Deletion of a Single-Copy tRNA Affects Microtubule Function in *Saccharomyces cerevisiae*

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ABSTRACT

rts1-1 was identified as an extragenic suppressor of *tub2-104*, a cold-sensitive allele of the sole gene encoding β-tubulin in the yeast, *Saccharomyces cerevisiae*. In addition, *rts1-1* cells are heat sensitive and resistant to the microtubule-destabilizing drug, benomyl. The *rts1-1* mutation is a deletion of approximately 5 kb of genomic DNA on chromosome *X* that includes one open reading frame and three tRNA genes. Dissection of this region shows that heat sensitivity is due to deletion of the open reading frame (*HIT1*). Suppression and benomyl resistance are caused by deletion of the gene encoding a $tRNA^{Arg}_{ACG}$ (*HSX1*). Northern analysis of *rts1-1* cells indicates that *HSX1* is the only gene encoding this tRNA. Deletion of *HSX1* does not suppress the *tub2-104* mutation by misreading at the AGG codons in *TUB2*. It also does not suppress by interfering with the protein arginylation that targets certain proteins for degradation. These results leave open the prospect that this $tRNA^{Arg}_{ACG}$ plays a novel role in the cell.

MICROTUBULES are elements of nearly all eukaryotic cells and play essential roles in chromosome segregation and organelle movement. They are composed of highly conserved αβ-tubulin dimers that assemble into a hollow circular 25-nm diameter filament. In the yeast, *Saccharomyces cerevisiae*, there is a single essential gene encoding β-tubulin called *TUB2* (NEFF *et al.* 1983); two genes, *TUB1* and *TUB3*, encode α-tubulins (SCHATZ *et al.* 1986). A number of cold-sensitive alleles of the yeast *TUB2* gene have been isolated (THOMAS, NEFF and BOTSTEIN 1985; HUFFAKER, HOYT and BOTSTEIN 1987) and shown to have diverse effects on microtubule assembly and function *in vivo* (HUFFAKER, THOMAS and BOTSTEIN 1988; PALMER *et al.* 1992; SULLIVAN and HUFFAKER 1992). Analyses of these mutants and wild-type cells that have been treated with the microtubule-destabilizing drug nocodazole (JACOBS *et al.* 1988) suggest that microtubules in yeast are primarily involved in mitotic events. Astral (or cytoplasmic) microtubules are required for nuclear migration and spindle orientation in the bud neck; spindle (or nuclear) microtubules are required for subsequent chromosome segregation.

Cells containing the *tub2-104* allele fail to grow at 14° and appear to have defects in both spindle orientation and spindle elongation (HUFFAKER, THOMAS and BOTSTEIN 1988). At the restrictive temperature, they arrest as a uniform population of large-budded cells. Each contains a replicated but undivided set of chromosomes randomly located within the mother cell. Immunofluorescence staining of microtubules indicates that these cells possess short spindle arrays and abnormally short astral microtubule arrays. The molecular basis of this defect is unknown. It may be that the altered β-tubulin causes an intrinsic defect in microtubule assembly. Alternatively, the mutation may produce microtubules that are unable to interact productively with other cellular components.

In an effort to identify cellular components that play a role in microtubule assembly and/or function, we have examined extragenic suppressors of the cold sensitivity of *tub2-104* cells. Here we describe the characterization of *rts1-1*, an unusual type of suppressor mutation. In essence, we show that suppression in this case is due to the complete deletion of a tRNA gene that is present in a single copy in the yeast genome. Several models describing how loss of this tRNA may correct a microtubule defect are considered.

MATERIALS AND METHODS

Strains and media: Yeast strains used in this study are listed in Table 1. Yeast media are essentially as described by SHERMAN (1991).

Isolation of the *rts1-1* mutation: The *rts1-1* mutation was originally isolated by J. THOMAS and the procedure used to obtain it is described in his thesis (THOMAS 1984). Because this text is not readily available, the strategy he used will be briefly outlined here. About 1500 spontaneous revertants of a *tub2-104* strain were isolated as colonies that were able to grow at 14°. These arose at a frequency of approximately 1 in 10^5 cells plated. Ninety-five showed some degree of

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TABLE 1

Yeast strains

Strain	Genotype
CUY10	*MATa his4-539 lys2-801 ura3-52*
CUY8	*MATα ade2-101 ura3-52*
CUY87	*MATa/MATα ade2-101/+ his4-539/+ lys2-801/+ ura3-52/ura3-52*
CUY51	*MATa tub2-104 his4-539 ura3-52*
CUY52	*MATα tub2-104 ade2-101 ura3-52*
CUY164	*MATa rts1-1 his4-539*
CUY165	*MATα rts1-1 ade2-101*
CUY428	*MATα rts1-1 leu2-3,112 ura3-52*
CUY166	*MATa rts1-1 tub2-104 his4-539*
CUY167	*MATα rts1-1 tub2-104 ade2-101*
CUY429	*MATa rts1-1 tub2-104 ade2-101 his4-539 ura3-52*
CUY430	*MATa hsx1::URA3 ade2-101 his4-539 lys2-801 ura3-52*
CUY431	*MATα hsx1::URA3 his4-539 ura3-52*
CUY432	*MATa hit1-Δ1::URA3 ade2-101 lys2-801*
CUY433	*MATα hit1-Δ1::URA3 ade2-101 lys2-801*
CUY434	*MATa hsx1::URA3 tub2-104 his4-539 ura3-52*
CUY435	*MATa hit1-Δ1::URA3 tub2-104 his4-539 lys2-801 ura3-52*
CUY409	*MATa/MATα ACT1::HIS3/+ tub2-Δ1::LEU2/+ ade2-101/ade2-101 his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 lys2-801/lys2-801 ura3-52/ura3-52*
CUY855	*MATa tub2-471::URA3 ade2-101 his3-Δ200 leu2-Δ1 lys2-801 ura3-52*
CUY436	*MATa hsx1::URA3 tub2-471::URA3 ade2-101 leu2-Δ1*
KMY207	*MATα ate1::TRP1 ade2-101 his3-Δ200 lys2-801 trp1-63 ura3-52*
JDY40	*MATα ubr1::HIS3 ade2-101 his3-Δ200 lys2-801 trp1-63 ura3-52*

temperature sensitivity for growth at 37°. For 29 of these, both suppression and temperature sensitivity were due to a mutation at a single genetic locus as determined by linkage analysis. One of these is an intragenic revertant of *tub2-104*. The other 28 were placed into 16 complementation groups based on their temperature sensitivity. One group has 8 members, 5 groups have 2 members and 10 groups have only a single member. Each member of the largest group is phenotypically identical. The *rts1-1* mutation is one of these originally termed r278.

DNA sequence analysis of the *RTS1* locus: The *RTS1* locus was cloned by complementation of the heat-sensitivity of an *rts1-1* strain. A strain carrying the *rts1-1* mutation and several auxotrophic markers (CUY428) was transformed (BECKER and GUARENTE 1991) with a yeast genomic library constructed in the yeast shuttle vector YCp50 (ROSE *et al.* 1987). Three independent transformants were identified that were able to grow at 37°. Plasmids from these strains were isolated (HOLM *et al.* 1986) and transformed into *Escherichia coli*. Restriction enzyme analysis indicated that the plasmids possessed overlapping regions of yeast genomic DNA. A 2.7-kb *Xho*I fragment, subcloned into YCp50 (pJT120), complemented both heat sensitivity and suppression conferred by the *rts1-1* mutation. After creating a nested set of deletions (HEINRICH 1991), double-stranded DNA sequencing was done using the Sequenase Version 1.0 DNA sequencing kit (U.S. Biochemical Corp., Cleveland, Ohio). DNA and RNA blotting and hybridizations were performed using random-primed ^{32}P-probes (BROWN 1991).

Disruption of the *HSX1* and *HIT1* genes: To disrupt the *HSX1* gene, the 2.7-kb *Xho*I fragment was cloned into the yeast integrating plasmid YIp5 to create pJT123. To remove the *Hin*dIII site from pJT123, it was digested with *Eco*RI and *Bam*HI. Blunt ends were created by Klenow fragment and the backbone was religated. The plasmid was then digested with *Mlu*I (which cuts at 21 bp from the 5′ end of the mature tRNA). Blunt ends were created with Klenow fragment and *Hin*dIII linkers were added. This fragment was ligated to a *Hin*dIII fragment containing the *URA3* gene to create pRR31.

To disrupt *HIT1*, the 2-kb *Pst*I-*Xho*I fragment was cloned into the integrating plasmid, pRS315 (SIKORSKI and HIETER 1989), and digested with *Nde*I. Blunt ends were created with Klenow fragment and *Hin*dIII linkers were added. This fragment was ligated to a *Hin*dIII fragment containing the *URA3* gene to create pRR69. In this construct, all but 10 N-terminal codons of *HIT1* are replaced by the *URA3* gene.

The *Kpn*I-*Pst*I fragment of pRR31 and the *Pst*I-*Xho*I fragment of pRR69 were transformed into wild-type diploid cells (CUY87) to disrupt *HSX1* and *HIT1*, respectively. Southern hybridization analysis of genomic DNA from the transformants indicated the constructions had properly integrated (data not shown). The diploids were sporulated and tetrads dissected. In each case, all four spores were viable and the *URA3* gene, which marked the disruptions, was present in two of the four spores.

Construction of *tub2-471*: A *Nar*I to *Sph*I genomic DNA fragment containing the wild-type *TUB2* gene was cloned into the *Sma*I site of pBluescript II KS+ (Stratagene) using *Sph*I linkers. The *URA3* gene was inserted into the downstream noncoding region of the *TUB2* locus at the *Bgl*II site. Mutant primers were annealed to ssDNA generated from this plasmid (pRR190) in CJ236 cells. The second strand was synthesized and mutant plasmids were selected by dut$^-$ung$^-$ mutagenesis (using the MutaGene kit; Bio-Rad, Richmond, California). The following primers were used sequentially to introduce three point mutations into the *TUB2* gene: TTGATCTCGAAGATTAGAGAAGAGTTTCCT to change codon 213 from AGG (Arg) to AGA (Arg), ACAACTTCATTGCATTATCCCGGC to change codon 241 from CGT (Arg) to CAT (His), and GACATC TGTCAAAGAACCTTAAAG to change codon 156 from AGG (Arg) to AGA (Arg). All mutations were verified by DNA sequencing. The resulting plasmid is called pAS64; the triple mutant allele is called *tub2-471*.

The *Sph*I fragment from pAS64, containing *tub2-471* and *URA3*, was transformed into the diploid yeast cell, CUY409. In this diploid, one copy of chromosome *VI* contains the wild-type *TUB2* gene and has the *HIS3* marker inserted just downstream of the tightly linked *ACT1* gene. On the other copy of chromosome *VI*, the *TUB2* coding region is entirely replaced by the *LEU2* marker. Ura$^+$ transformants were selected and screened for loss of the *LEU2* marker, to ensure that integration of *tub2-471* occurred on the copy of chromosome *VI* that originally contained the *TUB2* disruption. Diploids were sporulated and tetrads dissected. Two spores in each tetrad were Ura$^+$His$^-$ and two were Ura$^-$His$^+$, further demonstrating that integration of the *tub2-471* allele occurred at the *LEU2* disrupted copy of *TUB2*. The presence of the mutations in yeast was verified by sequencing the genomic DNA after polymerase chain reaction (PCR) amplification.

RESULTS

Phenotype of *rts1* cells: Cells containing the *tub2-104* allele fail to grow at 14°. They are also resistant

TABLE 2

Growth of wild-type and mutant haploid strains

Strain	Temperature			Benomyl (μg/ml)				
	14°	30°	37°	10	20	30	40	60
Wild-type	+	+	+	+	+	−	−	−
tub2-104	−	+	+	+	+	+	+	+
rts1-1	+/−	+/−	−	+	+	+	+/−	−
rts1-1 tub2-104	+/−	+/−	−	+	+	+	+	+
hsx1::URA3	+	+	+	+	+	+/−	−/+	−
hsx1::URA3 tub2-104	+	+	+	+	+	+	+	+
hit1-Δ1::URA3	+/−	+/−	−	+	+	−	−	−
hit1-Δ1::URA3 tub2-104	−	+/−	−	+	+	+	+	+
tub2-471	−	+	+	+	+	+	+	+
hsx1::URA3 tub2-471	+	+	+	+	+	+	+	+

Growth at each temperature was scored on YPD plates and is relative to the wild-type strain at the same temperature. Growth on YPD plus benomyl was scored at 30° and is relative to the wild-type strain on YPD without benomyl. Growth rate: + > +/− > −/+ > −.

to high levels of the microtubule destabilizing drug, benomyl. The *rts1-1* mutation was identified as a spontaneous suppressor of *tub2-104*'s cold-sensitive phenotype (THOMAS 1984). *rts1-1 tub2-104* cells grow at 14° but fail to grow at 37°; they grow somewhat slower than wild-type cells at both 14° and 30°. To determine whether these phenotypes were due to the same mutation, we crossed an *rts1-1 tub2-104* strain (RRY6) to a *tub2-104* strain (CUY52) and sporulated the diploid. All tetrads contained two cold-sensitive and two heat-sensitive spores. Segregants that grew at 14° failed to grow at 37° and vice versa. Thus, the *rts1-1* mutation represents an alteration at a single genetic locus that confers suppression of the cold-sensitive *tub2-104* allele and heat sensitivity. When the *rts1-1* mutation was crossed into a wild-type *TUB2* background, cells remained heat sensitive. In addition, *rts1-1* cells were substantially more resistant to benomyl than wild-type cells. (This latter phenotype could not be observed in *rts1-1 tub2-104* strains because *tub2-104* alone confers benomyl resistance). These results are summarized in Table 2.

rts1-1 cells displayed a partial cell cycle arrest following a shift to their restrictive temperature. After 5 hr at 37°, 60% of the cells in *rts1-1* cultures were large-budded and contained a single undivided nucleus. This phenotype is commonly observed for conditional-lethal *tub2* mutants (THOMAS, NEFF and BOTSTEIN 1985; HUFFAKER, THOMAS and BOTSTEIN 1988) and suggests that the *rts1-1* mutation causes a cell cycle block prior to anaphase. While this phenotype provided some of the motivation for studying *rts1-1*, it was subsequently found to be a composite phenotype caused by loss of several genes (see below).

Molecular analysis of the *rts1-1* mutation: Both heat sensitivity and suppression produced by the *rts1-1* mutation are recessive. Plasmids that complemented the heat sensitivity of an *rts1-1* strain were cloned from a yeast genomic library. Subcloning showed that complementing activity was contained in a 2.7-kb *Xho*I fragment. A plasmid carrying this fragment (pJT120) also complemented suppression by *rts1-1*; an *rts1-1 tub2-104* strain containing the plasmid did not grow at 14°. To demonstrate that the *RTS1* locus had been cloned, we showed that the *Xho*I fragment would direct integration of a plasmid at the *RTS1* locus. The *Xho*I fragment was subcloned into the yeast integrating plasmid, YIp5. This plasmid (pJT123) was digested at a single site within the *Xho*I fragment and transformed into an *RTS1*$^+$ *ura3-52* strain, selecting for Ura$^+$ transformants. One transformant was crossed back to an *rts1-1 ura3-52* strain and 10 tetrads dissected. All tetrads contained 2 heat-sensitive and 2 Ura$^+$ spores. Segregants that failed to grow at 37° were Ura$^-$ and vice versa. Thus, the *Xho*I fragment corresponds to the *RTS1* locus.

The 2.7-kb *Xho*I fragment was sequenced. It contains the gene encoding a $tRNA^{Arg}_{ACG}$ (GAFNER, ROBERTS and PHILIPPSEN 1983) and a 492-bp open reading frame. These genes have been previously designated *HSX1* and *HIT1*, respectively (KAWAKAMI *et al.* 1992). They lie in a region of chromosome *X* that contains five δ sequences (Figure 1). The 2.7-kb *Xho*I fragment lies between δ2 and δ3. Spontaneous chromosomal deletions arising from homologous recombination between these δ sequences have been characterized (ROTHSTEIN, HELMS and ROSENBERG 1987). Southern analysis showed that *rts1-1* cells also contain a chromosomal deletion (Figure 2). When probed with the *Xho*I fragment (pJT123), *rts1-1* cells lacked the 4-kb *Bgl*II fragment present in wild-type cells. This shows that *rts1-1* cells are missing at least the chromosomal DNA from the *Xho*I site in δ2 to the *Bgl*II site near δ3 that contains the *HSX1* and *HIT1* genes. To determine the extent of the deletion, we used an *Eco*RI-*Cla*I probe (pWJ51, see Figure 1) that corresponds to a region outside of the δ sequences (ROTHSTEIN, HELMS and ROSENBERG 1987). This probe identifies a 12-kb *Eco*RI fragment in wild-type cells and a 7-kb *Eco*RI fragment in *rts1-1* cells. Thus, the deletion is approximately 5 kb. Furthermore, digestion of genomic DNA with *Hin*dIII indicates that the single *Hin*dIII site between δ4 and δ5 is lacking in *rts1-1* cells. Taken together, these results suggest that all of the sequences internal to δ2 and δ5 are absent in *rts1-1* cells. Thus, *rts1-1* strains are also lacking two tRNA genes (a $tRNA^{Asp}$ and *SUP4*) known to be located between δ4 and δ5, in addition to the *HSX1* and *HIT1* genes.

Dissection of the *RTS1* locus: The 2.7-kb *Xho*I fragment was subcloned to determine which sequences complemented suppression of *tub2-104*'s cold sensitivity and which complemented heat sensitivity.

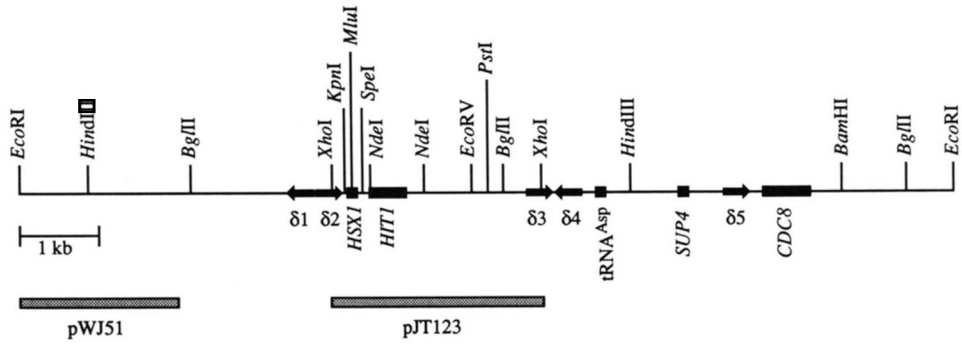

FIGURE 1.—Map of the *RTS1* locus. The restriction enzyme map and part of the DNA sequence of this region have been published previously (GAFNER, ROBERTS and PHILIPPSEN 1983; ROTHSTEIN, HELMS and ROSENBERG 1987). Boxes indicate coding regions of genes; arrows indicate δ elements and point in the direction of transcription. The bars beneath the map indicate the probes used for Southern and Northern blots.

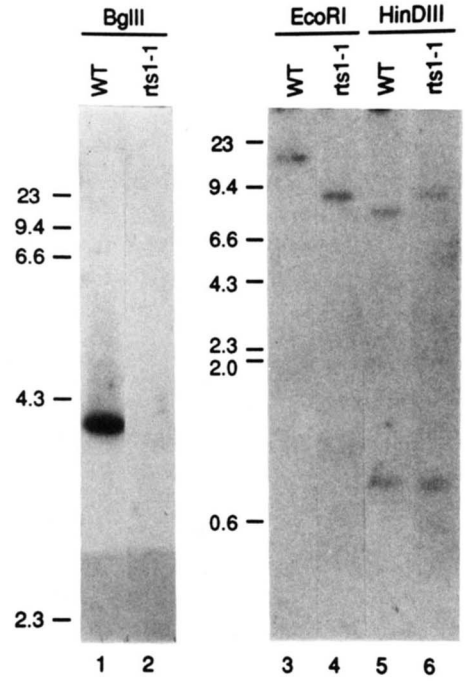

FIGURE 2.—Southern hybridization analysis of wild-type and *rts1-1* cells. Genomic DNA from wild-type (lanes 1, 3 and 5) and *rts1-1* (lanes 2, 4 and 6) cells was digested with either *Bgl*II (lanes 1 and 2), *Eco*RI (lanes 3 and 4) or *Hin*dIII (lanes 5 and 6), electrophoresed and blotted to nitrocellulose paper. The *Bgl*II digests were hybridized with the pJT123 probe; the *Eco*RI and *Hin*dIII digests were hybridized with the pWJ51 probe. Migration of DNA markers, in kilobases, are shown at left.

TABLE 3

Complementation of heat-sensitivity and suppression

Strain genotype	Plasmid genes	Growth 37°	Growth 14°
tub2-104 rts1-1	None	−	+
tub2-104 rts1-1	*HSX1, HIT1*	+	−
tub2-104 rts1-1	*HSX1*	−	−
tub2-104 rts1-1	*HIT1*	+	+

The results are summarized in Table 3. Both suppression and heat sensitivity are complemented by the entire *Xho*I fragment (pJT120). Suppression, but not heat sensitivity, is complemented by the *Xho*I to *Spe*I fragment that contains only *HSX1* (pRR23). Heat

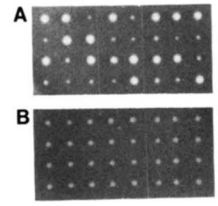

FIGURE 3.—Dissection of *hit1-Δ1::URA3/HIT1* and *hsx1::URA3/HSX1* diploids. Diploids were sporulated, the tetrads dissected onto YPD plates, and incubated at 30°. (A) Haploid segregants from a *hit1-Δ1::URA3/HIT1* diploid incubated for 5 days. The slow growing segregants are Ura$^+$. (B) Haploid segregants from a *hsx1::URA3/HSX1* diploid incubated for 3 days. Growth of Ura$^+$ and Ura$^-$ segregants was indistinguishable.

sensitivity, but not suppression, is complemented by the *Mlu*I to *Xho*I fragment that contains only *HIT1* (pRR4). These results suggest that suppression of *tub2-104* is caused by deletion of *HSX1* and heat sensitivity is caused by deletion of *HIT1*.

We tested this idea by disrupting the *HSX1* and *HIT1* genes independently as described in MATERIALS AND METHODS. Haploid cells containing a disruption of *HIT1* (*hit1-Δ1::URA3*) grew slower than wild-type cells at all temperatures below 30° and failed to grow at 37° (Table 2 and Figure 3A). These cells did not display a cell cycle arrest following a shift to their restrictive temperature. After 5 hr at 37°, the culture contained a normal population distribution of unbudded, small-budded and large-budded cells. *hit1-Δ1::URA3* did not suppress the cold sensitivity of *tub2-104* cells (Table 2). Haploid cells containing an *HSX1* disruption (*hsx1::URA3*) grew well at all temperatures (Table 2 and Figure 3B) and were more resistant to benomyl than wild-type cells. In addition, strains containing *hsx1::URA3* and *tub2-104* grew well at 14° (Table 2). Thus, the *HSX1* deletion suppresses the *tub2-104* defect and confers benomyl resistance; the *HIT1* deletion results only in heat sensitivity.

***HSX1* encodes a single-copy tRNA:** GAFNER, ROBERTS and PHILIPPSEN (1983) used DNA hybridization to show that the *HSX1* $tRNA^{Arg}_{ACG}$ is encoded by only a single gene in the yeast genome. Thus, it was quite surprising to find that this gene could be deleted without affecting cell growth. Further evidence that this $tRNA^{Arg}_{ACG}$ is encoded by only *HSX1* and has been

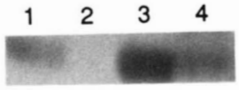

FIGURE 4.—Northern hybridization analysis. RNA was isolated from wild-type cells (lane 1), *rts1-1* cells (lane 2), wild-type cells containing *HSX1* on a high copy 2 μm plasmid (lane 3), and wild-type cells containing only the 2 μm plasmid (lane 4). Following electrophoresis, the RNA was transferred to nitrocellulose paper and hybridized with the pJT123 probe.

deleted in *rts1-1* mutants is shown in Figure 4. A Northern blot of equal amounts of RNA from four yeast strains was probed with the *Xho*I fragment. The four strains used were wild-type cells, *rts1-1* cells, wild-type cells containing *HSX1* on a high copy plasmid (pRR50), and wild-type cells containing only the vector (YEp352). The signal for the tRNA was similar in wild-type cells and wild-type cells containing the vector alone. The signal increased in overexpression strains but was absent in *rts1-1* cells. Thus, this $tRNA^{Arg}_{ACG}$ is not present in *rts1-1* cells nor is there a cross-hybridizing signal present in the total RNA of these cells.

Suppression is allele-specific: The original *rts1-1* mutation was crossed into several strains containing other cold-sensitive *tub2* alleles (*tub2-401*, *tub2-402*, *tub2-403*, *tub2-404*, *tub2-405*, *tub2-406*, *tub2-418*, *tub2-423*, *tub2-429*, *tub2-434* and *tub2-451*). *rts1-1 tub2-402* cells grew very slowly at 14°. The other *rts1-1 tub2* strains failed to grow at all at 14°. Similar results were obtained with *hsx1::URA3 tub2* strains. Thus, suppression by deletion of *HSX1* is allele-specific.

Suppression is not dependent on AGG codons in *TUB2*: *tub2-104* is a single base pair mutation that changes codon 241 from CGT (Arg) to CAT (His). It is difficult to imagine how the deletion of a gene encoding $tRNA^{Arg}_{ACG}$ could restore the wild-type amino acid at this position. However, the *TUB2* gene contains two AGG codons at positions 156 and 213. (The AGG codon at position 156 was originally published incorrectly as AAG; our unpublished results.) If the *HSX1* disruption suppresses *tub2-104* and confers benomyl resistance by interfering with some aspect of protein translation, then it seemed most likely that it would act through the two AGG codons in *tub2-104*. To test whether suppression relies on the AGG codons, we created *tub2-471* by oligonucleotide directed mutagenesis. *tub2-471* contains the *tub2-104* mutation at codon 241 and the more common Arg codon, AGA, at positions 156 and 213. Phenotypically, *tub2-471* cells are indistinguishable from *tub2-104* cells; they are cold-sensitive at 14° and resistant to high levels of benomyl. Like *tub2-104*, *tub2-471* is also suppressed by *hsx1::URA3* (Table 2). Thus, the presence of AGG codons in *tub2-104* is not required for suppression.

Suppression of the *tub2-104* defect is not via the arginylation pathway: Because the *HSX1* disruption did not appear to exert its effect by altering translation of *TUB2*, we examined the possibility that it suppresses *tub2-104* by altering some other cellular pathway. One known role for arginyl-tRNAs is in protein degradation. Degradation of many proteins is believed to occur via ubiquitination according to N-end rules (FINLEY and CHAU 1991). N-terminal amino acids such as Arg, Lys or His, are primary destabilizing residues. A protein with one of these amino acids at its N terminus can be directly conjugated with ubiquitin and subsequently degraded. The acidic amino acids, Asp or Glu, are termed secondary destabilizing residues. A protein with an acidic N-terminal residue can be targeted for ubiquitination following the addition of an arginine residue to its N terminus. Arginylation is catalyzed by an arginyl-tRNA-protein transferase that requires arginyl-tRNA as substrate. Although, the N terminus of β-tubulin is not acidic (the first 4 amino acids are Met, Arg, Glu, Ile), it seemed possible that the first two amino acids might be removed by proteases exposing an acidic N terminus. Alternatively, arginylation and ubiquitination of a microtubule-interacting protein might be affected.

If the *HSX1* disruption suppresses *tub2-104* by limiting arginylation, then mutations in other genes that block the arginylation pathway should also suppress *tub2-104*. In yeast, the arginyl-transferase is encoded by the *ATE1* gene (BALZI *et al.* 1990). *UBR1* encodes an enzyme that catalyzes the ubiquitination of proteins with destabilizing N-terminal residues (BARTEL, WUNNING and VARSHAVSKY 1990). Both of these genes can be deleted without affecting cell growth. The double mutants containing *tub2-104* and a disruption of either *ATE1* or *UBR1* were constructed and assayed for growth at 14°. The *ATE1* disruption is marked with *TRP1* and carried in a *trp1-63* cell. The *trp1-63* mutation causes cold sensitivity which complicated the analysis somewhat. We crossed KMY207 and CUY51, sporulated the diploid and analyzed tetrads. Only tetrads that had two Trp^+ and two Trp^- spores were useful, because only in these tetrads could we unambiguously identify both spores containing the *trp1-63* mutation. The two Trp^+ spores had to contain *ate1::TRP1* and lack the *trp1-63* mutation. The *tub2-104* mutation was identified by its benomyl resistance. Trp^+ benomyl resistant spores were scored for cold sensitivity. These failed to grow in the cold indicating that the *ATE1* disruption did not suppress the cold sensitivity of *tub2-104*. Similarly, the *UBR1* disruption also failed to suppress *tub2-104*. Therefore, suppression by deletion of *HSX1* is apparently unrelated to changes in the arginylation/ubiquitination pathway.

DISCUSSION

rts1-1 was identified as an extragenic suppressor of the cold sensitive mutation *tub2-104*. While *tub2-104*

cells fail to grow at 14°, *rts1-1 tub2-104* cells grow nearly as well as wild-type cells at 14°. The mutation also confers heat sensitivity; at 37° *rts1-1* cells display a mitotic arrest that is similar to the arrest phenotype of *tub2-104* cells at 14°. In addition, the *rts1-1* cells are substantially more resistant to benomyl than wild-type cells. Thus, the *rts1-1* allele confers three independent phenotypes each of which indicated that the mutation altered some aspect of microtubule function.

Molecular analyses showed that *rts1-1* cells lack nearly 5 kb of DNA on chromosome *X*. This deletion presumably arose via homologous recombination between δ sequences in this region of the genome. The deletion removes three tRNA genes and one open reading frame. Dissection of this region showed that heat sensitivity (and slower growth at permissive temperatures) is caused by deletion of the open reading frame, *HIT1*. However, cells containing a deletion of *HIT1* do not display the mitotic arrest phenotype observed for *rts1-1* cells. Presumably, deletion of sequences in addition to *HIT1* are required for the arrest phenotype, but we have not investigated this further. Suppression of *tub2-104* and benomyl resistance are caused by disruption of *HSX1*, which encodes a $tRNA^{Arg}_{ACG}$. Cells containing *tub2-104* and the *HSX1* disruption grow as well as wild-type cells at 14°. The *HSX1* disruption alone suppresses the *tub2-104* cold sensitivity better than the original *rts1-1* mutation, because the *HIT1* deletion in *rts1-1* cells slowed growth at 14°. Benomyl resistance is slightly less in strains containing only the *HSX1* disruption. Again, slower growth due to the *HIT1* deletion probably allowed *rts1-1* cells to survive on somewhat higher levels of benomyl.

Recently, KAWAKAMI *et al.* (1992) produced a collection of Ty element-induced heat-sensitive mutations in yeast. One of these, called *hit1-1*, contained a chromosomal deletion that removed both the *HSX1* and *HIT1* genes. They also disrupted both of these genes independently and obtained results similar to ours. Their *HSX1* deletion did not affect growth under normal conditions but did make cells heat sensitive on nonfermentable carbon sources (we also observed the latter phenotype in *hsx1::URA3* cells) and inhibited expression of the heat-shock protein hsp74. Their *HIT1* deletion produced heat-sensitive cells that were unable to grow on nonfermentable carbon sources at any temperature (we did not observe the latter in our *hit1-Δ1* strains).

The question we have addressed is how does deletion of a tRNA suppress a point mutation in the *TUB2* gene. Because *HSX1* appears to be the only gene encoding this particular $tRNA^{Arg}_{ACG}$, some other tRNA must be capable of reading the AGG codon in its absence. It is unlikely that the $tRNA^{Arg}_{ACA}$ could read this codon. Wobble decoding appears to be restricted in yeast (GUTHRIE and ABELSON 1982), consistent with the long standing observation that yeast ochre suppressors, unlike their prokaryotic counterparts, recognize UAA but not UAG nonsense codons (SHERMAN 1982). Thus, it is likely that yeast contain another $tRNA^{Arg}_{ACG}$ that is not homologous to the tRNA encoded by *HSX1*. Nonetheless, the *HSX1* disruption might significantly lower the levels of tRNA available to translate AGG codons. If this happens, then the error frequency at AGG codons would be expected to increase. In *E. coli*, imbalances among the aminoacyl-tRNA pools generate missense errors in translation, especially in *rel* mutants that are deficient in an error-damping function (GALLANT 1979).

The *TUB2* gene contains two AGG codons. We hypothesized that the *HSX1* disruption might suppress the *tub2-104* allele by causing specific translation errors at these codons. Precedent for this type of mechanism in yeast comes from the analysis of suppressors of a heat-sensitive allele of *CDC8* (SU, BELMONT and SCLAFANI 1990). A single C to T mutation in the anticodon of a $tRNA^{Glu}$ gene produces a missense suppressor tRNA capable of recognizing lysine codons. The resultant lysine to glutamate change presumably stabilizes the thermolabile Cdc8p. We tested our hypothesis by changing each of the two AGG codons in *tub2-104* to the more commonly used AGA codon. This new allele, *tub2-471*, was phenotypically identical to *tub2-104* and its cold sensitivity could still be suppressed by *hsx1::URA3*. Thus, suppression is not due to misreading at AGG codons in *TUB2*. We can not rule out the even more remote possibility that suppression is due to misreading at AGG codons in some other protein. However, it is unlikely that translational errors at AGG codons are common in cells lacking *HSX1* because they grow as well as wild-type cells.

In *E. coli*, aminoacyl-tRNA limitation also stimulates ribosomal frameshifts at "hungry" codons calling for the limiting species (WEISS *et al.* 1988). $tRNA^{Arg}_{ACG}$ should be a relatively rare tRNA in yeast because arginine is encoded by AGG only about 2% of the time (GUTHRIE and ABELSON 1982). Experiments on frameshifting in Ty elements suggest that $tRNA^{Arg}_{ACG}$ is limiting in yeast (BELCOURT and FARABAUGH 1990). Ty elements encode the two genes *TYA* and *TYB*. Expression of the *TYB* gene, which encodes the polymerase activity, requires frameshifting in the +1 direction. The mechanism of frameshifting involves ribosomal slipping on a 7-nucleotide site (CUU-AGG-C). The AGG codon is essential for slippage to occur; no other codon could be used in its place. The authors suggest that the ribosome encounters the AGG and pauses because of the low availability of $tRNA^{Arg}_{ACG}$ thus allowing the peptidyl-$tRNA^{Leu}$ to slip from the CUU reading frame into the UUA (+1) reading frame. In

agreement with this idea, overexpression of the *HSX1* gene reduces frameshifting at this site (XU and BOEKE 1990). It is possible that ribosomal frameshifting also controls the expression of a protein that plays a role in microtubule function. Altered levels of this protein may be responsible for suppression of the *tub2-104* mutation.

In addition to their role in translation, tRNAs are found as cofactors in a number of cellular processes, including the biosynthesis of heme and chlorophyll (JAHN, VERCAMP and SOLL 1992), reverse transcription of retroviral RNA (VARMUS and BROWN 1989), and ubiquitin-mediated protein degradation (FERBER and CIECHANOVER 1987). Recent evidence hints that they may play a role in transcription as well (HAPPEL and WINSTON 1992). Because ubiquitin-mediated protein degradation is known to occur in yeast and require an arginyl-tRNA, we examined the possibility that the tRNA deletion suppresses by interfering with this pathway. Proteins with secondary destabilizing at their N terminus require the addition of arginine prior to ubiquitination and subsequent degradation (FINLEY and CHAU 1991). Arginylation is catalyzed by an arginyl-tRNA-protein transferase, encoded by the *ATE1* gene in yeast. We reasoned that if the *HSX1* disruption suppresses the cold sensitivity of *tub2-104* by lowering the levels arginyl-tRNA substrate for this reaction, then removing the *ATE1* gene would likewise suppress this mutation. The fact that *ate1::TRP1 tub2-104* cells are unable to grow at 14° demonstrates that the *hsx1::URA3* does not suppress via this pathway.

The phenotypes of the *HSX1* disruption, suppression of a mutation that alters β-tubulin and increased resistance to the microtubule-destabilizing drug benomyl suggest that this $tRNA^{Arg}_{ACG}$ may be directly involved in microtubule function. There has been some controversy as to the role of RNA in centriole and kinetochore function (reviewed by BRINKLEY 1985). It has been suggested that centrioles and kinetochores contain RNA based on the observation that they specifically react with a stain for ribonucleoprotein that can be removed by treatment with RNase and perchloric acid (BIELEK 1978; RIEDER 1979). Similar results suggest that RNA is a component of the basal bodies of Tetrahymena, as well (HARTMAN, PUMA and GURNEY 1974). In addition, treatments which destroy RNA have been observed to abolish the ability of centrioles to initiate microtubule assembly (PETERSON and BERNS 1978). In yeast, however, there is no evidence that RNA is a component of the spindle pole body (the microtubule-organizing center in yeast) or centromere complex. Treatment of isolated spindle pole bodies with RNase does not alter this structure and only slightly diminishes the ability to nucleate microtubules (HYAMS and BORISY 1978). In this type of study, though, accessibility to RNase may be limited. Thus, it is difficult to rule out the possibility that the spindle pole bodies of yeast contain RNA, perhaps even this specific $tRNA^{Arg}_{ACG}$.

In summary, we have shown that deletion of the gene encoding a $tRNA^{Arg}_{ACG}$ suppresses a cold-sensitive allele of *TUB2* and confers resistance to the microtubule-destabilizing drug, benomyl. While we cannot rule out the possibility that this mutation acts as an informational suppressor, we have demonstrated that it does not act directly through the most logical candidate, the *TUB2* itself. This leaves open the prospect that this tRNA plays an additional role in the cell that is independent from its role in translation.

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