembly of chromatin (see, for example, HIRSCHHORN *et al.* 1992; WINSTON and CARLSON 1992), which in turn mediates the selective activation of promoters. Consistent with this possibility, JIANG and STILLMAN (1992) determined that a *tsf3/sin4* mutation alters the superhelical density of circular DNA molecules, implying that *TSF3/SIN4* affects the structure/function of chromatin. Further suggestive evidence for a chromatin role is provided by the fact that depletion of histone H4 activates expression from UAS-less promoters, similarly to *tsf* mutations (HAN and GRUNSTEIN 1988; GRUNSTEIN 1990). A third model can be envisioned where one or more *TSF1* to *TSF6* gene products comprises or regulates the activity of some component(s) of the basal transcription apparatus (*e.g.*, a subunit of RNA polymerase II or one of the general transcription factors TFIIA to TFIIH). A general negative regulatory factor designated NC2 has been characterized (MEISTERERNST and ROEDER 1991) which has a regulatory effect compatible with this model of *TSF1* to *TSF6* function. NC2 appears to compete with TFIIA, a general factor required for the establishment of a productive initiation complex, for binding to TFIID (MEISTERERNST and ROEDER 1991). Distinguishing among these models is now underway. We expect further studies on *TSF1* to *TSF6* will enhance our understanding of the mechanisms by which gene transcription may be controlled by complex upstream regulatory regions.

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