The *SNF2, SNF5* and *SNF6* Genes Are Required for Ty Transcription in *Saccharomyces cerevisiae*

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ABSTRACT

The *Saccharomyces cerevisiae SNF2, SNF5* and *SNF6* genes were initially identified as genes required for expression of *SUC2* and other glucose repressible genes. The Suc$^-$ defect in all three of these classes of mutants is suppressed by mutations in the *SPT6* gene. Since mutations in *SPT6* had also been identified as suppressors of Ty and solo δ insertion mutations at the *HIS4* and *LYS2* loci, we have examined Ty transcription in *snf2, snf5* and *snf6* mutants and have found that Ty transcription is abolished or greatly reduced. The *snf2, snf5* and *snf6* defect for Ty transcription, like the defect for *SUC2* transcription, is suppressed by *spt6* mutations. In contrast to other mutations that abolish or greatly reduce Ty transcription (in the *SPT3, SPT7* and *SPT8* genes), mutations in these *SNF* genes do not cause suppression of insertion mutations. This result suggests that the SNF2, SNF5 and SNF6 gene products act by a distinct mechanism from the SPT3, SPT7 and SPT8 gene products to promote transcription of Ty elements. This result also suggests that a reduction of Ty transcription is not always sufficient for activation of adjacent gene expression.

TY elements of *Saccharomyces cerevisiae* are members of a diverse set of transposable genetic elements. This set of transposons is called retrotransposons, since their mode of replication and transposition resembles that of mammalian retroviruses (for a review see BOEKE 1989). Ty1 and Ty2 elements, which constitute the vast majority of Ty elements in the *S. cerevisiae* genome, have been studied in great detail. These elements are approximately 6 kb long and are flanked by long terminal repeats (LTRs) called δ sequences (CAMERON, LOH and DAVIS 1979). Typical laboratory strains of *S. cerevisiae* contain 25–30 Ty1 elements and 10 Ty2 elements (BOEKE, 1989).

Ty transcription initiates in the 5′ δ and terminates in the 3′ δ (ELDER, LOH and DAVIS 1983). This transcription has been shown to be dependent on sites in the δ sequences and sequences within the internal (ε) portion of the Ty element (LIAO, CLARE and FARABAUGH 1987; ERREDE *et al.* 1985; ROEDER, ROSE and PEARLMAN 1985; FULTON *et al.* 1988). Several *trans*-acting factors have been shown to regulate Ty transcription (see BOEKE 1989 for a review), and some of the *cis*-acting sites are potential sites of action for these factors. Indeed, ERREDE and AMMERER (1989) have demonstrated that the STE12 gene product is part of a complex that binds to a transcriptional control element in Ty DNA. In addition, EISENMANN, DOLLARD and WINSTON (1989) have shown that mutations in *SPT15,* which encodes the TATA-binding factor TFIID, affect transcription from δ sequences.

Several studies have demonstrated that when Ty elements transpose into the 5′ noncoding region of a gene they are able to inhibit or otherwise alter gene expression (for examples see ERREDE *et al.* 1980; ROEDER *et al.* 1980; WILLIAMSON, YOUNG and CIRIACY 1981; SIMCHEN *et al.* 1984; EIBEL and PHILIPPSEN 1984). In all of the cases that have been examined, this change in gene expression occurs at the transcriptional level. For Ty and solo δ insertion mutations that inhibit adjacent gene expression, selection for suppressor mutations has identified a large number of genes designated *SPT* (SPT = suppressor of Ty; WINSTON *et al.* 1984, 1987; FASSLER and WINSTON 1988). In these cases, mutations in *SPT* genes restore functional transcription to the adjacent gene (SILVERMAN and FINK 1984; WINSTON, DURBIN and FINK 1984; CLARK-ADAMS and WINSTON 1987; CLARK-ADAMS *et al.* 1988; FASSLER and WINSTON 1988; EISENMANN, DOLLARD and WINSTON 1989). Based on their mutant phenotypes, *SPT* genes fall into several classes, including one class, *SPT3, SPT7, SPT8* and *SPT15,* that is required for normal Ty transcription (WINSTON, DURBIN and FINK 1984; WINSTON *et al.* 1987; D. EISENMANN, C. DOLLARD and F. WINSTON, unpublished results).

Another *SPT* gene, *SPT6,* initially identified by mutant alleles that caused suppression of solo δ insertion mutations (WINSTON *et al.* 1984), is the same as *SSN20,* a gene identified as important in the expression of the *SUC2* gene (NEIGEBORN, RUBIN and CARLSON 1986; CLARK-ADAMS and WINSTON 1987; NEIGEBORN, CELENZA and CARLSON 1987). Mutations in *SPT6* suppress mutations in *SNF2, SNF5* and *SNF6,* genes required for high level expression of *SUC2*

(NEIGEBORN and CARLSON 1984; NEIGEBORN, RUBIN and CARLSON 1986; ABRAMS, NEIGEBORN and CARLSON 1986; ESTRUCH and CARLSON 1990). Suppression by *spt6* mutations was gene specific among the set of *SNF* genes: *spt6* mutations suppressed mutations in *SNF2, SNF5* and *SNF6,* but did not suppress mutations in *SNF1* or *SNF4,* two other positive activators of *SUC2* (NEIGEBORN and CARLSON 1984; NEIGEBORN, RUBIN and CARLSON 1986). *spt6* mutations have also been shown to suppress some mutations in *cis*-acting elements in *SUC2* (NEIGEBORN, CELENZA and CARLSON 1987). In addition, *SPT6* is the same gene as *CRE2; cre2* mutations were isolated as mutations that alleviate glucose repression of *ADH2* (DENIS 1984, DENIS and MALVAR 1990).

Since *spt6* mutations suppress δ and Ty insertion mutations as well as *snf2, snf5* and *snf6* mutations, we have investigated whether these three *SNF* genes are themselves required for normal Ty transcription and/or Ty-mediated gene expression. In this paper, we present results that demonstrate that *SNF2, SNF5* and *SNF6* are required for Ty transcription and for transcription from solo δ elements. In addition, we present evidence that this defect in Ty transcription does not cause suppression of insertion mutations and is therefore distinct from the defect in Ty transcription caused by mutations in the *SPT3, SPT7* and *SPT8* genes.

MATERIALS AND METHODS

Yeast strains: The *S. cerevisiae* strains used in this study are listed in Table 1. All strains are derived from strain S288C (*MATα gal2*) and are from our collection with the exception of strain MCY2006 (ESTRUCH and CARLSON 1990). The three insertion mutations at the *HIS4* locus, *his4–912δ, his4–917δ* and *his4–917,* have been described previously (FARABAUGH and FINK 1980; ROEDER *et al.* 1980). Insertion mutations at the *LYS2* locus, *lys2–61* and *lys2–128δ,* have also been described (SIMCHEN *et al.* 1984). The mutations *spt3–101, spt3–202* and *spt3Δ203::TRP1* are all *spt3* null alleles (WINSTON and MINEHART 1984; WINSTON, DURBIN and FINK 1984; HAPPEL 1989).

Media: Rich media (YPD), synthetic complete media lacking a specific amino acid (drop-out media; for example, SC-his), minimal media (SD), and sporulation media were made as described by SHERMAN, FINK and LAWRENCE (1978). For growth in minimal media, SD media was supplemented with required amino acids as described by SHERMAN, FINK and LAWRENCE (1978). GNA presporulation media contains (per liter) 10 g yeast extract, 30 g nutrient broth, and 50 g dextrose. Solid media contained 2% agar.

General genetic methods: Standard methods for mating, sporulation, and tetrad analysis were used (MORTIMER and HAWTHORNE 1969; SHERMAN, FINK and LAWRENCE 1978). Germination of *snf spt* mutants was improved by dissecting tetrads immediately after sporulation and by germinating spores at 23° on GNA media. Yeast cells were transformed by the lithium acetate method (ITO *et al.* 1983). Suppression of insertion mutations was scored both on supplemented SD media and on drop-out media (SC-his and SC-lys). In general, growth on both types of media was similar. For some *spt snf* double mutants, however, the supplemented SD media was a more permissive media than the drop-out media.

RNA isolation and Northern hybridization analysis: Cells for RNA isolation were grown in supplemented SD medium to 1.5–2.0 × 10^7 cells/ml, and yeast RNA was isolated as described by CARLSON and BOTSTEIN (1982). Blotting and hybridization with DNA probes was performed using the dextran sulfate method described in the GeneScreen protocols (New England Nuclear). RNA was cross-linked onto GeneScreen by exposure to UV radiation (1200 μW/cm², 2 min; CHURCH and GILBERT 1984). Hybridization with RNA probes was performed according to protocols provided by Stratagene (San Diego) with Bluescribe vectors.

Hybridization probes: ^{32}P-Labeled DNA probes were prepared using a Boehringer Mannheim nick translation kit. Plasmids used as DNA probes were as follows: pFW45, a *HIS4* internal 1.2-kb *BglII-SalI* restriction fragment in pBR322 (WINSTON *et al.* 1984); B161, an internal 1.2-kb *BglII* restriction fragment from Ty1 in pBR322 that hybridizes to both Ty1 and to Ty2 mRNA (R. SUROSKY, B-K. TYE and G. R. FINK, unpublished data); pFR2, containing the *PYK1* gene on a 3-kb *HindIII* fragment (kindly provided by P. SINHA); pCC69, an *ACT1* internal 1.6-kb *HindIII-BamHI* fragment in pBR322 (C. D. CLARK-ADAMS and F. WINSTON, unpublished data); and pYST138, containing a 0.24-kb *BglII-KpnI* restriction fragment of *TUB2* (SOM *et al.* 1988). ^{32}P-Labeled RNA hybridization probes were made with the Bluescribe system (Stratagene). Plasmid pAH99 (from which antisense *HIS4* [^{32}P]RNA probe was made) was constructed by cloning an *EcoRI-SalI* fragment from an M13 mp18 vector (YANISCH-PERRON, VIEIRA and MESSING 1985) that contained the 1.1-kbp *PvuII-SalI* fragment from the 5′ region of the *HIS4* gene (generously provided by KIM ARNDT) into the *EcoRI-SalI* sites of the M13 SK bluescribe vector (Stratagene). Plasmid pAH99 was digested with *HhaI* such that transcription initiated at the T7 promoter and extended to the *HhaI* site at +16 bp downstream of the *HIS4* +1 of transcription.

RNase protection: RNase protection was performed according to the procedure described by MELTON *et al.* (1984). For each sample, total RNA was hybridized with a ^{32}P-labeled anti-sense RNA probe and digested with 25 μg/ml RNase A and 6.5 μg/ml RNase T, for 15 min at room temperature. Protected transcripts were analyzed on a 5% polyacrylamide, 8 M urea sequencing gel. Either 1 μg of RNA (for strain S288C) or 7 μg of RNA (for strains FW1237 and MS1) was used for each RNase protection assay.

RESULTS

***SNF2, SNF5* and *SNF6* are required for Ty transcription:** Mutations in *SPT6* were isolated as suppressors of δ insertion mutations (WINSTON *et al.* 1984; FASSLER and WINSTON 1988). In addition, *spt6* mutations were isolated as suppressors of *snf2* and *snf5* mutations and were also found to suppress *snf6* mutations (NEIGEBORN, RUBIN and CARLSON 1986). These results suggested that *SNF2, SNF5* and *SNF6* may be required for normal expression of Ty elements. Therefore, we examined Ty transcription in several *snf* mutants (Figure 1). Interestingly, *snf2* and *snf5* null mutations abolish full length Ty transcription and *snf6–719,* a leaky mutation (ESTRUCH and CARLSON 1990), greatly reduces full length Ty transcrip-

TABLE 1

Yeast strains

Strain	Genotype
S288C	*MATα gal2 mal1-1*
0349	*MATα lys2-801 his4-539 ura3-52 snf1-Δ3*
MS1	*MAT***a** *his4-912δ lys2-128δ ura3-52 snf2-Δ1::HIS3*
0348	*MATα lys2-801 ura3-52 snf4-Δ1*
0347	*MAT***a** *his4-539 ade2-101 ura3-52 snf5-5::URA3*
0346	*MAT***a** *his4-539 ade2-101 ura3-52 snf6-719*
MS35	*MATα his4-912δ lys2-128δ ura3-52 snf2-Δ1::HIS3 spt4Δ::URA3*
MS6	*MATα snf2-Δ1::HIS3 spt5-194 his4-912δ lys2-128δ*
MS42	*MATα his4-912δ lys2-128δ ura3-52 snf2-Δ1::HIS3 spt6-140*
MS82	*MATα his4-917 lys2-128δ ura3-52 leu2*
MS84	*MAT***a** *spt6-140 spt3Δ203::TRP1 lys2-128δ his4-917 leu2 ura3-52*
MS85	*MAT***a** *spt6-140 lys2-128δ his4-917 ura3-52 leu2*
FW948	*MATα spt3-202 his4-917δ lys2-173R2 ura3-52 cry1*
FW1237	*MAT***a** *ura3-52 his4-912δ lys2-128δ*
FY2	*MATα ura3-52*
A704	*MAT***a** *ura3-52 snf2-Δ1::HIS3*
A726	*MATα ura3-52 snf5-5::URA3*
MCY2006	*MATα ura3-52 snf6-Δ2*
FY44	*MAT***a** *his4-912δ ura3-52 trp1Δ63*
FW822	*MATα his4-917δ ura3-52 trp1Δ1 lys2-61*
FW1337	*MATα lys2-128δ his4-917 trp1Δ1 leu2*
A739	*MATα spt3Δ203::TRP1 his4-912δ ura3-52 leu2Δ1*
FY51	*MAT***a** *spt3Δ203::TRP1 his4-917δ ura3-52 trp1Δ63 leu2Δ1*
A635	*MAT***a** *spt3-202 his4-917 ura3-52 leu2 lys2-128δ*
A742	*MAT***a** *spt3Δ203::TRP1 his4-912δ lys2-128δ leu2Δ1 ura3-52*
FW791	*MAT***a** *spt3-101 lys2-61 his4-917δ ura3-52*
A706	*MAT***a** *snf2-Δ1::HIS3 his4-912δ ura3-52*
A730	*MATα snf2-Δ1::HIS3 his4-917δ ura3-52*
A617	*MATα snf2-Δ1::HIS3 his4-917 lys2-128δ ura3-52*
A731	*MATα snf2-Δ1::HIS3 lys2-128δ ura3-52*
A755	*MAT***a** *snf2-Δ1::HIS3 lys2-61 his4-917δ ura3-52*
A701	*MATα snf5-5::URA3 his4-912δ lys2-128δ ura3-52*
FW1509	*MAT***a** *snf5-5::URA3 his4-917δ lys2-61 ura3-52*
A618	*MAT***a** *snf5-5::URA3 his4-917 lys2-128δ ura3-52 leu2*
FW1499	*MATα snf5-5::URA3 lys2-61 ura3-52*
A746	*MATα snf2-Δ1::HIS3 spt3Δ203::TRP1 his4-912δ ura3-52*
A744	*MATα snf2-Δ1::HIS3 spt3Δ203::TRP1 his4-917δ ura3-52*
FW1798	*MATα snf2-Δ1::HIS3 spt3Δ203::TRP1 his4-917 lys2-128δ ura3-52*
A754	*MATα snf2-Δ1::HIS3 spt3-101 lys2-61 ura3-52*
A748	*MATα snf5-5::URA3 spt3Δ203::TRP1 his4-912δ lys2-128δ ura3-52*
FW1531	*MATα snf5-5::URA3 spt3Δ203::TRP1 his4-917δ lys2-61 ura3-52 trp1Δ1*
FW1533	*MAT***a** *snf5-5::URA3 spt3Δ203::TRP1 lys2-61 his4-917δ trp1Δ1*
MS39	*MATα snf2-Δ1::HIS3 spt6-140 lys2-128δhis4-912δ ura3-52*

tion. However, Ty transcription is not affected by *snf1* or *snf4* mutations. Thus, the three *SNF* genes that are required for Ty transcription, *SNF2, SNF5* and *SNF6,* are the same as those that, when mutant, are suppressed by *spt6*. The second, shorter Ty transcript seen in Figure 1 (ELDER, LOH and DAVIS 1983; WINSTON, DURBIN and FINK 1984) may be reduced in some *snf* mutants; however, due to variability in the levels observed for this transcript, no strong conclusion can be made. (In this paper, Ty1 plus Ty2 transcription is referred to as "Ty transcription." Transcription of α-factor stimulated Ty3 elements (VAN ARSDELL, STETLER and THORNER 1987; CLARK *et al.* 1988) is unaffected by these *snf* mutations (M. S. SWANSON and F. WINSTON, unpublished results) and transcription of Ty4 elements (STUCKA, LOCHMULLER and FELDMANN 1989) has not been examined in these *snf* mutants).

Analysis of Ty transcription in *spt snf* mutants: Previous work demonstrated that *snf2, snf5* and *snf6* mutants are defective for *SUC2* transcription (ABRAMS, NEIGEBORN and CARLSON 1986; ESTRUCH and CARLSON 1990). Suppression of these *snf* mutations by *spt6* mutations occurs at the transcriptional level (NEIGEBORN, CELENZA and CARLSON 1987; ESTRUCH and CARLSON 1990). In addition, we have recently shown that mutations in two other *SPT* genes, *SPT4* and *SPT5,* also partially suppress the Suc$^-$ phenotype of *snf2* mutants (M. S. SWANSON and F. WINSTON, unpublished results). Therefore, we examined

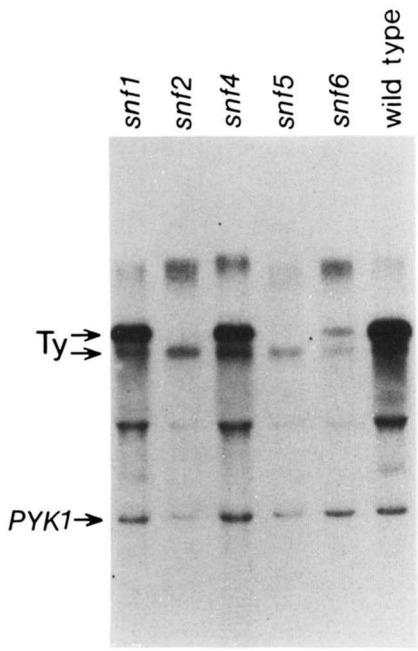

FIGURE 1.—Northern analysis of transcription of Ty elements in *snf* mutants. Total RNA was isolated from strains (from left to right): 0349, MS1, 0348, 0347, 0346 and S288C, and hybridized with ^{32}P-labeled B161 (Ty) and pFR2 (*PYK1*) DNA. By measurement of *PYK1* hybridization the amount of RNA run in each lane differed by less than twofold. The diffuse band above the Ty RNAs is observed in Northerns probed with a Ty probe; its identity is unknown, but it may be caused by hybridization to DNA.

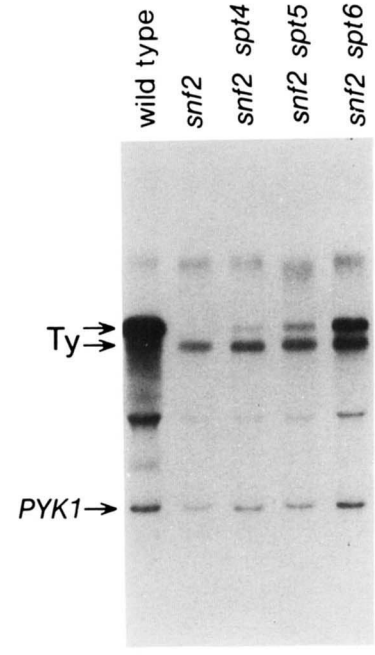

FIGURE 2.—Northern analysis of transcription of Ty elements in *snf spt* double mutants. Total RNA was isolated from strains (from left to right): S288C, MS1, MS35, MS6, MS42 and hybridized with ^{32}P-labeled B161 (Ty) and pFR2 (*PYK1*) DNA.

Ty transcription to determine whether *spt4*, *spt5* and *spt6* mutations also suppress the Ty transcription defect of *snf2* mutants. The results demonstrate that mutations in all three of these *SPT* genes restore Ty transcription in a *snf2* mutant, although to different extents: the *spt4* null mutation suppresses weakly, an *spt5* mutation suppresses at an intermediate level, and an *spt6* mutation is a strong suppressor (Figure 2). (Since *SPT5* and *SPT6* are essential for growth (NEIGEBORN, CELENZA and CARLSON 1987; CLARK-ADAMS and WINSTON 1987; SWANSON, MALONE and WINSTON 1991), we could not use *spt5* and *spt6* null alleles in these experiments.) Since both of the *snf2* transcription defects (for *SUC2* and Ty transcription) are suppressed by *spt6*, as well as *spt4* and *spt5*, suppression of these defects probably occurs by a similar mechanism.

Another group of genes, *SPT3*, *SPT7* and *SPT8*, has previously been shown to be required for Ty transcription (WINSTON, DURBIN and FINK 1984; WINSTON *et al.* 1987). Since *spt6* mutations strongly suppress the Ty transcription defect caused by a *snf2* null mutation, we examined whether they also suppress the Ty transcription defect caused by an *spt3* null mutation. The results (Figure 3) demonstrate that there is only weak suppression of *spt3* by *spt6*, suggesting that the *spt3* and *snf2* transcription defects are distinct from each other.

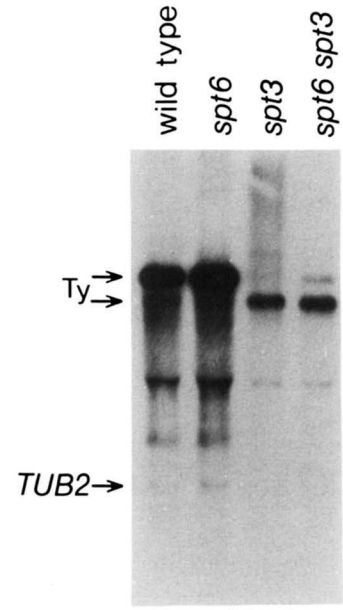

FIGURE 3.—Northern analysis of transcription of Ty elements in *spt3 spt6* double mutants. Total RNA was isolated from strains (from left to right): MS82, MS85, FW948, and MS84 and hybridized with ^{32}P-labeled B161 (Ty) and pYST138 (*TUB2*).

***snf* mutations do not confer Spt$^-$ phenotypes:** Previous work had shown that *spt3*, *spt7*, and *spt8* mutations cause suppression of Ty and δ insertion mutations (WINSTON, DURBIN and FINK 1984; WINSTON *et al.* 1984, 1987). In the previous section, we demonstrated that, like *SPT3*, *SPT7* and *SPT8*, the *SNF2*, *SNF5* and *SNF6* genes are also required for Ty transcription. Therefore, we tested whether *snf2* and *snf5*

TABLE 2

Suppression of insertion mutations in *snf*, *spt* and *snf spt* mutants

Relevant genotype	Insertion mutation				
	his4-912δ	*his4-917δ*	*his4-917*	*lys2-128δ*	*lys2-61*
Wild type	cs	−	−	−	cs
spt3	+	+	+	+	+
snf2	−	−	−	−	−
snf5	−	−	−	−	−
spt3 snf2	∓	±	−	−	−
spt3 snf5	∓	±	−	−	−
spt6 snf2	+	ND	ND	+	ND

Ty and δ insertion phenotypes in *snf* mutants. Phenotypes of strains containing the *lys2-61* and *his4-912δ* insertion mutations were scored at 23° and 37° and were the same at both temperatures unless otherwise indicated. + indicates growth; ± indicates weak growth; ∓ indicates very weak growth; − indicates no growth; and cs indicates no growth at 23° and weak growth at 37°. Phenotypes were scored on minimal media lacking either lysine or histidine as compared to growth on a completely supplemented minimal plate.

mutations suppress Ty and δ insertion mutations. Our results (Table 2) demonstrate that neither *snf2* nor *snf5* mutations suppress any of the Ty or solo δ insertion mutations tested. In fact, *snf2* and *snf5* mutations had the opposite effect on two cold-sensitive insertion mutations, *lys2–61* and *his4–912δ*. Normally these insertion mutations confer auxotrophies at low temperature (23°; Lys$^-$ for *lys2–61* and His$^-$ for *his4–912δ*) but not at high temperature (37°). Mutations in *SPT* genes suppress the auxotrophic defects at low temperature. In *snf2* or *snf5* backgrounds, *his4–912δ* and *lys2–61* conferred auxotrophies at all temperatures tested, including 37° (Table 2). The result that *snf2* and *snf5* mutations do not confer Spt$^-$ phenotypes again suggests that they play a different role from *SPT3, SPT7* and *SPT8* in Ty transcription.

To determine the epistasis relationship between these *snf* and *spt* mutations, *spt3 snf2* and *spt3 snf5* double mutants were constructed and tested for suppression of Ty and δ insertion mutations. The results demonstrate that *snf2* and *snf5* mutations are epistatic to *spt3* mutations for suppression of some, but not all insertion mutations tested (Table 2). For suppression of *lys2–61, lys2–128δ* and *his4–917,* both *snf2* and *snf5* were epistatic to *spt3*; for suppression of *his4–917δ* and *his4–912δ,* intermediate phenotypes were observed. All *spt snf* double mutants were extremely sick and double mutant spores germinated at a poor frequency during strain constructions. These results demonstrate that, at least in some cases, *SNF2* and *SNF5* function is required for *spt3*-mediated suppression.

***snf2* mutations alter transcription of *his4–912δ*:** Based on the *snf2 his4–912δ* phenotype (nonconditional His$^-$) it seemed likely that *snf2* was affecting transcription of genes adjacent to solo δ insertion mutations as well as transcription of Ty elements.

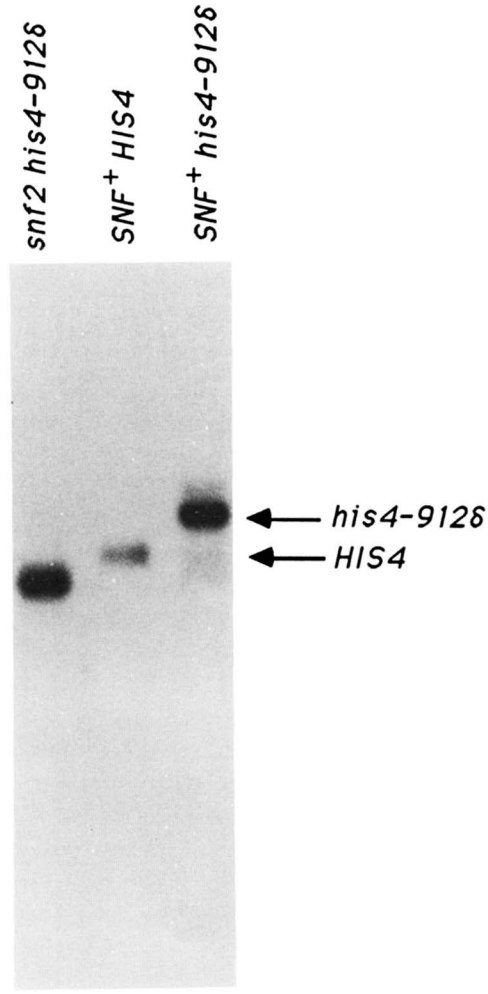

FIGURE 4.—Northern analysis of transcription of the *his4–912δ* insertion allele in a *snf2* mutant. Total RNA was isolated from strains (left to right): MS1, S288C, FW1237. Hybridization to the *PYK1* gene shows that equal amounts of RNA were run in lanes 1 and 3 (data not shown). Lane 2 contains approximately tenfold less RNA than other lanes.

Therefore, transcription of *his4–912δ* was examined in *snf2* mutants. Strains that contain the *his4–912δ* mutation and that are grown at 23° or 30° initiate transcription primarily in the 912δ sequence, producing a transcript that is nonfunctional, presumably due to translational starts and stops upstream of the *HIS4* ATG (SILVERMAN and FINK 1984; HIRSCHMAN, DURBIN and WINSTON 1988). Northern analysis demonstrates that in the *snf2 his4–912δ* double mutants, the pattern of *his4–912δ* transcription is altered (Figure 4). In such strains, there are no transcripts the same length as those that initiate at the δ or at the *HIS4* wild-type initiation site; instead, a novel nonfunctional *HIS4* transcript that is shorter than the wild-type *HIS4* transcript is produced.

To determine the nature of the change in the *HIS4* transcripts in *snf2 his4–912δ* strains, RNase protection experiments were performed to determine the 5′ end of the novel *his4–912δ* transcript. One major pro-

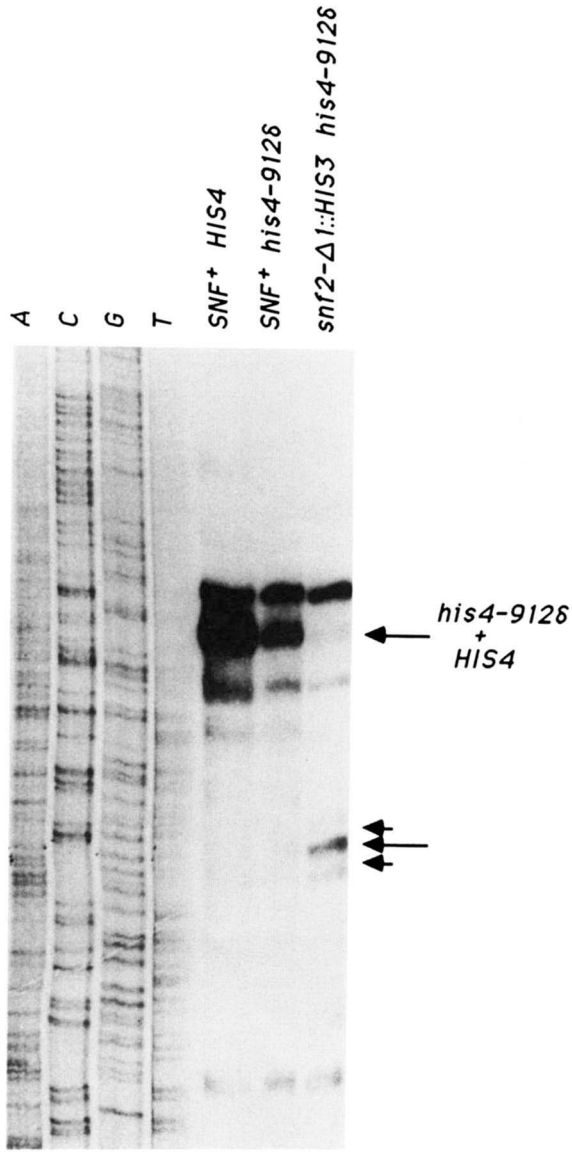

FIGURE 5.—RNase protection of *HIS4* transcripts in a *snf2 his4–912δ* mutant. Total RNA was hybridized with a ^{32}P-labeled ribonucleotide probe that contained anti-sense *HIS4* sequences from +532 to +16 nucleotides from the +1 of transcription initiation (*Sal*I to *Hha*I). Total RNA from strains (left to right): S288C, FW1237, MS1. Either 1 μg of RNA (S288C) or 7 μg of RNA (FW1237 and MS1) was used in each RNase protection assay. Transcripts that initiated at either the wild-type *HIS4* or the 912δ initiation sites will give rise to a protected band of the identical size since the probe only extends to +16 with respect to the wild type *HIS4* initiation site. The upper band is due to undigested riboprobe. A major protected fragment is marked by the large arrow. The DNA sequence run adjacent to the RNase samples was used to approximate the size of the protected transcripts. The position of the 5′ end of the new HIS4 transcript was calculated based on the fact that RNA migrates 5–10% slower than DNA under the conditions used (SAMBROOK, FRITSCH and MANIATIS 1989). In the RNase experiments, several protected RNAs appeared in all lanes and were present without the addition of yeast RNA (not shown). These protected fragments were specific for the individual probe used in the experiment and may be due to secondary structures in the antisense RNA probe that were resistant to RNase digestion. Several other transcripts (marked by small arrows) are weakly protected. Some of these putative weak initiation sites may be artifacts due to non-uniform synthesis of the probe, although the vast majority of the antisense RNA probe was of uniform length as judged by the intensity of a single band upon electrophoresis (not shown).

tected fragment in *snf2 his4–912δ* strains corresponds to initiation at approximately 70 bp downstream of the wild-type *HIS4* transcription initiation site, placing it at a site approximately 7 bases 3′ of the ATG of the *HIS4* coding region (Figure 5). Therefore, in *snf2* strains, *his4–912δ* transcription fails to initiate at either the previously identified δ or *HIS4* transcription initiation sites; instead, transcription apparently initiates at a new site, internal to the *HIS4* coding region.

Altered transcription of *his4–912δ* in *snf2* mutants could be due to altered use of transcription signals in either the δ or *HIS4* sequences. Since total Ty transcription is severely reduced in *snf2* strains, it seems likely that most of the *snf2* effect on *his4–912δ* is caused via the transcription signals in the δ sequence. To address directly if *snf* mutations alter use of the *HIS4* transcription signals, we analyzed *HIS4*$^+$ transcription in wild type, *snf2, snf5,* and *snf6* strains. The results demonstrate that, while there is a mild reduction in *HIS4* transcript levels, there are no *HIS4* transcripts of altered size, as detected by Northern analysis (Figure 6). Therefore, the *snf2* effects on the transcription pattern of *his4–912δ* are dependent on the presence of δ sequences, rather than *HIS4* sequences.

DISCUSSION

In this paper, we have described transcriptional defects conferred by mutations in *SNF2, SNF5* and *SNF6*. These mutations abolish transcription of Ty1 and Ty2 elements as well as transcription from at least some solo δ elements. These results, taken together with previous results demonstrating the requirement of these genes for normal expression of *SUC2* and other genes (CARLSON, OSMOND and BOTSTEIN 1981; NEIGEBORN and CARLSON 1984; ABRAMS, NEIGEBORN and CARLSON 1986; ESTRUCH and CARLSON 1990; LAURENT, TREITEL and CARLSON 1990; MOEHLE and JONES 1990), suggest that *SNF2, SNF5* and *SNF6* are required for normal levels of transcription of a large set of genes in yeast. In addition to our results, recent work has shown that *TYE3* and *TYE4,* which are required for Ty-mediated expression of *ADH2* (CIRIACY and WILLIAMSON 1981), are identical to *SNF2* and *SNF5,* and that *tye3* and *tye4* mutants have very low levels of Ty transcripts (M. CIRIACY, personal communication).

In addition to *SNF2, SNF5* and *SNF6,* the *SPT3, SPT7* and *SPT8* genes are also required for transcription of Ty elements and from solo δ sequences (WINSTON *et al.* 1984, 1987). The results in this paper show that these two sets of genes almost certainly act by

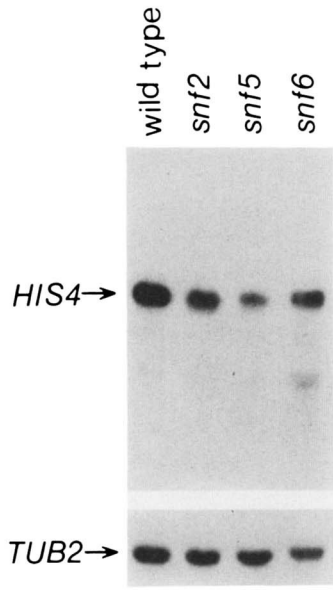

FIGURE 6.—Northern analysis of transcription of HIS4. Total RNA was isolated from strains (left to right) FY2, A704, A726 and MCY2006 and hybridized to the *HIS4* probe pFW45 and the *TUB2* probe, pYST138.

different mechanisms to promote Ty transcription. First, unlike the *spt3, spt7* and *spt8* mutations, the *snf2, snf5* and *snf6* mutations do not suppress Ty or solo δ insertion mutations. Second, although mutations in all six of these genes eliminate δ-initiated transcription, *snf2* mutants differ from *spt3* mutants with respect to their patterns of *his4–912δ* transcription: *spt3 his4–912δ* mutants produce wild type length *HIS4* transcripts (SILVERMAN and FINK 1984), while *snf2 his4–912δ* strains produce a shorter nonfunctional *HIS4* transcript (Figure 4). Third, *spt6* mutations strongly suppress the Ty transcription defect caused by *snf2, snf5* and *snf6* mutations, but only weakly affect the Ty transcription defect caused by *spt3* mutations. Finally, unlike *snf2, snf5* and *snf6* mutants, *spt3* mutants are not defective for *SUC2* expression (M. S. SWANSON and F. WINSTON, unpublished results).

The effect of a *snf2* mutation on transcription initiation at *his4–912δ* has yielded some insight into possible mechanisms of action for the SNF2, SNF5 and SNF6 gene products. Previous work suggested that the two promoters at *his4–912δ* (the δ and *HIS4* promoters) are in competition with each other and that a decrease in initiation from the δ initiation site allows an increase in transcription at the wild-type *HIS4* initiation site (HIRSCHMAN, DURBIN and WINSTON 1988). This result has been observed for both *cis*- and *trans*-acting mutations (SILVERMAN and FINK 1984; WINSTON, DURBIN and FINK 1984; EISENMANN, DOLLARD and WINSTON 1989). In *snf2* mutants, a different pattern is observed: the decrease in the δ-initiated transcript is accompanied by the production of an aberrant shorter transcript whose 5′ end is internal to the *HIS4* coding sequence (Figures 4 and 5). No initiation from the wild-type *HIS4* site is detectable, suggesting that transcription initiation at the normal *HIS4* +1 is blocked in a *snf2* mutant. Since *snf2* is at least partially suppressed by *spt6* for both the Ty transcription defect (Figure 2) and the inhibition of *his4–912δ* expression (Table 2), this "block" in initiation at the normal *HIS4* +1 may be conferred by either the SPT6 gene product itself or by something controlled or modified by SPT6. Whatever the mechanism, our experiments have shown that the *snf2* effect on *his4–912δ* transcription causes differential changes in transcription initiation at three sites: a decrease from the normal δ and *HIS4* initiation sites and an increase from a new site within the *HIS4* coding sequence.

The suppression of *snf2, snf5* and *snf6* by *spt6* mutations suggests that the *SNF2, SNF5* and *SNF6* genes may act by affecting chromatin structure. The mutant phenotypes conferred by *spt6* mutations are very similar to those caused by altered dosage of the genes that encode histones H2A and H2B, including suppression of *snf2, snf5,* and *snf6* mutations (CLARK-ADAMS *et al.* 1988; CLARK-ADAMS 1988; S. A. BROWN, J. N. HIRSCHHORN and F. WINSTON unpublished results). In addition, as for histone genes, increased or decreased *SPT6* gene dosage causes suppression of insertion mutations and of certain *snf* mutations (CLARK-ADAMS and WINSTON 1987; NEIGEBORN, CELENZA and CARLSON 1987; CLARK-ADAMS *et al.* 1988; CLARK-ADAMS 1988). Recent results of LAURENT, TREITEL and CARLSON (1990) have suggested that *SNF5* encodes a transcriptional activator. One model that can account for these results is that *SPT6* normally represses transcription via maintaining chromatin in a repressing structure. The role of *SNF2, SNF5,* and *SNF6,* then, would be to counteract repression by *SPT6*. Further genetic and biochemical characterization of the *SPT6, SNF2, SNF5,* and *SNF6* functions will help to elucidate their roles in transcription in yeast.

Analysis of the effect of *snf* mutations on the phenotype of Ty insertion mutations has also shown that reduction in the level of Ty transcription is not always sufficient for suppression of Ty insertion mutations. Previously, several experiments suggested that Ty and δ insertion mutations that inhibit or activate adjacent gene transcription do so by promoter competition (BOEKE, STYLES and FINK 1986; CONEY and ROEDER 1988; HIRSCHMAN, DURBIN and WINSTON 1988). For example, mutations in δ sequences that reduce δ-initiated transcription do result in increased adjacent gene transcription for both a Ty insertion mutation (CONEY and ROEDER 1988) and a solo δ insertion mutation (HIRSCHMAN, DURBIN and WINSTON 1988). In contrast, *snf2* and *snf5* mutations eliminate Ty and

solo δ-initiated transcription, yet they do not suppress insertion mutations. While in the case of *his4–912δ*, a *snf2* mutation does allow production of a nonfunctional *HIS4* transcript, *snf2 his4–917* mutants do not produce *HIS4* transcripts (A. M. HAPPEL and F. WINSTON, unpublished). This result shows that a reduction of Ty transcription does not always cause suppression of Ty insertion mutations. This result is also consistent with the model of repression of transcription initiation over a region of DNA, perhaps via altered chromatin structure, in *snf2, snf5* and *snf6* mutants.

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