Ty Element-Induced Temperature-Sensitive Mutations of *Saccharomyces cerevisiae*

Koichi Kawakami,* Brenda K. Shafer,† David J. Garfinkel,† Jeffrey N. Strathern† and Yoshikazu Nakamura*

* *Department of Tumor Biology, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108, Japan, and*
† *Laboratory of Eukaryotic Gene Expression, Frederick Cancer Research and Development Center, ABL-Basic Research Program, Frederick, Maryland 21701-1201*

Manuscript received September 16, 1991
Accepted for publication April 24, 1992

ABSTRACT

Temperature-sensitive mutants of *Saccharomyces cerevisiae* were isolated by insertional mutagenesis using the *HIS3* marked retrotransposon Ty*H3HIS3*. In such mutants, the Ty*HIS3* insertions are expected to identify loci which encode genes essential for cell growth at high temperatures but dispensable at low temperatures. Five mutations were isolated and named *hit* for *h*igh *t*emperature growth. The *hit1-1* mutation was located on chromosome *X* and conferred the *pet* phenotype. Two *hit2* mutations, *hit2-1* and *hit2-2*, were located on chromosome *III* and caused the deletion of the *PET18* locus which has been shown to encode a gene required for growth at high temperatures. The *hit3-1* mutation was located on chromosome *VI* and affected the *CDC26* gene. The *hit4-1* mutation was located on chromosome *XIII*. These *hit* mutations were analyzed in an attempt to identify novel genes involved in the heat shock response. The *hit1-1* mutation caused a defect in synthesis of a 74-kD heat shock protein. Western blot analysis revealed that the heat shock protein corresponded to the *SSC1* protein, a member of the yeast hsp70 family. In the *hit1-1* mutant, the Ty*HIS3* insertion caused a deletion of a 3-kb DNA segment between the δ1 and δ4 sequences near the *SUP4* locus. The 1031-bp wild-type *HIT1* DNA which contained an open reading frame encoding a protein of 164 amino acids and the AGG arginine tRNA gene complemented all *hit1-1* mutant phenotypes, indicating that the mutant phenotypes were caused by the deletion of these genes. The pleiotropy of the *HIT1* locus was analyzed by constructing a disruption mutation of each gene in vitro and transplacing it to the chromosome. This analysis revealed that the *HIT1* gene essential for growth at high temperatures encodes the 164-amino acid protein. The arginine tRNA gene, named *HSX1*, is essential for growth on a nonfermentable carbon source at high temperatures and for synthesis of the *SSC1* heat shock protein.

TEMPERATURE-sensitive mutants of *Saccharomyces cerevisiae* have been isolated to identify genes required for cell division cycle, macromolecular synthesis and other essential processes. These temperature sensitive mutations are thought in most cases to be missense mutations in essential genes (reviewed by PRINGLE 1975). On the other hand, some genes are essential for growth only at high temperatures; for instance, *PET18* (TOH-E and SAHASAHI 1985), *SSN6* (SCHULTZ and CARLSON 1987), *SGP2* (*DPR1*) (NAKAYAMA, ARAI and MATSUMOTO 1988), *VPS33* (BANTA *et al.* 1990), *VPS34* (HERMAN and EMR 1990) and *MFT1* (GARRETT *et al.* 1991). However, a genetic approach to identify such genes has not been performed.

S. cerevisiae cells respond to temperature shift from 22° to 37° by synthesis of a set of proteins (MILLER, XUONG and GEIDUSCHEK 1979) called heat shock proteins (hsps). Yeast hsp genes and hsp-related genes have been identified mostly on the basis of high homology to the hsp genes of other organisms or its own hsp genes (reviewed by LINDQUIST and CRAIG 1988). Analyses of mutations of these hsp genes have suggested that the heat shock response affects growth at high temperatures within a normal growth range. For instance, the *ssa1 ssa2* strain which carries mutations in two hsp70-related genes cannot grow at 37° (CRAIG and JACOBSEN 1984). However, roles of the heat shock response in high temperature growth are generally unclear. In *Escherichia coli*, the *rpoH* gene product, the σ^{32} subunit of the prokaryotic RNA polymerase holoenzyme, regulates the heat shock transcription and is required for growth at high temperatures but dispensable at low temperatures (YURA *et al.* 1984). This fact encouraged us to isolate *S. cerevisiae* factors carrying the similar character.

In this paper we sought to identify *S. cerevisiae* genes essential for cell growth at high temperatures but dispensable at low temperatures. The major problem in identifying mutations in such genes is to distinguish them from temperature sensitive mutations in genes essential at all temperatures. To enrich for mutations

of the desired type, we used the retrotransposon Ty*1* as an insertional mutagen. It was our expectation that the insertion of the Ty element is more likely to generate null alleles than temperature-sensitive alleles. In *E. coli*, the *htrA* gene, a gene essential for growth only at high temperatures, has been isolated by screening temperature-sensitive mutants generated by random insertion of a prokaryotic transposon, Tn*10* (LIPINSKA *et al.* 1989).

The Ty*1* element of *S. cerevisiae* is a retrotransposon with a broad insertion specificity (reviewed by BOEKE 1988). Ty insertion can disrupt genes causing their inactivation or can alter expression of genes by inserting into their regulatory regions. By placing the Ty element under the control of the *GAL1* promoter, the transposition of Ty elements can be regulated and increased to high levels (BOEKE *et al.* 1985). The usefulness of the Ty element as an insertional mutagen has been increased by constructing an element that carries selectable markers (GARFINKEL *et al.* 1988). This Ty element mutagenesis system has been used to isolate α-factor resistant mutants (WEINSTOCK *et al.* 1990; MASTRANGELO *et al.* 1992).

Here we report the isolation of temperature-sensitive mutants of *S. cerevisiae* by Ty mutagenesis and the identification of genes essential for cell growth at high temperatures. One of the *S. cerevisiae* mutants isolated here, *hit1-1*, had a defect in synthesis of a heat shock protein. Analysis of the mutant revealed genes involved in high temperature growth and the heat shock response.

MATERIALS AND METHODS

Yeast strains, media and genetic methods: The genotypes of yeast strains used in this study are described in Table 1. Standard genetic methods and media have been described (SHERMAN, FINK and HICKS 1986).

Plasmids and phages: The plasmid pGTy*H3HIS3* has been described previously (GARFINKEL *et al.* 1988). A YCp50-yeast genomic library is a kind gift from M. ROSE (ROSE *et al.* 1987). The plasmids p18-2 and p18-4 were isolated from this library. The subclone plasmids p18-4S and p18-4C, were constructed by digestion of the p18-4 DNA with *Sma*I and *Cla*I, respectively, and self-ligation. The plasmid p18-4Δ15 was constructed by ligating the 1031-bp wild-type *HIT1* DNA generated by digestion of a subclone plasmid with exonuclease III between the *Eco*RI and *Hin*d III sites of YCp50. pKK39 carrying the disruption mutation of the *HIT1* gene was constructed by deleting the *Hin*cII-*Spe*I segment from p18-4Δ15, converting the junction into a *Cla*I site and inserting the 1-kb *HIS3* DNA from pGTy*H3HIS3* into the *Cla*I site. pKK60 carrying the tRNA disruption mutation was constructed by converting the *Mlu*I site of p18-4Δ15 into a *Cla*I site and inserting the 1-kb *HIS3* DNA. Plasmids pIC4 and pIC12 carrying the *PET* and *MAK* genes and the gene required for high temperature growth of the *PET18* locus were generously provided by I. CHIU. pYS10 carrying the *PET18* DNA (TOH-E and SAHASHI 1985) was generously provided by A. TOH-E. Plasmids carrying the *URA2* (SOUCIET *et al.* 1987) and the *RAD52* (ADZUMA, OGAWA and OGAWA 1984) genes were generously provided by T. BURKETT. The pAL18 (KANEKO *et al.* 1989) plasmid carrying the *PEP4* gene (AMMERER *et al.* 1986; WOOLFORD *et al.* 1986) was generously provided by S. HARASHIMA. The VI-2F8 phage carrying the *CDC4* gene (YOCHEM and BYERS 1987) was generously provided by S. YOSHIKAWA (unpublished data).

A λEMBL4- and a λZAP-genomic libraries were constructed by digesting the *hit1-1* mutant KK80 DNA with *Eco*RI and ligating into the same site of the λEMBL4 (FRISCHAUF *et al.* 1983) and the λZAP (SHORT *et al.* 1988) phages. λKK1 and λKK2 were isolated from the λEMBL4-genomic library and the λZAP-genomic library by plaque hybridization using a *HIS3* DNA probe, respectively.

Isolation of temperature sensitive *hit* mutants: Yeast DG662 cells were mutagenized by induction of the galactose regulated Ty*H3HIS3* element as described previously (GARFINKEL *et al.* 1988) and seven independent pools of the mutagenized cells were constructed. These cells were grown on SC-his plates and incubated at 20°. After formation of about 300 small colonies per plate, colonies were replica-plated on two YPD plates. One was incubated at 20° for about 3 days and the other was incubated at 37° for 24 hr to screen temperature sensitive colonies.

Protein labeling, electrophoresis and immunological analysis: Pulse labeling of yeast heat shock proteins (CRAIG and JACOBSEN 1984) and two-dimensional gel electrophoresis of the labeled yeast protein was conducted as described previously (O'FARRELL 1975; MILLER, XUONG and GEIDUSCHEK 1979; IIDA and YAHARA 1984) with slight modifications. Yeast cells were grown at 23° in a SC-met or SC-met-ura medium, and shifted to 37° at a cell density of about 1 × 10^7 cells/ml. Samples of 0.5 ml cells were labeled before and after the temperature shift-up with 15 μCi [^{35}S]methionine (Amersham) for 15 min. The labeling was terminated by adding 12.5 μl 10 mg/ml cycloheximide and chilling on ice. When samples were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, the cell pellets were resuspended in 100 μl 10% (w/v) trichloroacetic acid (TCA) and broken by vigorous mixing with 0.1 g glass beads (425–600 μm, Sigma), the resulting TCA-precipitates were washed with acetone, suspended in 50-μl sample buffer (10% glycerol; 0.12 M Tris-HCl, pH 6.8; 5% v/v β-mercaptoethanol; 2% SDS) and boiled for 10 min. When samples were subjected to two-dimensional gel electrophoresis, the same cell pellets were washed with 0.25 ml of 20 mM Tris-HCl (pH 8.8)/2 mM $CaCl_2$, vortexed for 4 × 30 s with 0.1 g of the glass beads and 2.5 μl of 100 mM phenylmethylsulfonyl fluoride (PMSF), suspended in 200 μl of Tris-HCl/$CaCl_2$ and transferred to new tubes. The lysates were mixed with 10 μl of 1 mg/ml micrococcal nuclease, incubated for 5 min on ice, then mixed with 20 μl of 2% SDS/10% 2-mercaptoethanol, 1 mg/ml pancreatic DNase I, 2 mg/ml RNase A and 0.5 M Tris-HCl (pH 7.0)/50 mM $MgCl_2$, incubated for 5 min on ice, lyophilized, resuspended in 100 μl of lysis buffer. The gels were dried and analyzed by autoradiography.

Western blotting was conducted by a standard method (SAMBROOK, FRITSCH and MANIATIS 1989). The *SSC1* antibody was generously provided by E. CRAIG. The antibodies hybridizing to the filter were detected with SuperScreen immunoscreening system (Amersham) and 3,3′-diaminobenzidine tetrahydrochloride.

PCR analysis of the *HIT1* locus: Oligonucleotides, K1; GTACAAATCTGGATTTTCTG, K2; GCATTATGTATCATTGCAGC, K3; ACTTACACGATGGAAATCAA and K4; CTCGAATGATAATGGAAGGA, were synthesized to analyze the structure of the *HIT1* region. The positions and the directions of these oligonucleotides are

TABLE 1

***S. cerevisiae* strains**

Strain	Genotype	Source
YH8	*MATα his3Δ200 leu2Δ trp1Δ1 ura3-167*	GARFINKEL *et al.* (1988)
DG662	YH8 harboring pGTy*H3HIS3*	GARFINKEL *et al.* (1988)
JSS104-4A	*MATa ade2-101 his3Δ200 lys2-801 ura3-52*	Laboratory stock
JSS104-21B	*MATa ade2-101 his3Δ200 gal*	Laboratory stock
JSS108-2C	*MATa his3Δ200 lys2-801 tyr7-1 ura3-52*	Laboratory stock
JSS110-5C	*MATa arg4-17 his3Δ200 tyr7-1 ura3-52*	Laboratory stock
KK16	*MATα his3Δ200 lys9* and/or *lys2 ura3 met10 ade1 pet9*	Laboratory stock
KK80	*MATa his3Δ200 lys2-801 trp1Δ1 ura3 hit1-1*	Ts18 × JS108-2C
KK88	*MATα his3Δ200 lys2-801 trp1Δ1 ura3 hit1-1*	KK80 × YH8
KK90	*MATa arg4-17 his3Δ200 leu2Δ trp1Δ1 tyr7-1 ura3 hit2-1*	Ts2 × JSS110-5C
KK91	*MATa ade2-101 his3Δ200 leu2Δ lys2-801 ura3 hit2-2*	Ts7 × JSS104-4A
KK93	*MATa his3Δ200 lys2-801 ura3 hit3-1*	Ts12 × JSS104-4A
KK94	*MATa his3Δ200 lys2-801 ura3 hit4-1*	Ts22 × JSS104-4A
KK106	*MATa ade2-101 his3Δ200 leu2Δ trp1Δ1 ura3 hit2-2*	KK91 × YH8
KK107	*MATa leu2Δ trp1Δ1 his3Δ200 ura3 hit3-1*	KK93 × YH8
KK110	*MATa leu2Δ trp1Δ1 his3Δ200 ura3 hit4-1*	KK94 × YH8
KK111	*MATa arg4-17 his3Δ200 leu2Δtrp1Δ1 ura3 hit2-1*	KK90 × YH8
KK126	*MATα his3Δ200 leu2Δ trp1Δ1 ura3 hit4-1*	KK94 × YH8
KK205	*MATa leu2 trp1 his3 ura3 hsx1::HIS3*	This work
KK208	*MATa leu2 trp1 his3 ura3*	This work
SH3021	*MATα cdc26-1 his3 leu2 trp1 ura3*	S. HARASHIMA
KA311A-11C	*MATa his3 leu2 trp1 ura3 smr1::LEU2 smp2::URA3*	K. IRIE
LM65A-9C	*MATα ade4 his5 cdc65-1*	PRENDERGAST *et al.* (1990)

indicated in Figure 5. The polymerase chain reaction (PCR) was performed as described previously (SAIKI *et al.* 1988). PCR using K1 and K2 amplified a 913-bp DNA from p18-2, p18-4 and DG662 DNAs containing the complete δ3 and δ4 sequences. PCR using K3 and K4 amplified a 1021-bp DNA from p18-2 DNA containing the complete δ1 and δ2 sequences but amplified about a 700-bp DNA from DG662 and *hit1-1* DNAs.

Construction of disruption mutations: DNA fragments were amplified from pKK39 (*hit1::HIS3*) and pKK60 (*hsx1::HIS3*) by PCR using oligonucleotides 608 (ACAACGATAATTCTTATTTC) and 609 (ATTGATTTCCATCGTGTAAG) which were designed to make the longest DNA fragment without the δ2 sequence (Figure 7). Haploid yeast cells were transformed by the amplified fragments and the His$^+$ colonies were selected on SC-his plates at 23°. The disruption mutations were confirmed by Southern blot analysis.

DNA manipulations: Plaque hybridization and Southern hybridization were conducted by standard methods (DAVIS, DIBNER and BATTEY 1986). Yeast DNA preparation and transformation were done as described previously (SHERMAN, FINK and HICKS 1986). pUC118 and pUC119 (VIEIRA and MESSING 1987) were used for subcloning. DNA sequence was determined by the dideoxy-chain-termination method (SANGER, NICKLEN and COULSON 1977; TABOR and RICHARDSON 1987). Chromosomal DNAs were prepared and analyzed by contour-clamped homogeneous electric field (CHEF) gel electrophoresis (CARLE and OLSON 1985; CHU, VOLLRATH and DAVIS 1986).

RESULTS

Temperature-sensitive *hit* mutants: The haploid yeast strain DG662 harboring a plasmid pGTy*H3HIS3* (GARFINKEL *et al.* 1988) was mutagenized by the induction of Ty transposition. From 2.5 × 10^5 His$^+$ colonies, 24 temperature sensitive (Ts$^-$) colonies were isolated by replica-plating. In 16 of these mutants, the His$^+$ phenotypes were independent of the plasmid, indicating that Ty*HIS3* insertions had occurred somewhere in the genome. One mutant was sterile and therefore was not subjected to the genetic analysis. The 15 Ts$^-$ *MATα* strains were mated with wild type *MATa* strains at 20°. All diploid strains grew at 37°, indicating that the Ts$^-$ phenotypes were recessive. The diploids were sporulated at 20°, and tetrads were analyzed for growth on YPD plates at 37°. For 10 of the 15 diploid strains, the Ts$^-$ and the His$^+$ phenotypes segregated independently. We interpret these strains as cases where the insertion of the Ty*H3HIS3* element was not associated with the Ts$^-$ mutation. The Ts1 and Ts5 strains are examples of this class (Table 2). Note that in Ts1 there are significantly more His$^+$ than His$^-$ segregants suggesting that there were two or more Ty*HIS3* insertions. In 5 out of 15 cases (the crosses of the Ts2, Ts7, Ts12, Ts18, and Ts22 strains), all Ts$^-$ segregants were His$^+$ (Table 2), suggesting that the Ty*HIS3* insertion caused a mutation conferring the temperature sensitivity. These five mutants were derived from independent pools of the mutagenized yeast cells.

***hit1*:** The cross between Ts18 and JSS108-2C gave poor spore viability, but the correlation of the His$^+$ and Ts$^-$ phenotypes indicates that the temperature sensitivity results from a mutation caused by the insertion of Ty*HIS3* (Table 2). The mutation in Ts18 was designated *hit1-1* for *h*igh *t*emperature growth.

TABLE 2

Linkage of His$^+$ and Ts$^-$ in temperature-sensitive mutants

Cross	No. of asci analyzed	Spore phenotype			
		His$^+$Ts$^+$	His$^+$Ts$^-$	His$^-$Ts$^+$	His$^-$Ts$^-$
Unmarked Ts strains					
Ts1 × JSS110-5C	22	19	29	12	11
Ts5 × JSS104-4A	10	11	10	9	9
hit1					
Ts18 × JSS108-2C	10	0	8	15	0
KK80 × YH8	13	0	26	26	0
hit2					
Ts2 × JSS110-5C	19	0	28	26	0
Ts7 × JSS104-4A	10	0	20	20	0
hit3					
Ts12 × JSS104-4A	10	8	20	12	0
KK93 × YH8	6	0	12	12	0
hit4					
Ts22 × JSS104-4A	10	9	7	4	0
KK94 × YH8	8	0	16	16	0

Asci were dissected and all viable spores were analyzed for His$^+$ and Ts$^-$. The genotypes of the Ts strains are the same as YH8 except Ty*HIS3* insertions and the temperature sensitive mutations.

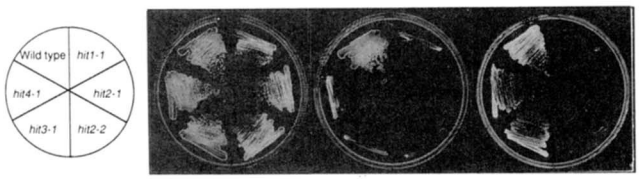

FIGURE 1.—Growth of the *hit* mutants on YPD and YPG. KK88 *hit1-1*, KK111 *hit2-1*, KK106 *hit2-2*, KK107 *hit3-1* and KK110 *hit4-1* were streaked on YPD and YPG plates. YPD plates were incubated for 4 days at 20° or for 2 days at 37°. The YPG plate was incubated for 7 days at 20°.

To confirm the segregation patterns of *hit1-1*, a *MAT***a** *hit1-1* segregant (KK80) from Ts18 × JSS108-2C was backcrossed to the unmutagenized parental strain YH8. This backcross gave good spore viability and showed 2:2 segregation for His$^+$Ts$^-$:His$^-$ Ts$^+$ (Table 2). The *hit1-1* mutant formed smaller colonies even at a permissive temperature than the wild type and did not form colonies on YPG plates at any temperature (Figure 1). This Gly$^-$ phenotype was correlated to the His$^+$, Ts$^-$ phenotype in the above tetrad analysis, indicating that the Ty insertion in the *hit1-1* mutant caused a chromosomal *pet* mutation.

Mutations caused by the Ty*HIS3* insertion can be assigned to chromosomes by the CHEF electrophoretic system and Southern hybridization using the *HIS3* probe (GARFINKEL *et al.* 1988). In the *hit1-1* mutant, the *HIS3* probe hybridized to chromosome *X* (Figure 2A). This chromosome assignment was confirmed by hybridization using the *URA2* (SOUCIET *et al.* 1987) probe and the physical analysis of the *hit1-1* allele described below. Southern hybridization analysis of the *Eco*RI- or *Bam*HI-digested genomic DNA

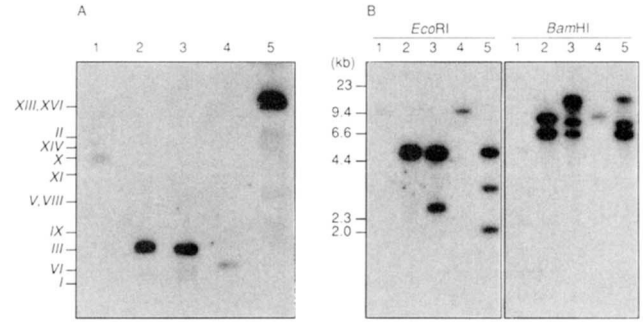

FIGURE 2.—Southern hybridization analysis of the *hit* mutants using the *HIS3* probe. Chromosome DNAs were prepared from KK88 *hit1-1* (lane 1), KK111 *hit2-1* (lane 2), KK106 *hit2-2* (lane 3), KK107 *hit3-1* (lane 4) and KK110 *hit4-1* (lane 5). The *HIS3* probe DNA was prepared from the pGTy*H3HIS3* plasmid and labeled with [^{32}P]dCTP. (A) Chromosomal DNAs were separated on a 1% agarose gel using a CHEF electrophoretic system, transferred to a nylon membrane, and the resulting blot was hybridized with the *HIS3* probe. Chromosome numbers are shown on the left. The positions of chromosome *VI*, *X*, *XIII* and *XVI* were confirmed by hybridization of the same blot with chromosome-specific probes, *CDC4*, *URA2*, *PEP4* and *RAD52*, respectively. (B) Chromosomal DNAs were digested with *Eco*RI or *Bam*HI, separated on a 1% agarose gel, and then blotted. The resulting filter was hybridized with the *HIS3* probe. The 5-kb *Eco*RI-band in lanes 2, 3 and 5 correspond to the predicted junction fragment from a marked Ty repeat. The other size *Eco*RI-bands are junction to the target sequence. The 7-kb *Bam*HI-bands and the other *Bam*HI bands in lanes 2, 3 and 5 represent the *HIS3* marked and unmarked Tys in the multimeric array. The 5-kb *Eco*RI-band in lanes 2, 3 and 5 is at least a doublet.

revealed a single Ty*HIS3* insertion in the *hit1-1* mutant (Figure 2B).

hit2*:** The crosses Ts2 × JSS110-5C and Ts7 × JSS104-4A gave good spore viability, showed 2 His$^+$:2 His$^-$ segregation, and all of the Ts$^-$ spore clones were His$^+$ (Table 2). A cross between a Ts2 *MAT*α strain and a Ts7-derived *MATa** strain yielded a diploid that did not grow at 37° (data not shown), suggesting that these mutations were allelic. We designated the Ty*H3HIS3*-induced mutations in these strains *hit2-1* and *hit2-2*. Both *hit2* mutants did not grow on YPG plates (Figure 1). The Gly$^-$ phenotype was inseparable from the His$^+$, Ts$^-$ phenotype in the genetic analysis, indicating both Ty*HIS3* insertion mutations caused chromosomal *pet* mutations.

CHEF gel analysis using the *HIS3* probe identified the site of these *hit2* mutations as on chromosome *III* (Figure 2A). Although the 2:2 segregation for His$^+$:His$^-$ indicated the Ty*HIS3* insertion at a single locus, Southern hybridization analysis revealed multiple Ty*HIS3* elements in both *hit2* mutants (Figure 2B). The difference in the structure of *hit2-1* and *hit2-2* mutations reflects the independence of their origin. Multimeric arrays of Ty*HIS3* insertions at the *HML*α locus and other loci have been identified when Ty-induced α-pheromone-resistant mutants were isolated (WEINSTOCK *et al.* 1990). The 5-kb *Eco*RI bands in the *hit2* mutants (Figure 2B) correspond to the

TABLE 3

Meiotic mapping of the *hit2*, *hit3* and *hit4* mutations

Gene pair	Ascus type[a] PD	NPD	T	Map distance (cM)[b]
A. *hit2*				
hit2/leu2	15	0	5	13
hit2/MAT	17	0	3	7.5
leu2/MAT	13	0	7	18
B. *hit3*				
hit3/met10	13	0	6	16
hit3/cdc26	22	0	0	<0.02
C. *hit4*				
hit4/smp2	10	3	12	60
hit4/cdc65	15	0	18	27

[a] PD, parental ditype; NPD, nonparental ditype; T, tetratype.

[b] Map distances were calculated using the equation described by SHERMAN, FINK and HICKS (1986): $100/2 \times [(T + 6NPD)/(PD + NPD + T)]$. (A) Ts 2 *hit2-1* × JSS110-5C and KK90 *hit2-1* × JSS104-21B. (B) KK107 *hit3-1* × KK16 and × SH3021. (C) KK126 *hit4-1* × KA311A-11C and KK110 × LM65A-9C. In each experiment, only tetrads with four viable spores were included. All of these asci showed 2:2 segregation for pair-markers. *smp2* is allelic to *pet-ts2858* (K. IRIE, personal communication).

predicted junction fragment from a marked Ty repeat. The *Eco*RI bands of the other sizes are junction to the target sequence. The 7-kb and the other *Bam*HI bands in the *hit2* mutants (Figure 2B) are indicative of the *HIS3* marked and unmarked Tys in the multimeric array. Therefore we assume that the *hit2* mutations are caused by insertion of Ty multimers at a single locus on chromosome *III*.

The *hit2-1* mutation was mapped between *leu2* and *MAT*, 7.5 cM centromere proximal to *MAT* (Table 3). The map position of *hit2-1* is similar to that of *pet18* mutations. Cells carrying *pet18* mutations have phenotypes similar to the *hit2* mutants in that they are temperature sensitive in addition to being unable to grow on glycerol as a carbon source (LEIBOWITZ and WICKNER 1978). To test whether the *hit2* mutations affect the *PET18* locus, the *hit2-1* and *hit2-2* strains were transformed by plasmids carrying the wild type *PET18* DNA, pIC4 and pIC12 (I. CHIU, personal communication). The Ts^- phenotype of the *hit2* mutants was complemented by these plasmids but the *pet* phenotype was not (data not shown). The inability to complement the *pet* phenotype by the pIC4 and pIC12 plasmids can be explained by the fact that *pet18* mutants are [rho^0] mutants (LEIBOWITZ and WICKNER 1978). Previous molecular characterization has demonstrated that several *pet18* mutations are deletions of the *PET18* locus (TOH-E and SAHASHI 1985). A 3-kb *PET18* DNA containing a gene essential for growth at high temperatures hybridized to neither the *hit2-1* nor *hit2-2* DNA (Figure 3). From these data, we concluded that in the *hit2* mutants the insertion of Ty multimers at the *PET18* locus was accompanied by the deletion of genes encoded in this region.

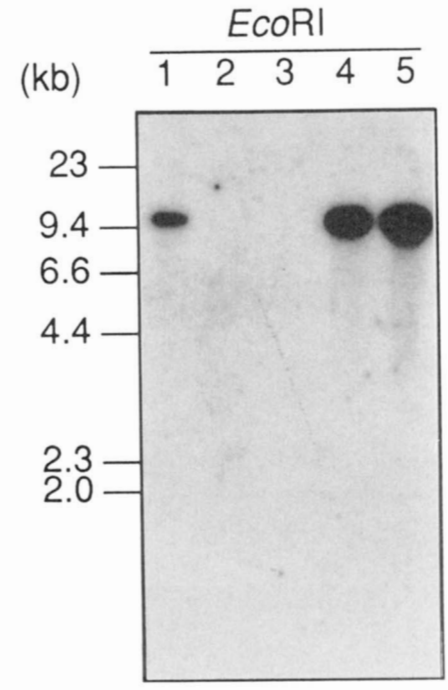

FIGURE 3.—Southern hybridization analysis of the *hit* mutants using the *PET18* probe. The same filter as used in Figure 2B was hybridized with the *PET18* probe, ^{32}P-labeled pYS10 DNA.

hit3: The cross between Ts12 and JSS104-4A yielded more His^+ than His^- spores indicating that there were multiple (probably two) Ty*HIS3* insertions (Table 2). The Ts^- phenotype segregated 2:2 in these tetrads and all of the Ts^- clones were His^+. A segregant KK93 containing only the Ty*HIS3* responsible for the Ts^- phenotype was identified by backcross to strain YH8 (Table 2). This mutation was designated *hit3-1*. Southern hybridization and CHEF gel analyses of a *hit3-1* segregant, KK107, indicated a single Ty*HIS3* insertion on chromosome *VI* (Figure 2, A and B). Further genetic analysis revealed that *hit3* was mapped 16 cM from *met10* (Table 3). After a shift to the nonpermissive temperature, cells with the *hit3-1* mutation showed a cell cycle-specific arrest. Microscopic observation showed that most of the arrested cells had large buds characteristic of the G2 phase of the cell cycle. Two *cdc* mutations, *cdc14* and *cdc26*, have been mapped close to the *met10* locus (MORTIMER and SCHILD 1980). The *hit3-1* mutant was crossed to the *cdc14* and *cdc26* mutants and the diploids were analyzed for growth at 37°. The *hit3/cdc14* diploid grew at 37° but the *hit3/cdc26* diploid did not. The tetrad analysis of the *hit3/cdc26* diploid indicated that the *hit3-1* and *cdc26* mutations are allelic (Table 3).

hit4: The initial genetic analysis of the mutation in Ts22 gave poor spore viability and an excess of His^+ over His^- spores (Table 2). As above, a segregant KK94 was identified which when backcrossed to strain YH8 gave good viability and 2 His^+Ts^-:2 His^-Ts^+ segregation (Table 2). The mutation was designated *hit4-1*. Southern hybridization analysis of the *hit4-1*

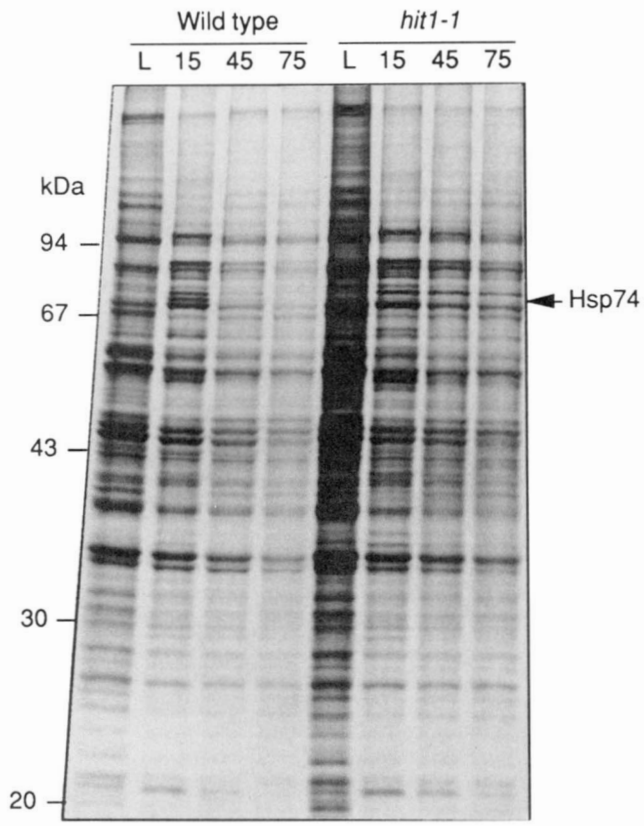

FIGURE 4.—Synthesis of heat shock proteins in the wild type and the *hit1-1* mutant cells. The DG662 (Wild type) and the Ts18 (*hit1-1*) strains were pulse-labeled at 23° (lane L) or 15, 45, and 75 min after shift to 37° (lanes 15, 45 and 75, respectively), and analyzed by SDS-polyacrylamide gel electrophoresis as described in MATERIALS AND METHODS. An arrow indicates the position of the Hsp74 band disappeared in the *hit1-1* mutant.

mutant revealed multiple Ty*HIS3* elements (Figure 2B). This result together with the above genetic analysis suggested that the *hit4-1* mutation was the result of a Ty multimer insertion at a single locus. CHEF gel analysis identified the marked Ty element on chromosome *XIII* or *XVI* (Figure 2A). Then the *hit4* mutant was crossed to several strains carrying chromosome *XIII* and *XVI* markers (Table 3). *hit4-1* showed linkage to *cdc65* (PRENDERGAST *et al.* 1990) of chromosome *XIII*, but little or no linkage to *smp2* (allelic to *pet-ts2858*; K. IRIE, personal communication).

Heat shock protein synthesis in the *hit1-1* mutant: To test whether the *hit* mutations affect the heat shock response, synthesis of the heat shock proteins (hsps) in the *hit* mutants was analyzed by the pulse-labeling experiment. In comparison with the hsp synthesis in the wild-type cells, one of the hsps whose molecular mass is about 74 kD (Hsp74) was not synthesized in the *hit1-1* mutant (Figure 4). In other *hit* mutants, no significant change was found (data not shown). A diploid strain formed by mating the *hit1-1* mutant with the wild-type strain synthesized the Hsp74 protein, indicating that the Hsp74$^-$ phenotype is recessive (data not shown). A tetrad generated from the diploid showed 2:2 segregation for Hsp74$^-$His$^+$Ts$^-$:Hsp74$^+$His$^-$Ts$^+$ (data not shown), suggesting that the Hsp74$^-$ phenotype is also caused by the *hit1-1* Ty*HIS3* insertion mutation. The Hsp74 protein was identified as the *SSC1* protein (CRAIG, KRAMER and KOSIC-SMITHERS 1987) by immunological analysis described below (Figure 8). The *SSC1* gene has been mapped on chromosome *X* (CRAIG, KRAMER and KOSIC-SMITHERS 1987). To determine whether the mutation affected the Hsp74 (*SSC1*) gene itself or a gene involved in the Hsp74 synthesis, the *hit1-1* mutation was studied further.

Cloning of the mutant and wild-type *HIT1* loci: The plasmids which complemented the Ts$^-$ phenotype of the *hit1-1* mutant, KK80, were isolated from a YCp50-yeast genomic DNA library. The p18-2 and p18-4 plasmids thus isolated carried 8.8-kb and 10.7-kb chromosomal segments, respectively. Restriction enzyme mapping, Southern hybridization and DNA sequencing analyses revealed that both plasmids included the *SUP4* region of chromosome *X* which has been published previously (GAFNER, DE ROBERTIS and PHILIPPSEN 1983; ROTHSTEIN, HELMS and ROSENBERG 1987; Figure 5). Three subclones were constructed from p18-4, which carried the 3.5-kb (p18-4S), 2-kb (p18-4C) and 1031-bp (p18-4Δ15) *HIT1* DNA fragments (Figure 5). The *hit1-1* mutant KK80 was transformed by these plasmids and the resulting Ura$^+$ transformants grew on both YPD and YPG plates at 20° and 37° and synthesized the Hsp74 (*SSC1*) protein upon heat shock (Figure 6).

The 3′ end of the Ty*HIS3* insertion in *hit1-1*: The *HIS3* marker gene is located close to the 3′δ element in Ty*HIS3* (GARFINKEL *et al.* 1988). Therefore a recombinant phage, λKK1, carrying 12 kb of the *Eco*RI fragment which hybridized to the *HIS3* probe (Figure 2B) was isolated from the KK80 λEMBL4-genomic library. This DNA fragment was expected to contain the *HIS3* marker gene and DNA flanking one side of the insertion. Several subclone plasmids were constructed from λKK1 and analyzed by DNA sequencing. The DNA sequence of the flanking region corresponded to that near the *SUP4* locus (Figure 5). This result was consistent with the CHEF gel assignment of *hit1* to chromosome *X* (Figure 2A) and the complementation of *hit1-1* by p18-2 and p18-4. The sequencing analysis revealed several interesting features of the *hit1-1* mutation. The 3′ end of the Ty*HIS3* insertion occurred into the δ3 sequence and the δ4 sequence was precisely deleted relative to the published sequence in this interval (Figure 5). The last 74-bp DNA of 3′δ of the inserted Ty*HIS3* element was derived from another δ element. The presence of the complete δ4 and δ3 sequence in the parental strain

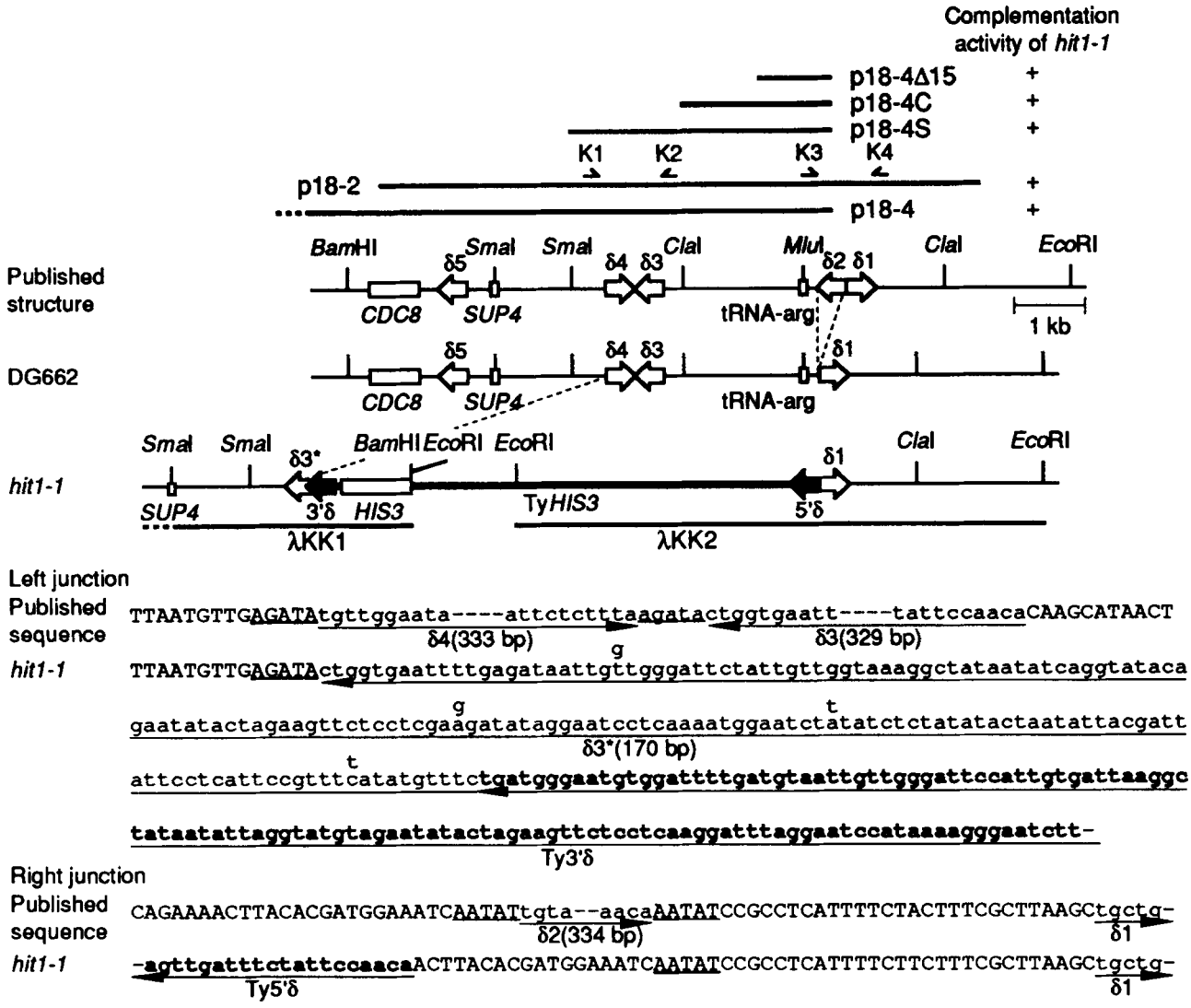

FIGURE 5.—The structure of the *SUP4* and *HIT1* region and the *hit1-1* mutant. The restriction enzyme map and part of DNA sequence of this region have been published previously (GAFNER, DE ROBERTIS and PHILIPPSEN 1983; ROTHSTEIN, HELMS and ROSENBERG 1987). DNA segments carried by p18-2, p18-4, p18-4S, p18-4C, p18-4Δ15, λKK1 and λKK2 are indicated by bold bars. The positions and the directions of oligonucleotides K1, K2, K3 and K4 used in this study are indicated by small arrows. Open boxes indicate the coding region of genes (*CDC8*, *SUP4*, the arginine tRNA gene and the *HIS3* gene carried by the inserted Ty). Open arrows indicate solo δ sequences and their directions correspond to the orientation of transcription. The absence of the δ2 sequence in DG662, the parental strain of *hit1-1* (see text), was confirmed by the PCR analysis (see MATERIALS AND METHODS). The Ty*HIS3* element in *hit1-1* is shown in bold. Black arrows indicate Tyδs. δ3* indicates 170 bp 3′ part of δ3. DNA sequences of δs are written in lower cases and arrows under sequences indicate their directions. The published δ3 sequence differs from δ3* by 4-bp mismatches indicated above the sequence lines. Ty*HIS3*δ sequences are written boldly. In the published sequence, 5 bp duplications found at both sides of the δ2 and δ4 sequences are underlined. In complementation activity of *hit1-1*, + means complementation of the Ts$^-$, *pet*, Hsp74$^-$ phenotype.

DG662 was confirmed by PCR analysis (see MATERIALS AND METHODS).

The 5′ end of the Ty*HIS3* insertion in *hit1-1*: The *hit1-1* mutant was analyzed by Southern hybridization using part of p18-2 or p18-4 as a probe. Unexpectedly, the ^{32}P-labeled 2-kb *Cla*I-*Mlu*I DNA fragment between the δ2 and δ3 sequences did not hybridized to the *hit1-1* genomic DNA (data not shown), suggesting the deletion of this region. Therefore, a DNA probe centromere proximal to the δ1 sequence was prepared from p18-2 and a recombinant phage, λKK2, which carried the 7.5-kb *Eco*RI fragment containing the 5′ end of Ty*HIS3* and DNA flanking the other side of the insertion was isolated from the KK80 λZAP-genomic library by plaque hybridization (Figure 5). Sequencing analysis of subclone plasmids constructed from λKK2 revealed that the 5′ end of the Ty*HIS3* insertion was located 51 bp from the δ1 sequence and the deletion of the δ2 sequence. The absence of the δ2 sequence in the parental strain DG662 used for the mutagenesis was confirmed by the PCR analysis (see MATERIALS AND METHODS). We think that this is a polymorphism between strains.

A 5-bp duplication of the target sequence which accompanied most Ty insertions (FARABAUGH and FINK 1980; GAFNER and PHILIPPSEN 1980) was not

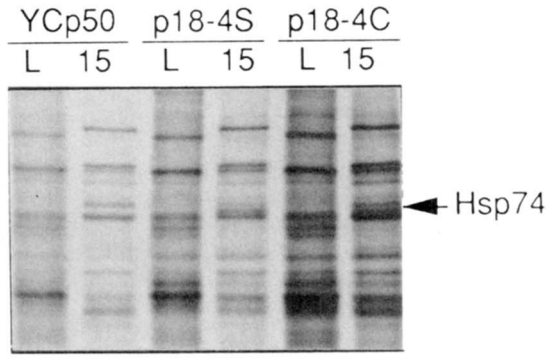

FIGURE 6.—Complementation of the Hsp74$^-$ phenotype. The *hit1-1* mutant KK80 was transformed by the vector plasmid, YCp50, and the plasmids carrying the wild-type *HIT1* DNA, p18-4S and p18-4C. The Ura$^+$ transformants were grown in a SC-ura-met medium, labeled at 23° (lane L) and 15 min after shift to 37° (lane 15) and analyzed by SDS-polyacrylamide gel electrophoresis. p18-4Δ15 also complemented the Hsp74$^-$ phenotype (data not shown).

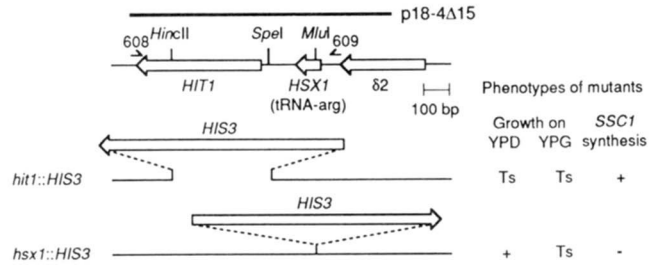

FIGURE 7.—Disruption of the *HIT1* gene and the *HSX1* gene (the AGG arginine tRNA gene). Open arrows indicate the positions and the directions of the δ2 sequence, the *HSX1* tRNA gene, the *HIT1* gene and the *HIS3* gene used for gene disruption. The DNA segment carried by p18-4Δ15 is shown as a bold bar. The lower two lines indicate the chromosome structures of the disruption mutants, *hit1::HIS3* and *hsx1::HIS3*. These mutant DNA fragments were constructed on plasmids, pKK39 and pKK60, amplified by RCR using oligonucleotide 608–609 and used for transformation. The disruption mutants were analyzed for growth on YPD and YPG plates at 23°, 30°, and 37° and by the pulse-labeling experiment. The phenotypes of each mutant are shown on the right side. Ts means no growth at 37°.

found in the *hit1-1* Ty insertion (Figure 5). Thus, the ends of the Ty element were inserted into two different sites and resulted in a deletion of nearly 3 kb. Whether there were multiple steps involved in generating the *hit1-1* allele cannot be determined from this analysis.

Dissection of the *HIT1* locus: The cloning and sequencing analyses established that the *hit1-1* mutation did not affect the Hsp74 (*SSC1*) gene itself but affected an unknown trans-acting factor. The complementation analysis indicated that the 1031 bp DNA carried by p18-4Δ15 plasmid encoded a gene (or genes) required for growth at high temperatures, growth on glycerol and the Hsp74 synthesis. The DNA segment contained an open reading frame encoding a protein of 164 amino acids and the AGG arginine tRNA gene which has been reported previously (GAFNER, DE ROBERTIS and PHILIPPSEN 1983). Southern hybridization using the whole tRNA gene revealed that the *hit1-1* mutant contained no copy of this gene (data not shown). To analyze the function of each gene more precisely, a disruption mutation of each gene was constructed (Figure 7). The disruption of the open reading frame caused the Ts$^-$ phenotype on both YPD and YPG plates. On the other hand, the tRNA mutant did not form colonies on YPG plates at 37° but formed smaller colonies on YPD plates at 37° than the parent strain (Figure 7). The heat shock protein synthesis in each disruption mutant was analyzed by the pulse-labeling experiment. The labeled Hsp74 protein was detected in the disruption mutant of the open reading frame but not in the tRNA mutant (Figures 7 and 8). Thus, the original *hit1-1* mutant phenotypes except the *pet* phenotype at low temperatures were reproduced by the disruption mutations of these genes. Therefore the open reading frame was designated *HIT1*, a gene essential for growth at high temperatures, and the arginine tRNA gene was designated *HSX1*, a gene essential for the heat shock protein synthesis.

Identification of Hsp74 as the *SSC1* protein: The Hsp74 protein was characterized by two-dimensional gel electrophoresis and was identified as a spot which was localized between pH 5 and pH 6 because the spot disappeared in the *hsx1::HIS3* disruption mutant (Figure 8A). Since most of the known hsp70 proteins are acidic, we tested whether the Hsp74 protein was a member of yeast hsp70 family by Western blot analysis using antibodies against known hsp70s. As shown in Figure 8B, the antibody recognizing the *SSC1* protein cross-reacted with Hsp74. The *SSC1* protein was slightly labeled in the *hsx1::HIS3* mutant. This may indicate the basal level expression of the *SSC1* gene (see DISCUSSION).

DISCUSSION

We isolated the temperature-sensitive *hit1*, *hit2*, *hit3* and *hit4* mutants of *S. cerevisiae* by insertional mutagenesis using a Ty*H3HIS3* transposon. The *hit1-1* mutation conferred temperature sensitivity and the *pet* phenotype. In the *hit1-1* mutant, the Ty*HIS3* element was inserted near the *SUP4* locus of chromosome *X* and resulted in the deletion of about 3 kb of DNA. The *HIT1* gene which is essential for growth at high temperatures and the AGG arginine tRNA gene (named *HSX1* in this study) were identified in this region, and the *hit1-1* mutation deleted both of them. The *hit2* mutants contained the Ty insertion mutation that resulted in the deletion of chromosomal DNA at the *PET18* locus of chromosome *III*. The *PET18* locus has been shown to encode a *PET* gene, a *MAK* gene and a gene required for growth over 37° (LEIBOWITZ and WICKNER 1978; TOH-E and SAHASHI 1985). Several *pet18* mutations are deletions of these genes

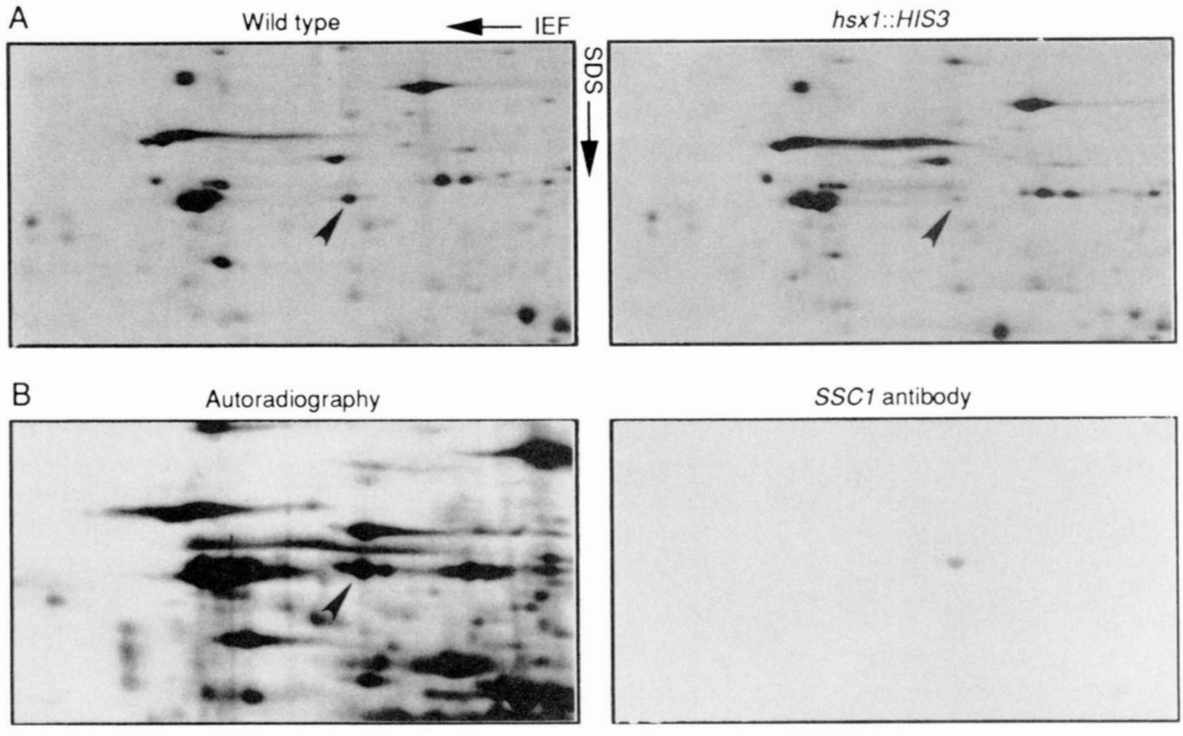

FIGURE 8.—Identification of the Hsp74 protein as the *SSC1* protein. (A) Total proteins of the wild-type strain (KK208) and the *hsx1::HIS3* mutant (KK205) were pulse-labeled 15 min after shift to 37°. The labeled proteins were separated with isoelectric focusing gels (IEF) in the first dimension and with SDS-polyacrylamide gel (SDS) in the second dimension. Arrows on panels indicate the spot almost disappeared in the *hsx1::HIS3* mutant, which corresponds to the Hsp74 protein. (B) The labeled proteins of the wild type were electrophoresed, blotted to a PVDF filter (Immobilon, Millipore) and analyzed by autoradiography (left) and hybridization to the *SSC1* antibody (right). An arrow on the left panel indicates the position of the *SSC1* protein identified on the right panel and to that of the disappeared protein in (A).

(TOH-E and SAHASHI 1985). The *hit3-1* mutation was located on chromosome *VI* and allelic to the *cdc26* mutation. The *hit3-1* mutant showed the same phenotype as the *cdc26* mutant; *i.e.*, arrest in the G2 phase at the restrictive temperature (HARTWELL *et al.* 1973). Recently, disruption of the *CDC26* gene has been shown to cause the same defect as the original *cdc26* mutant (H. ARAKI, personal communication). Therefore we assume that in the *hit3-1* mutant the Ty*HIS3* insertion disrupted the *CDC26* gene that is dispensable at low temperatures but essential at high temperatures. The *CDC26* gene product may stabilize a temperature sensitive factor which is essential for cell cycle. The *hit4-1* mutation was located on chromosome *XIII*. Detailed characterization of the affected gene has not been done yet.

Insertions of multiple Ty elements at the *hit2* and *hit4* locus were observed. The precise structures of the Ty insertions have not been analyzed but preliminary Southern analysis suggests tandem arrays of Ty like those observed when this mutagenesis system was used for isolation of α-factor resistant mutations (WEINSTOCK *et al.* 1990). In contrast, the *hit1-1* mutant contained a single Ty*HIS3* insertion with several interesting features. First, the 5′ end of the Ty*HIS3* element landed close to the δ1 sequence and its 3′ end was located in the δ3 sequence, about 2.5 kb apart from the δ1 sequence in the parent strain. Second, the δ4 sequence which existed in the parent strain was lost in *hit1-1*. Third, a 5-bp duplication of the target site which is usually observed at the ends of Ty insertion (FARABAUGH and FINK 1980; GAFNER and PHILIPPSEN 1980) was not found in *hit1-1*. We cannot distinguish whether this deletion was made by the ends of a single Ty element acting at different sites or by recombination between two independently inserted Ty elements.

The arginine tRNA gene, which encodes a minor AGGtRNA^{Arg}, has been thought to be essential since the yeast haploid genome contains only a single copy of this tRNA gene (GAFNER, DE ROBERTIS and PHILIPPSEN 1983). Chromosomal rearrangements involving the δ sequences of this region have been characterized previously (ROTHSTEIN, HELMS and ROSENBERG 1987). They inserted the *URA3* gene near the *SUP4* gene between the δ4 and δ5 sequences and identified several deletions including the *SUP4* gene by selecting Ura$^-$. However they have failed to isolate a deletion of DNA between the δ2 and δ3 sequences which corresponded to the *hit1-1* deletion. This observation was rationalized by assuming that the minor arginine tRNA in this region was essential. The *hit1-1* mutant contained no copy of this tRNA gene, indicating that the arginine tRNA gene is not an essential gene. The observed growth defect of the

hit1-1 mutant may explain the previous failures to isolate a deletion between the δ2 and δ3 sequences. Recently, this arginine tRNA gene has been identified as having a role in the translational frame shift involved in Ty expression (BELCOURT and FARABAUGH 1990). The frameshift occurs at the sequence CUUAGGC presumably because ribosomes become starved for the rare AGG arginyl tRNA. XU and BOEKE (1990) demonstrated that this arginine tRNA gene on a high copy number plasmid inhibits Ty transposition presumably because abundant AGG tRNA leads to failure to frameshift. Experiments are in progress to determine how the tRNA disruption mutation affects Ty frameshifting and transposition and to determine how the AGG codons are decoded in the absence of the AGG arginyl tRNA.

The defect in synthesizing the *SSC1* protein was associated with the original *hit1-1* deletion mutation. Gene disruption experiments revealed that the Ssc1$^-$ phenotype was caused by the deletion of the AGG arginine tRNA gene (the *HSX1* gene) alone, which is located upstream of the *HIT1* gene. Thus, the *HSX1* arginine tRNA gene is essential for the heat shock induced synthesis of the *SSC1* protein. The *SSC1* gene is an essential member of the yeast hsp70 family (CRAIG, KRAMER and KOSIC-SMITHERS 1987), encodes a mitochondrial protein (CRAIG *et al.* 1989) and is involved in translocation of nuclear-encoded precursor proteins into mitochondria and in folding of imported proteins in the matrix (KANG *et al.* 1990). Since the disruption of the *SSC1* gene confers lethality (CRAIG, KRAMER and KOSIC-SMITHERS 1987), we infer that the basal level expression of the *SSC1* gene is not affected by the *hsx1* tRNA disruption mutation. The *hsx1::HIS3* mutant showed temperature sensitive *pet* phenotype. The defect in the *SSC1* synthesis may be a component of this phenotype. The hsp synthesis in yeast has been thought to be regulated by the heat shock transcription factor (HSTF) that binds the DNA sequence named heat shock element (HSE) located upstream of the *HSP* genes (WIEDERRECHT, SETO and PARKER 1988; SORGER and PELHAM 1988). The transcript of the *SSC1* gene is, however, moderately abundant at low temperatures and is not greatly induced upon heat shock (CRAIG, KRAMER and KOSIC-SMITHERS 1987; MORISHIMA *et al.* 1990; K. KAWAKAMI, unpublished data). The analysis is in progress to determine whether the *SSC1* gene expression is regulated translationally by the *HSX1* tRNA gene and minor AGG arginine codons, which appear three times in the *SSC1* coding region.

The identification of genes that are essential at high temperatures is difficult because such mutations must be distinguished from temperature sensitive alleles of genes that are essential at all temperatures. The use of a movable genetic element as an insertional mutagen should generate few conditional alleles of essential genes. Our results show the success of this strategy. Five Ty*HIS3* induced temperature sensitive mutations were identified from a screen of 2.5×10^5 mutagenized cells. Given that several genes (see Introduction) that would have satisfied the screen have been identified, the low recovery of mutants may reflect the observation that Ty elements give a nonrandom distribution of insertion events (NATSOULIS *et al.* 1989). On the other hand, four of the five mutations are in genes (*PET18*, *CDC26* and *HIT1* identified by this study) known to be essential only at high temperatures. A null allele of *HIT4* has not yet been made. These observations, combined with recent improvements in protocols for Ty mutagenesis (CURCIO and GARFINKEL 1991), indicate that Ty mutagenesis may be an effective means of identifying conditionally essential genes as opposed to conditional alleles of essential genes.

We thank E. CRAIG for the *SSC1* antibody and discussion, M. ROSE and K. WEINSTOCK for a yeast genomic library, T. BURKETT, I. CHIU, A. TOH-E, S. TANAKA, S. YOSHIKAWA, K. IRIE, G. JOHNSTON and S. HARASHIMA for plasmids and strains, H. ARAKI, I. CHIU and K. IRIE for personal communications and K. EGAWA for continuous encouragement. This work was supported in part by grants from The Ministry of Education, Science and Culture, Japan, and by the National Cancer Institute, Department of Health and Human Services, under Contract No. NO1-CO-74101 with Advanced Bio-Science Laboratories, Inc.

LITERATURE CITED

ADZUMA, K., T. OGAWA and H. OGAWA, 1984 Primary structure of the *RAD52* gene in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:** 2735–2744.

AMMERER, G., C. P. HUNTER, J. H. ROTHMAN, G. C. SAARI, L. A. VALLS and T. H. STEVENS, 1986 *PEP4* gene of *Saccharomyces cerevisiae* encodes proteinase A, a vacuolar enzyme required for processing of vacuolar precursors. Mol. Cell. Biol. **6:** 2490–2499.

BANTA, L. M., T. A. VIDA, P. K. HERMAN and S. D. EMR, 1990 Characterization of yeast Vsp33p, a protein required for vacuolar protein sorting and vacuole biogenesis. Mol. Cell. Biol. **10:** 4638–4649.

BELCOURT, M. F., and P. J. FARABAUGH, 1990 Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. Cell **62:** 339–352.

BOEKE, J. D., 1988 Retrotransposons, p. 59–103 in *RNA Genetics, Retroviruses, Viloids, and RNA Recombination*, edited by E. DOMINGO,. J. HOLLAND and P. AHLQUIST. CRC Press, Boca Raton, Fla.

BOEKE, J. D., D. J. GARFINKEL, C. A. STYLES and G. R. FINK, 1985 Ty elements transpose through an RNA intermediate. Cell **40:** 491–500.

CARLE, G. F., and M. V. OLSON, 1985 An electrophoretic karyotype for yeast. Proc. Natl. Acad. Sci. USA **82:** 3756–3760.

CHU, G., D. VOLLRATH and R. W. DAVIS, 1986 Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234:** 1582–1585.

CRAIG, E. A., and K. JACOBSEN, 1984 Mutations of the heat inducible 70 kilodalton genes of yeast confer temperature sensitive growth. Cell **38:** 841–849.

CRAIG, E. A., J. KRAMER and J. KOSIC-SMITHERS, 1987 *SSC1*, a

member of the 70-kDa heat shock protein multigene family of *Saccharomyces cerevisiae*, is essential for growth. Proc. Natl. Acad. Sci. USA **84:** 4156–4160.

CRAIG, E. A., J. KRAMER, J. SHILLING, M. WERNER-WASHBURNE, S. HOLMES, J. KOSIC-SMITHERS and C. M. NICOLET, 1989 *SSC1*, an essential member of the yeast HSP70 multigene family, encodes a mitochondrial protein. Mol. Cell. Biol. **9:** 3000–3008.

CURCIO, M. J., and D. J. GARFINKEL, 1991 Single-step selection for Ty*1* element retrotransposition. Proc. Natl. Acad. Sci. USA **88:** 936–940.

DAVIS, L. G., M. D. DIBNER and J. F. BATTEY, 1986 *Basic Methods in Molecular Biology*. Elsevier, New York.

FARABAUGH, P. J., and G. R. FINK, 1980 Insertion of the eukaryotic transposable element Ty*1* creates a 5-base pair duplication. Nature **286:** 352–356.

FRISCHAUF, A-M., H. LEHRACH, A. POUSTKA and N. MURRAY, 1983 Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170:** 827–842.

GAFNER, J., E. M. DE ROBERTIS and P. PHILIPPSEN, 1983 Delta sequences in the 5′ non-coding region of yeast tRNA genes. EMBO J. **2:** 583–591.

GAFNER, J., and P. PHILIPPSEN, 1980 The yeast transposon Ty*1* generates duplications of target DNA on insertion. Nature **286:** 414–418.

GARFINKEL, D. J., M. F. MASTRANGELO, N. J. SANDERS, B. K. SHAFER and J. N. STRATHERN, 1988 Transposon tagging using Ty elements in yeast. Genetics **120:** 95–108.

GARRETT, J. M., K. K. SINGH, R. A. VONDER HAAR and S. D. EMR, 1991 Mitochondrial protein import: isolation and characterization of the *Saccharomyces cerevisiae MFT1* gene. Mol. Gen. Genet. **225:** 483–491.

HARTWELL, L. H., R. K. MORTIMER, J. CULOTTI and M. CULLOTI, 1973 Genetic control of the cell division cycle in yeast. V. Genetic analysis of *cdc* mutants. Genetics **74:** 267–286.

HERMAN, P. K., and S. D. EMR, 1990 Characterization of *VPS34*, a gene required for vacuolar protein sorting and vacuole segregation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:** 6742–6754.

IIDA, H., and I. YAHARA, 1984 Durable synthesis of high molecular weight heat shock proteins in G_0 cells of the yeast and other eukaryotes. J. Cell. Biol. **99:** 199–207.

KANEKO, Y., A. TOH-E, I. BANNO and Y. OSHIMA, 1989 Molecular characterization of a specific *p*-nitrophenylphosphatase gene, *PHO13*, and its mapping by chromosome fragmentation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **220:** 133–139.

KANG, P.-J., J. OSTERMANN, J. SHILLING, W. NEUPERT, E. A. CRAIG and N. PFANNER, 1990 Requirement for hsp70 in the mitochondrial matrix for translocation and folding of precursor proteins. Nature **348:** 137–143.

LEIBOWITZ, M. J., and R. B. WICKNER, 1978 *pet18*: a chromosomal gene required for cell growth and for the maintenance of mitochondrial DNA and the killer plasmid of yeast. Mol. Gen. Genet. **165:** 115–121.

LINDQUIST, S., and E. A. CRAIG, 1988 The heat shock proteins. Annu. Rev. Genet. **22:** 631–677.

LIPINSKA, B., O. FAYET, L. BAIRD and C. GEORGOPOULOS, 1989 Identification, characterization, and mapping of the *Escherichia coli htrA* gene, whose product is essential for bacterial growth only at elevated temperatures. J. Bacteriol. **171:** 1574–1584.

MASTRANGELO, M. F., K. G. WEINSTOCK, B. K. SHAFER, A.-M. HEDGE, D. J. GARFINKEL and J. N. STRATHERN, 1992 Disruption of a silencer domain by a retrotransposon. Genetics **131:** 519–529.

MILLER, M. J., N-H. XUONG and E. P. GEIDUSCHEK, 1979 A response of protein synthesis to temperature shift in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **76:** 5222–5225.

MORISHIMA, N., K.-I. NAKAGAWA, E. YAMAMOTO and T. SHIBATA, 1990 A subunit of yeast site-specific endonuclease *Sce*I is a mitochondrial version of the 70-kDa heat shock protein. J. Biol. Chem. **265:** 15189–15197.

MORTIMER, R. K., and D. SCHILD, 1980 Genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44:** 519–571.

NAKAYAMA, N., K.-I. ARAI and K. MATSUMOTO, 1988 Role of *SGP2*, a suppressor of a *gpa1* mutation, in the mating-factor signaling pathway of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:** 5410–5416.

NATSOULIS, G., W. THOMAS, M.-C. ROGHMANN, F. WINSTON and J. D. BOEKE, 1989 Ty*1* transposition in *Saccharomyces cerevisiae* is nonrandom. Genetics **123:** 269–279.

O'FARRELL, P. H., 1975 High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250:** 4007–4021.

PRENDERGAST, J. A., L. E. MURRAY, A. ROWLEY, D. R. CARRUTHERS, R. A. SINGER and G. C. JOHNSTON, 1990 Site selection identifies new genes that regulate *Saccharomyces cerevisiae* cell proliferation. Genetics **124:** 81–90.

PRINGLE, J. R., 1975 Induction, selection, and experimental uses of temperature-sensitive and other conditional mutants of yeast. Methods Cell. Biol. **12:** 233–272.

ROSE, M. D., P. NOVICK, J. H. THOMAS, D. BOTSTEIN and G. R. FINK, 1987 A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:** 237–243.

ROTHSTEIN, R., C. HELMS and N. ROSENBERG, 1987 Concerted deletions and inversions are caused by mitotic recombination between delta sequences in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 1198–1207.

SAIKI, R. K., D. H. GELFAND, S. STOFFEL, S. J. SCHARF, R. HIGUCHI, G. T. HORN, K. B. MULLIS and H. A. ERLICH, 1988 Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:** 487–491.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

SCHULTZ, J. and M. CARLSON, 1987 Molecular analysis of *SSN6*, a gene functionally related to the *SNF1* protein kinase of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 3637–3645.

SHERMAN, F., G. R. FINK and J. B. HICKS, 1986 *Laboratory Course Manual for Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

SHORT, J. M., J. M. FERNANDEZ, J. A. SORGE and W. D. HUSE, 1988 λZAP, a bacteriophage λ expression vector with in vivo excision properties. Nucleic Acids Res. **16:** 7583–7600.

SORGER, P. K., and H. R. B. PELHAM, 1988 Yeast heat shock factor is an essential DNA-binding protein that exhibits temperature-dependent phosphorylation. Cell **54:** 855–864.

SOUCIET, J.-L., S. POTIER, J.-C. HUBERT and F. LACROUTE, 1987 Nucleotide sequence of the pyrimidine specific carbamoyl phosphate synthetase, a part of the yeast multifunctional protein encoded by the *URA2* gene. Mol. Gen. Genet. **207:** 314–319.

TABOR, S., and C. C. RICHARDSON, 1987 DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. Proc. Natl. Acad. Sci. USA **84:** 4767–4771.

TOH-E, A., and Y. SAHASHI, 1985 The *PET18* locus of *Saccharomyces cerevisiae*: a complex locus containing multiple genes. Yeast **1:** 159–171.

VIEIRA, J., and J. MESSING, 1987 Production of single-stranded plasmid DNA. Methods Enzymol. **153:** 3–11.

WEINSTOCK, K. G., M. F. MASTRANGELO, T. J. BURKETT, D. J.

GARFINKEL and J. N. STRATHERN, 1990 Multimeric arrays of the yeast retrotransposon Ty. Mol. Cell. Biol. **10:** 2882–2892.

WIEDERRECHT, G., D. SETO and C. S. PARKER, 1988 Isolation of the gene encoding the *S. cerevisiae* heat shock transcription factor. Cell **54:** 841–853.

WOOLFORD, C. A., L. B. DANIELS, F. J. PARK, E. W. JONES, J. N. VAN ARSDELL and M. A. INNIS, 1986 The *PEP4* gene encodes an aspartyl protease implicated in the posttranslational regulation of *Saccharomyces cerevisiae* vacuolar hydrolases. Mol. Cell. Biol. **6:** 2500–2510.

XU, H., AND J. D. BOEKE, 1990 Host genes that influence transposition in yeast: the abundance of a rare tRNA regulates Ty*1* transposition frequency. Proc. Natl. Acad. Sci. USA **87:** 8360–8364.

YOCHEM, J., and B. BYERS, 1987 Structural comparison of the yeast cell division cycle gene *CDC4* and a related pseudogene. J. Mol. Biol. **195:** 233–245.

YURA, T., T. TOBE, K. ITO and T. OSAWA, 1984 Heat shock regulatory gene (*htpR*) of *Escherichia coli* is required for growth at high temperature but is dispensable at low temperature. Proc. Natl. Acad. Sci. USA **81:** 6803–6807.

Communicating editor: M. CARLSON