A Mutation in *GRS1*, a Glycyl-tRNA Synthetase, Affects 3′-End Formation in *Saccharomyces cerevisiae*

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ABSTRACT

3′-end formation is a complex and incompletely understood process involving both *cis*-acting and *trans*-acting factors. As part of an effort to examine the mechanisms of transcription termination by RNA polymerase II, a mutant hunt for strains defective in 3′-end formation was conducted. Following random mutagenesis, a temperature-sensitive strain exhibiting several phenotypes consistent with a role in transcription termination was isolated. First, readthrough of a terminator increases significantly in the mutant strain. Accordingly, RNA analysis indicates a decrease in the level of terminated transcripts, both *in vivo* and *in vitro*. Moreover, a plasmid stability assay in which high levels of readthrough lead to high levels of plasmid loss and transcription run-on analysis also demonstrate defective termination of transcription. Examination of polyadenylation and cleavage by the mutant strain indicates these processes are not affected. These results represent the first example of a transcription termination factor in *Saccharomyces cerevisiae* that affects transcription termination independent of 3′-end processing of mRNA. Complementation studies identified *GRS1*, an aminoacyl-tRNA synthetase, as the complementing gene. Sequence analysis of *grs1-1* in the mutant strain revealed that nucleotides 1656 and 1657 were both C to T transitions, resulting in a single amino acid change of proline to phenylalanine. Further studies revealed *GRS1* is essential, and the *grs1-1* allele confers the temperature-sensitive growth defect associated with the mutant strain. Finally, we observed structures with some similarity to tRNA molecules within the 3′-end of various yeast genes. On the basis of our results, we suggest Grs1p is a transcription termination factor that may interact with the 3′-end of pre-mRNA to promote 3′-end formation.

TRANSCRIPTION termination requires the dissociation of the tertiary complex formed by the DNA template, the transcribing RNA polymerase, and the nascent RNA. Accurate termination is required for wild-type levels of gene expression as readthrough transcription into an adjacent gene results in reduced expression of the downstream transcriptional unit (Proudfoot 1986; Eggermont and Proudfoot 1993; Springer *et al.* 1997; Greger *et al.* 1998) and may also result in decreased expression of the affected gene (Zaret and Sherman 1982; Whitelaw and Proudfoot 1986).

The investigation of transcription termination by RNA polymerase II is complicated by the 3′-end processing of the pre-mRNA, which is coupled to the termination of transcription (Hyman and Moore 1993; Russo 1995; McCracken *et al.* 1997; Birse *et al.* 1998). Once the RNA polymerase II transcribes past the polyadenylation site, three significant and interrelated reactions occur: pre-mRNA cleavage, polyadenylation, and termination. The cleavage reaction results in the production of two RNAs; these are the upstream cleavage product, containing the functional mRNA, and the downstream cleavage product that has no coding capacity and is rapidly degraded *in vivo*. The upstream product is further processed by the addition of a tract of adenylate residues that comprises the poly(A) tail (reviewed in Colgan and Manley 1997; Preker *et al.* 1997). The downstream product is subject to termination of transcription, an event that defines the end of transcription.

Numerous *trans*-acting factors have been identified in yeast as having roles in 3′-end formation, including the ~20 gene products that have direct roles in the biochemical processing of pre-mRNAs *in vitro* (Wahle and Keller 1996; reviewed in Keller and Minvielle-Sebastia 1997). Some of these factors, including Rna14p, Rna15p, and Pcf11p, are known to be important for both RNA processing and transcription termination, thus providing a mechanistic bridge between the key events involved in 3′-end formation (Birse *et al.* 1998). Other genes have also been identified by the genetic characterization of mutations that affect 3′-end formation. For example, *REF2* was discovered using a screen for mutations that lead to inefficient recognition of wild-type polyadenylation signals (Russnak *et al.* 1995). Recently, *Ptf1*, the gene for the protein processing protein *cis*-/*trans*-prolyl isomerase was identified in a genetic

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screen for factors involved in transcription termination by RNA polymerase II in *Saccharomyces cerevisiae* (Hani *et al.* 1999). An additional set of mutations affecting 3′-end formation was isolated as a suppressor of the *pap1-1* mutation, including a mutation in the second-largest subunit of RNA polymerase III (Briggs and Butler 1996) and a mutation in *RRP6*, a gene involved in 5.8S rRNA biosynthesis (Briggs *et al.* 1998), suggesting unexpected overlap of function of proteins not expected to play a role in RNA polymerase II 3′-end formation. Finally, mammalian 3′-end formation proteins involved in cleavage and polyadenylation interact with the C-terminal domain of the largest subunit of RNA polymerase II, providing a mechanism, in principle, for linking transcription and RNA processing *in vivo* (McCracken *et al.* 1997; Steinmetz 1997). Thus, although many of the factors involved in 3′-end processing and transcription termination are being defined, an understanding of how these factors are integrated with each other and the actual events that lead to 3′-end formation *in vivo* is still unclear. Here we report the novel finding that a mutation in *GRS1*, the probable gene for glycyl-tRNA synthetase, also affects 3′-end formation in *S. cerevisiae*.

MATERIALS AND METHODS

Reagents: Culture media components were obtained from Difco (Detroit). Amino acids, 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-Gal) and *o*-nitrophenyl-β-d-galactosidase (ONPG), as well as most other chemicals, were from Sigma (St. Louis). T4 DNA ligase was obtained from New England Biolabs (Beverly, MA). Most restriction enzymes, T3, SP6, and T7 polymerases, RNasin, calf intestine alkaline phosphatase, and Taq DNA polymerase were obtained from Promega (Madison, WI). All reagents were used according to manufacturer's specifications. Radiolabeled nucleotides were obtained from Dupont/NEN (Boston). Sequenase enzyme was obtained from Amersham (Arlington Heights, IL). Nylon transfer membrane was from Micron Separations (Westborough, MA).

Plasmids: The plasmids, pL101 and pT7T3*ADH2* (Hyman *et al.* 1991), pL501 (Hyman and Moore 1993), pT7T3URA3 (Chen *et al.* 1996), YCplac111 and YCplac211 (Gietz *et al.* 1992), pBEVY (Miller *et al.* 1998), pCYC (Butler and Platt 1988), pGCYC and pGcyc-512 (Birse *et al.* 1998), and pBM272 (Johnston and Davis 1984; Rose *et al.* 1987) have been previously described. YCpMM3 is identical to YRp14/CEN4/H4ARS (Hieter *et al.* 1985). p366 (ATCC 77163) and the yeast genomic library (ATCC 77162) were obtained from ATCC.

pGTP and pGTM were constructed by amplifying an *ADH2* 3′-end fragment by PCR of pT7T3*ADH2* with primers (5′GA**AGATCT**GACACTTCTAAATAAGCGG and 5′GA**AGATCT**GGCATGCGAAGGAAAATGAG) that created *Bgl*II sites (in boldface) on both ends of the PCR fragment. Following digestion with *Bgl*II, the fragment was cloned into the *Bam*HI site of pBM272, creating pBM272 derivatives with the *ADH2* 3′-end downstream of the *GAL1* promoter in both orientations (plus and minus relative to the *GAL1* promoter). These *GAL1/ADH2* 3′-end fusions (+ and −) were used as templates for PCR with primers (5′GA**AGATCT**TCGCCCCATTATCTTAGCC and 5′GCCAGCAACCGCACC), which created a *Bgl*II site (in boldface) on the 5′-end of the PCR fragment. Following digestion of the PCR product with *Bgl*II and *Hin*dIII and the vector, YCpMM3, with *Bam*HI and *Hin*dIII, the fragments were ligated to generate pGTP and pGTM. pGTP contains the plus orientation of *ADH2* and pGTM contains the minus orientation.

YCplac111/*TFC1* was obtained by digesting the genomic library clone, p366/711, with *Nsi*I and ligating the 3212-bp gel-purified restriction fragment into the *Pst*I site of YCplac111. Digesting YCp111/*TFC1* with *Xmn*I and *Sma*I and ligating the resulting fragment into the *Sma*I site of YCp111 generated YCp111/YBR124W. YCp111/*MRPL36* was constructed by digesting p366/57 with *Eco*RV and ligating the 1479-bp product into the *Sma*I site of YCp111. p366/57Δ*tfc1* was generated by digesting the genomic library clone, p366/57, with *Sac*I and religating the plasmid to delete the region of *TFC1* between the two *Sac*I sites. p366/76Δ*GRS*1 was generated by digestion of the genomic library clone, p366/76, with *Msc*I and religating the plasmid to delete the region of *GRS1* between the two *Msc*I sites that flank *GRS1*. A third *Msc*I site, located within the vector p366, is blocked by dam methylation. To construct YCp111/*grs1-1*, the *GRS1* gene was cloned from genomic DNA preparations of both the wild-type (YPH499) and mutant (2-1-1) strains after introducing flanking *Bam*HI and *Xba*I restriction sites (in boldface) by PCR, using the primers LEH285 (5′ CG**GGATCC**AGTGTAGAAGATATC) and LEH286 (5′ GC**TCTAGA**GATTTCCGCACTTC). For each independent PCR reaction (four or more reactions from both strains), the PCR fragments and vectors were digested with *Bam*HI and *Xba*I and cloned into the *Xba*I and *Bam*HI site of the centromeric vectors YCplac111 and pT7T319U. The PCR-derived *GRS1*-containing plasmids were transformed into 2-1-1 and temperature sensitivity at 37° was assessed. Strains that contained plasmid derivatives that did not complement the temperature sensitivity were identified, and plasmid YCp111/*grs1-1* isolated from the strains. The *grs1-1* allele was excised from YCp111/*grs1-1* as a *Pst*I/*Eco*RI fragment and cloned into *Pst*I/*Eco*RI cut YIplac211, creating YIp211/*grs1-1*. As a control, an identical protocol was used to remove a wild-type version of *GRS1* from the genome of W303a creating the plasmids YCp111/*GRS1* and YIp211/*GRS1*. All plasmids used in the genetic study are shown in Table 1.

UV mutagenesis and screening strategy: YPH499 was transformed with the reporter plasmid, pL101, using the lithium acetate method (Ausubel *et al.* 1994). In excess of 45,000 YPH499 cells with pL101 were plated to complete minimal dextrose media plates without uracil, at ~1000 cells per plate. The plates were then immediately subjected to UV light at a dosage of 675 J/m^2, resulting in 65–80% cell death, and incubated at 24° for 3–5 days, until colonies were fully formed. The colonies were replica plated to complete minimal galactose media without uracil and with X-Gal (CUXG; Ausubel *et al.* 1994) and incubated at 37°, 24°, and 16°. Colonies that produced β-galactosidase were patched to complete minimal glucose media plates without uracil and grown at 24°. The strains were screened on X-Gal plates at 37°, 24°, and 16°. Strains that consistently produced β-galactosidase on the CUXG indicator plates at any of the three temperatures were chosen for further study.

Elimination of plasmid-based mutations: Candidate strains were cured and retransformed with the pL101 plasmid using a scaled down lithium acetate protocol (Firmenich and Redding 1993; Ausubel *et al.* 1994). The retransformed strains were then screened on X-Gal plates for β-galactosidase production at 37°, 24°, and 16°. Temperature-sensitive growth at any of these temperatures was monitored.

Northern analysis: Cells were grown to saturation in complete minimal dextrose media without uracil and the saturated cultures were used to inoculate complete minimal galactose media without uracil. These induced cells were grown to satu-

TABLE 1

Plasmids used in genetic study

Name	Description	Source
pL101	β-Galactosidase reporter	L. E. Hyman
pGTP	Plasmid loss reporter/+ orientation	This study
pGTM	Plasmid loss reporter/− orientation	This study
YCPlac111	Centromeric vector (*LEU2* marked)	D. Gietz
YIPlac211	Integrative vector (*URA3* marked)	D. Gietz
YCP111/GRS1	YCPlac111 with *GRS1* insert	This study
YCP111/MRPL36	YCPlac111 with *MRPL36* insert	This study
YCP111/TFC1	YCPlac111 with *TFC1* insert	This study
YCP111/YBR124w	YCPlac111 with YBR124w insert	This study
p366	Vector (centromeric *LEU2* marked)	ATCC
p366/library	p366 with genomic library	ATCC
p366/76	p366 with complementing library fragment	ATCC
p366/711	p366 with complementing library fragment	ATCC
p366/57	p366 with complementing library fragment	ATCC
p366/76ΔGRS1	p366/76 with *GRS1* deleted	This study
T7T3GRS1+	T7T3 with *GRS1* insert	This study
T7T3grs1-1	T7T3 with *grs1-1* insert	This study
YCP111/grs1-1	YCP111 with *grs1-1* insert	This study
YIP211/grs1-1	YIPlac211 with *grs1-1* insert	This study
YIP211/GRS1	YIPlac211 with *GRS1* insert	This study

ration (OD_{600} of 1.5–2.0), reinoculated, and harvested when the OD_{600} was 0.5–1.0. For temperature-shift studies, cells were grown to an OD_{600} between 0.15 and 0.5, split, and grown at the appropriate temperatures for 7–24 hr. Total RNA was prepared using the hot phenol/glass beads method. Samples were electrophoresed on 1.5%-agarose/formaldehyde gel, and Northern analysis performed. [α-^{32}P]UTP-labeled *ADH2* and *URA3* probes were made and hybridization was completed. All procedures for RNA analysis were as previously described (Hyman *et al.* 1991; Hyman and Moore 1993; Ausubel *et al.* 1994).

Plasmid loss analysis: Strains transformed with either plasmid pGTP or pGTM were grown in complete minimal dextrose media without uracil and inoculated into YPD or YPGal (Ausubel *et al.* 1994). At various time points, approximately every 2–4 hr, cells were counted using a hemacytometer and ~500 cells were plated to YPD. The total number of colonies and the number of red or non-red colonies (white or sectored) were determined. Percent plasmid maintenance was calculated as percent non-red/total colonies. For temperature-shift experiments, the YPD or YPGal cultures were grown to early log phase (OD_{600} of 0.2) and split to the permissive and nonpermissive temperatures.

Random spore analysis: Strain 2-1-1 transformed with pL101 was mated to YPH500 transformed with YCplac211, and diploids (2-1-1/500) were selected on complete minimal dextrose plates without uracil or leucine. Sporulation was induced by growth on plates supplemented with histidine, adenine, lysine, and tryptophan (Ausubel *et al.* 1994). One of the spore progeny (2-1-1-B) was grown in nonselective media to induce plasmid loss. The strain was retransformed with pL101 and backcrossed to YPH500; diploids (2-1-1-B/500) were selected, as above. Following sporulation, random spore analysis was performed as described in Ausubel *et al.* (1994). Plates containing dispersed spores were replica plated to X-Gal plates and screened at 30° and 37° for growth and β-galactosidase production.

Yeast extracts and *in vitro* transcription: Yeast nuclear extracts were prepared from 2-1-1 and BJ926 by the methods of Guthrie and Fink (1991), Lue *et al.* (1991), and Lue and Kornberg (1987), except the final OD_{600} of 2-1-1, which was 2.9. Briefly, dense cell cultures were prepared and harvested. Spheroplasts were prepared from the cultures using digestion with yeast lytic enzyme, and, following a period of recovery by growth in rich media, the cells were washed extensively. The cells were then homogenized using conditions that preserved the intact nuclei. The nuclei were recovered by centrifugation and the nuclear proteins were prepared. Protein concentration was determined by Bradford Assay (Bio-Rad, Hercules, CA). Transcription reactions were carried out as previously described (Lue and Kornberg 1987; Lue *et al.* 1991). The transcripts were analyzed on a 1.5%-agarose/formaldehyde gel.

Polyadenylation and cleavage extracts: Yeast whole-cell extracts were prepared according to published procedures (Butler and Platt 1988; Chen and Moore 1992). The full-length CYC1 precursor was made by *in vitro* transcription using SP6 polymerase and the pCYC template digested with *Pvu*II. Radiolabeled RNA substrate was gel purified from a 5% polyacrylamide gel with 8.3 m urea. Approximately 70,000 cpm were added to the extracts. Processing reactions were performed at 30° for 30 min. Heat-treated extracts were incubated at 37° for 5 min prior to addition of RNA substrate. dATP was substituted for ATP for the cleavage-only reactions. Results were visualized on a 5% denaturing acrylamide gel.

Transcription run-on: Examination of transcription run-on was completed as described in Birse *et al.* (1998) with the following modifications: YCM101 and W303a were used and the temperature shift was for 2 hr at 37°. Briefly, cells (containing the plasmid pGCYC or pGcyc-512) grown on complete minimal galactose plates without tryptophan were used to inoculate liquid media that was incubated at 30°. The cultures were grown to early log phase (OD_{600} was 0.05–0.1) and harvested. For temperature-shift experiments, the cells were resuspended in media and grown at 37° for ~2 hr. The samples were washed in cold water and treated with Sarkosyl. The resulting cell pellets were used in a transcription reaction. Total RNA was isolated from the cells and hybridized overnight

TABLE 2

Yeast strains used in this study

Strain	Genotype	Source
S. cerevisiae		
W303a	*MAT***a** *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 leu2-Δ1*	
W303α	*MATα ura3-52 lys-2-801^{amber} ade2-101^{ochre} trp1-Δ63 leu2-Δ1*	
W303a/α	*MAT***a**/α *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 leu2-Δ1*	
YPH499	*MAT***a** *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 his3-Δ200 leu2-Δ1*	Stratagene
YPH500	*MATα ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 his3-Δ200 leu2-Δ1*	Stratagene
YPH501	*MAT***a**/α *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 his3-Δ200 leu2-Δ1*	Stratagene
2-1-1	Derivative (mutant) of YPH499	This study
2-1-1/500	2-1-1 X YPH500	This study
2-1-1/W303	2-1-1 X W303α	This study
2-1-1-B	2-1-1/500 spore product (*MAT***a**)	This study
2-1-1-B/500	2-1-1-B X YPH500	This study
YCM101	*MAT***a** *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 leu2-Δ1 grs1-1*	This study
YCM102	*MAT***a** *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 leu2-Δ1 grs1::grs1-1/URA3*	This study
YCM103	*MATα ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 leu2-Δ1 grs1-Δ1*	This study
	*MAT***a** *ura3-52 lys2-801^{amber} ade2-101^{ochre} trp1-Δ63 leu2-Δ1 GRS1*	
BJ926	*MATα trp1 prc1-126 prb1-1122 pep4-3 can1 gal2*	Yeast Genetic
	*MAT***a** *his1 prc1-126 prb1-1122 pep4-3 can1 gal2*	Stock Center

(24°) to a slot blot containing M13 probes specific for regions of *CYC1* gene. After being washed, the filters were analyzed by autoradiography.

Complementation: Strain 2-1-1 was transformed with 0.5 μg of yeast genomic library ATCC 77162, plated to complete minimal dextrose plates without uracil, and incubated at 37°. Eight independent transformations were completed and at least 20 genome equivalents of library DNA were screened. The transformants that appeared at the restrictive temperature were selected and the recovery of growth at 37° was confirmed. Plasmids were recovered from strains with restored growth and amplified in *Escherichia coli* (DH5α; Ausubel *et al.* 1994). Restriction analysis was used to determine the number of independent genomic library inserts. DNA was sequenced by the double-stranded dideoxy sequencing technique with Sequenase using primers that flanked the *Bam*HI genomic library fragment insertion site of p366 (5′GCCGGCCAC GATGCG and 5′GGCGACCACACCCG). The sequence was then used in a BLAST search (Altschul *et al.* 1990; Cherry *et al.* Saccharomyces Genome Database, 1997) and the ends of the genomic inserts were defined.

Generation and analysis of *GRS1* null strain: Generation of a *grs1*Δ/GRS1 derivative of W303a/α was completed using the kan/lox method (Guldener *et al.* 1996), with primers specific for the region ~100 bp upstream and downstream of the *GRS1* locus (LEH290, 5′CGTATTAGTGGACAGTACATAA TCATATCTTGCAATACGTATCAGCTGAAGCTTCGTACGC and LEH289, CAGTAGACGATTTTACTCTCAGATTGTTAA AAAATCGGTTAAGCATAGGCCACTAGTGGATCTG). The genotype of G418 resistant colonies was determined using PCR with primers specific for the kanamycin cassette (Guldener *et al.* 1996), paired with LEH289 or LEH290. The resulting heterozygotic strain (YCM103) was sporulated and dissected. The spore products were analyzed on YPD and YPD plus G418 media at 30° and 37° to determine if a haploid *grs1* null strain was viable.

Generation of integrant: YIP211/*grs1-1* and YIP211/*GRS1* were transformed into yeast, and potential integrants were selected on the basis of the ability to grow on complete minimal dextrose media without uracil (Ausubel *et al.* 1994). Integration of the plasmid sequence harboring the *URA3* gene and the *grs1-1* allele from the 2-1-1 background (resulting in strain YCM102) was confirmed by Southern analysis (not shown). After growth on 5-fluoroorotic acid, strains that had recombined between the two tandemly arrayed *GRS1* alleles (*grs1-1* and *GRS1*) were selected (Guthrie and Fink 1991; Ausubel *et al.* 1994). Multiple strains containing a single copy of the mutant *grs1* allele (renamed as YCM101) were selected on the basis of temperature sensitivity at 37°. To confirm our previous genetic linkage data, YCM101 transformed with pL101 was mated to W303α transformed with p366, and diploids were selected on complete minimal dextrose plates without uracil or leucine. The resulting diploids were dissected and the phenotypes of the resulting segregants were examined on YPD, complete minimal dextrose plates without uracil and X-gal indicator plates at the permissive (30°) and nonpermissive (37°) temperatures.

DNA sequencing: The T7T319U derivatives constructed from the mutant background (above) were used for DNA sequencing. Independently derived clones were sequenced and compared to clones derived from the wild-type strain. DNA was sequenced by the double-stranded dideoxy sequencing technique with Sequenase (USB/Amersham).

Yeast strains and methods: All mutant strains are derivatives of the *S. cerevisiae* strain YPH499 or W303 (Table 2). Yeast cells were cultured by standard techniques (Sherman *et al.* 1986; Guthrie and Fink 1991; Ausubel *et al.* 1994).

RESULTS

Mutagenesis of YPH499 to create defects in *trans*-acting factors involved in transcription termination: As part of an effort to examine the mechanisms of transcription termination by RNA polymerase II, a mutant hunt for strains defective in 3′-end formation was conducted. The reporter plasmid pL101 was used as the primary indicator of increased readthrough transcription (Figure 1A). pL101 contains a galactose inducible promoter that drives transcription into an intron-imbed-

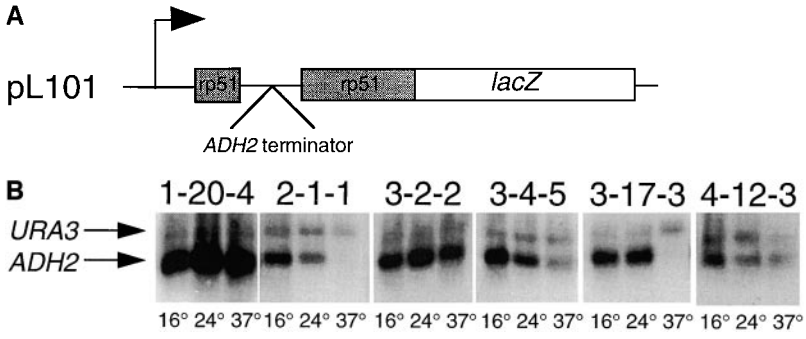

Figure 1.—Reporter construct used in analysis of *trans*-acting factors. (A) *LacZ* reporter construct. When the plasmid pL101 is transformed into mutant or wild-type cells, varying levels of readthrough can be detected by both a color assay, based on β-galactosidase production from the *LacZ* gene, and Northern analysis, based on hybridization of an antisense *ADH2* 3′-end specific probe. (B) Northern analysis of mutant strains. Cells containing the pL101 reporter plasmid were grown in complete minimal galactose media minus uracil and leucine at the temperature indicated. Total RNA (5 μg) was run on a 1.5%-agarose/formaldehyde gel. Following transfer, transcripts were detected using a radiolabeled antisense *ADH2* 3′-end specific probe with *URA3* as a loading control. Total RNA from a nonmutant strain (3-2-2) is shown for comparative purposes.

ded transcription termination signal placed upstream of the *lacZ* gene. Consequently, β-galactosidase production serves as an indication of the efficiency of transcription termination (Hyman *et al.* 1991). In wild-type cells termination occurs within the intron because of recognition of the *ADH2* 3′-end formation signal, and the cells do not produce β-galactosidase, thus producing white colonies on indicator plates. However, when readthrough of the termination signal occurs, the *LacZ* gene downstream from the intron is transcribed and, following removal of the termination signal by splicing, the cells produce β-galactosidase, thus producing blue colonies on indicator plates.

Using this reporter in an analysis of ~46,000 UV-mutagenized *S. cerevisiae* cells, we sought to identify mutations that give poor 3′-end formation at a permissive temperature (24°) but are nonviable at a nonpermissive temperature (16° or 37°). Approximately 300 β-galactosidase-producing strains were identified and analyzed.

Additional screening of potential mutants to eliminate false positives: To eliminate false positives that were expected to arise because of plasmid-borne mutations (*i.e.*, *cis*-mutations of the *ADH2* 3′-end signal), each putative mutant strain was cured of the original plasmid, retransformed with pL101, and retested for β-galactosidase production. Subsequently, to reduce the pool of the 120 remaining strains, only those candidates that exhibited a temperature-sensitive phenotype in addition to increased β-galactosidase production were selected for continued investigation, leaving 20 strains for RNA analysis.

Northern analysis: Total RNA was prepared from these 20 candidates containing pL101 and examined by Northern blotting using the *ADH2* 3′-end sequence as a probe. The amount of hybridization to this probe correlates to the level of correctly terminated transcripts produced by the reporter plasmid. Readthrough transcripts do not contain the *ADH2* 3′-end sequence, as this sequence is located within a functional intron. Consequently, the readthrough transcripts are not detected by the *ADH2* 3′-end specific probe (Hyman *et al.* 1991). Of the 20 remaining strains, we observed different classes of mutations; some strains had no obvious defect in RNA synthesis (1-20-4, 3-2-2), while others had subtle (4-12-3, 3-4-5) or severe defects (2-1-1, 3-17-3; Figure 1B).

Analysis of mutants by plasmid loss assay: To increase the rigor of the screen and eliminate mutations that are due to indirect effects, a second complementary reporter system based on a plasmid instability assay (Snyder *et al.* 1988) was used. Mutant phenotypes that may result from indirect effects on RNA metabolism, including splicing, transport, stability, and translational efficiency, are eliminated by the plasmid loss analysis using pGTP and pGTM (described below and in Figure 2A; Koshland *et al.* 1985; Snyder *et al.* 1988). As this assay does not rely on any translated gene product, only increased readthrough transcription should be detected by the reporter system. When readthrough of the *ADH2* terminator sequence in pGTP or pGTM occurs, it is directed into an ARS (autonomous replicating sequence). Consequently, ARS activity is impaired, resulting in the loss of the plasmid and the plasmid marker, *SUP11*. *SUP11* is a tRNA suppressor that suppresses the nonsense mutation *ade2-101*. Failure to suppress the mutant *ade2* allele results in accumulation of a red pigment. Thus, a transcription termination defect leads to increased levels of transcription into the ARS and, consequently, increased numbers of red (or sectored) colonies. The pGTP construct terminates transcription in a wild-type strain; however, *trans*-acting mutations that affect the transcription termination reaction lead to readthrough into the ARS, resulting in plasmid destabilization. The plasmid pGTM that will consistently fail to terminate transcription because of a *cis*-acting sequence mutation serves as a control. Of the four mutants examined with the plasmid loss assay, both phenotypes consistent with a defect in transcription termination (2-1-1) and inconsistent with a termination defect (4-16-1) were observed.

***In vitro* transcription termination:** We focused on the 2-1-1 strain because it displayed defects in growth at the permissive temperature that were exacerbated at higher temperatures, β-galactosidase production, and RNA ex-

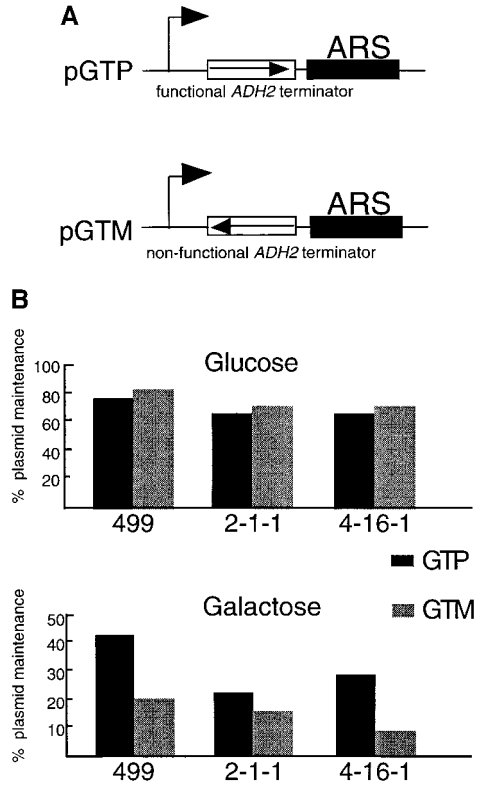

Figure 2.—Plasmid loss analysis. (A) Plasmid loss reporter constructs. When the plasmid constructs (pGTP and pGTM), which contain both *sup11* and *URA3* as selectable markers, are transformed into wild-type and mutant cells, readthrough can be evaluated on the basis of rates of plasmid loss. The *ADH2* 3′-end sequence in either the plus (right arrow) or minus (left arrow) orientation was placed between a galactose-inducible promoter and the H4ARS. Only in a transcription termination competent strain will the *ADH2* terminator prevent readthrough into the H4ARS sequence and maintain the plasmid, allowing production of *sup11* and formation of white colonies. (B) Graphic representation of plasmid loss rate. YPH499, 2-1-1, and 4-16-1 cells transformed with pGTP or pGTM were grown in rich media containing either dextrose (glucose) or galactose. At various time intervals, samples were plated to YPD. Once colonies formed, red colonies *vs.* non-red (white and sectored) colonies were scored, and the percent plasmid maintenance was calculated. The data shown represent the average of at least three independent samples.

pression. Additionally, this strain exhibited equal rates of plasmid loss regardless of the presence of a functional terminator in the plasmid loss assay (Figure 2B). To confirm the 3′-end formation defect, we extended this analysis to include a more direct assay that examines transcription termination *in vitro* by nuclear extracts prepared from 2-1-1. The plasmid pBEVY (Miller *et al.* 1998) served as an ideal template for the *in vitro* transcription reaction, in that it allows evaluation of transcripts produced from two different promoters (*GPD* and *ADH1*) directed into two different 3′-end sequences (*ADH1* and *ADH2*, respectively; Figure 3A). Extracts from strain 2-1-1 showed two significant differences from wild extracts. First, the level of specific terminated transcription product decreased as the temperature increased (compare lanes 1–4 to 5–8 in Figure 3B). Second, a high molecular weight, heterogeneous "smear" accumulated as the temperature increased. This is shown most clearly in Figure 3B, lanes 9 (wild type) and 10 (mutant), with a longer exposure of the 37° samples shown in lanes 4 and 8. The presence of the longer transcripts, coupled with the strong evidence of transcriptional readthrough by both plasmid loss analysis and Northern analysis, indicates these observations are consistent with a defect in transcription termination. Similar results are observed using a transcription template with a different promoter and a different 3′-end sequence as shown in Figure 3C. Taken together these results demonstrate that the transcription termination defect of the 2-1-1 strain is not dependent on the context of the transcription reaction. Additionally, extracts prepared from strain 2-1-1 were examined kinetically to determine if the extracts were completely deficient in processing or if slower transcription kinetics were evident. When transcription was examined in the mutant extracts at various time points during a 2-hr period, defects in producing the wild-type pattern of transcription products were evident at all time points examined (data not shown).

Genetic analysis of 2-1-1: On the basis of the evidence of a termination defect in strain 2-1-1, genetic studies were initiated. After 2-1-1 was mated to the wild-type strain, YPH500, the resulting strain was able to grow at 37°, thus demonstrating the 2-1-1 mutation is recessive. Unfortunately genetic analysis of strain 2-1-1 has been hampered by poor sporulation efficiency of the diploid and low spore viability. Consequently, random spore analysis was completed to establish linkage between the temperature-sensitive growth phenotype and overproduction of β-galactosidase from the pL101 reporter. Temperature sensitivity was present in approximately half of the >100 segregants examined, indicating 2:2 segregation of this phenotype. In addition, β-galactosidase production from pL101 at 30° was evident in all segregants that were temperature sensitive but none of these segregants were wild type for growth at 37°. Thus the data indicate that the termination defect and the growth defect are closely linked and that these phenotypes resulted from mutation in a single gene.

Cloning of the gene responsible for the mutant phenotypes of 2-1-1: A centromere-based library was used to identify the wild-type gene corresponding to the 2-1-1 mutation. We recovered 13 transformants that grew at the nonpermissive temperature in a screen of ~20 genome-equivalents of DNA. Restriction analysis and sequencing of the complementing plasmids from these strains demonstrated that 12 were overlapping members of one of three identical plasmid clones (p366/57, p366/76, and p366/711; Figure 4). These plasmids restored the wild-type growth phenotype (Figure 5A), wild-type levels of β-galactosidase phenotype

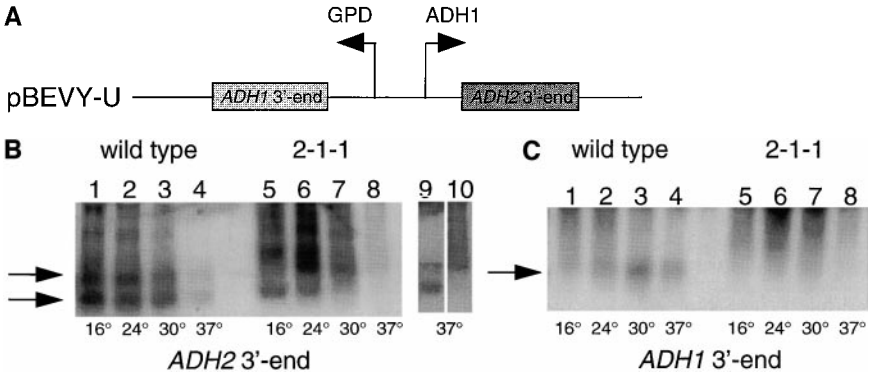

Figure 3.—*In vitro* transcription termination of strain 2-1-1. (A) Templates used for *in vitro* transcription. Termination of transcripts is driven by the *ADH1* promoter within the *ADH2* 3′-end at both permissive and nonpermissive temperatures, as well as within the *ADH1* 3′-end, driven by the *GPD* promoter. (B) Results of *in vitro* transcription using the *ADH2* 3′-end containing template or (C) the *ADH1* 3′-end containing template. *In vitro* transcription was completed at four temperatures (16°, 24°, 30°, and 37°). Following electrophoresis in a 1.5%-agarose/formaldehyde gel, transcripts were detected with an antisense probe specific for the *ADH2* 3′-end or an *ADH1* 3′-end-specific probe. The extra panel in B displays a longer exposure of the 37° reactions. Arrows point to the expected terminated transcripts.

(Figure 5B), and a wild-type RNA phenotype (Figure 5, C). Thus, a single segment of DNA complemented all three phenotypes, supporting our genetic evidence that the phenotypes resulted from mutation of a single gene. A search of the yeast database (BLAST) revealed that all three complementing fragments were from a region of chromosome II containing four potential genes: *GRS1*, a putative glycyl-tRNA synthetase; *MRPL36*, a mitochondrial ribosomal protein; *TFC1*, the 95-kD subunit of *TFIIIC*, a RNA polymerase III transcription factor; and *YBR124w*, an open reading frame of unidentified function (Figure 4).

The *GRS1* gene complements the temperature sensitivity and transcription termination defects of strain 2-1-1: The four genes contained in the overlapping fragment were individually cloned into a yeast centromeric vector, creating YCp111/*TFC1*, YCp111/*MRPL36*, YCp111/*YBR124w*, and YCp111/*GRS1* (Figure 4). Of these, YCp111/*GRS1* was able to support growth at 37° (Figure 4 and Figure 6A), whereas the other three plasmids could not do so. In a complementing approach, we constructed deletions of single genes from the original complementing clones yielding the plasmids p366/76Δ*GRS1* and p366/57Δ*TFC1* (Figure 4). Deletion of the *GRS1* gene from the genomic library fragment in p366 resulted in a plasmid that could not support growth of the 2-1-1 strain at 37°; deletion of the *TFC1* gene, by contrast, did not affect the complementing activity of p366/57 (Figure 6A). Thus, upon subcloning of the original plasmids, as well as deletion analysis, we determined that the *GRS1* gene, which codes for the probable glycyl-tRNA synthetase (Feldmann *et al.* 1994), could complement the growth defects of 2-1-1 (Figure 6A). The designation of Grs1p as a synthetase is based on significant homology to other glycl-tRNA synthetases, notably the human glycyl-tRNA synthetase with 60%

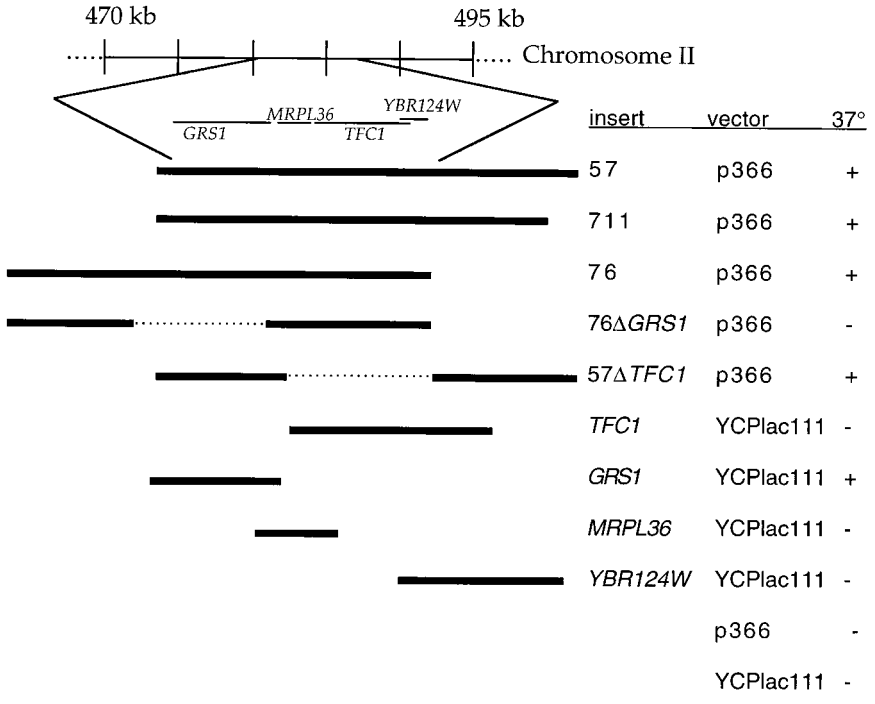

Figure 4.—A map of the genomic inserts used to determine which gene complements the temperature-sensitive phenotype of mutant 2-1-1. The 7-kb insert that has complementing activity is shown in detail. The three overlapping fragments isolated in our original cloning (57, 76, 711), as well as the inserts derived from chromosome II used in clones to verify the complementing activity of *GRS1* (76Δ*GRS1*, 57Δ*TFC1*, *TFC1*, *GRS1*, *MRPL36*, and *YBR124W*) are shown. The ability of the inserts to complement the 37° growth defect in the plasmid host p366 or YCplac111 is shown.

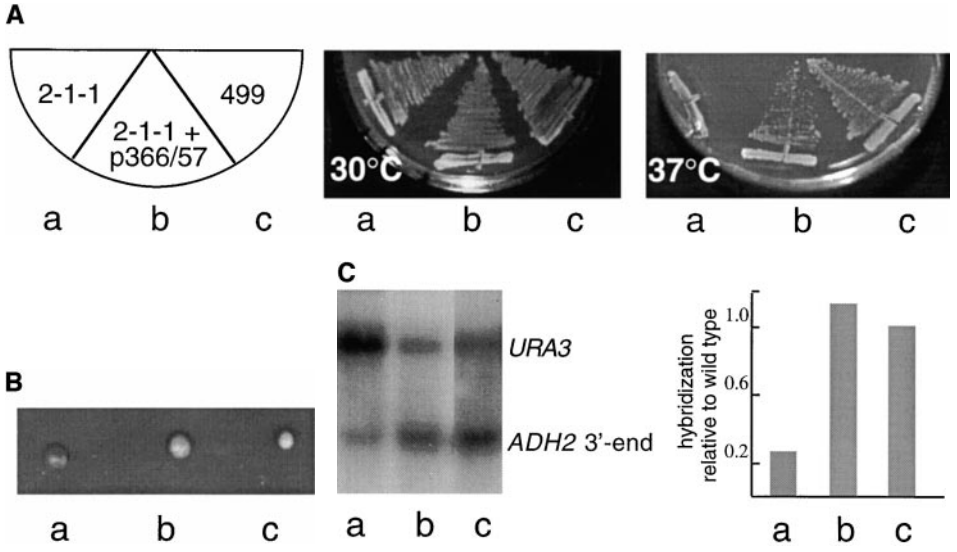

Figure 5.—Complementation of defects in strain 2-1-1 by complementing clones. (A) Complementation of growth defect. An approximately equal number of cells were streaked onto minimal media and incubated at the indicated temperatures. (B) Complementation of β-galactosidase activity. Cells containing the pL101 reporter plasmid were grown in complete minimal galactose media without uracil and leucine at the permissive temperature (24°). During early log the cultures were shifted to various temperatures (30° and 37°) and grown for several additional generations (3–4 generations) until the OD_{600} was 1.0–1.5. Cells were collected by centrifugation, and equal volumes of cells dispensed onto an X-Gal containing indicator plate. (C) RNA defect. Northern analysis of 5 μg of total RNA prepared from cells shifted from permissive to nonpermissive temperature, as described in B. Transcripts were detected using radiolabeled antisense probes to *ADH2* 3′-end and *URA3* (as a loading control). Lane a contains 2-1-1 plus pL101 and p366; lane b contains 2-1-1 plus pL101 and p366/57; lane c contains 499 plus pL101 and p366. The graph shows results of quantitation obtained by Phosphoimager analysis.

identity to Grs1p (Freist *et al.* 1996). As the only potential nonmitochondrial glycyl-tRNA synthetase in the yeast genome database, Grs1p is very likely to function in this role; however, to date, a direct examination of synthetase activity has not been reported.

The *grs1-1* allele confers the temperature-sensitive growth defect associated with 2-1-1: Two approaches were used to demonstrate that *GRS1* is responsible for the mutant phenotypes observed in strain 2-1-1. First, we cloned the *GRS1* gene from genomic DNA from wild-type and mutant strains into a centromeric plasmid. Eight clones from independent PCR reactions were obtained. The ability of the plasmid versions of *GRS1* to complement the temperature-sensitive phenotype of strain 2-1-1 was determined and the results showed that the *GRS1*-containing clones derived from the wild-type strain were able to complement the temperature sensitivity, whereas the eight clones derived from the mutant strain were unable to do so (data not shown). This demonstrates that the 2-1-1 strain contains a mutant allele of the *GRS1* locus, *grs1-1*.

Second, we asked whether the mutant phenotype of the 2-1-1 strain could be conferred by the *grs1-1* allele that was recovered. We integrated the *grs1-1* allele from 2-1-1 into a wild-type strain (W303a) at the *GRS1* locus, creating YCM102, and selected for recombination events that deleted the wild-type *URA3* gene located between the two alleles. The strains harboring the *grs1-1* allele (YCM101) were identified by screening for temperature sensitivity at 37° (Figure 6B). To further confirm that *grs1-1* is the mutant allele, YCM101 was mated to W303α, diploids were selected, and tetrad dissection was completed. The resulting segregants were transformed with the β-galactosidase reporter plasmid, pL101, and examined at both permissive and nonpermissive temperatures. In all eight segregants examined, the spore products that were temperature sensitive on YPD at 37° also expressed increased levels of β-galactosidase at 30°, and the spore products that grew at the nonpermissive temperature did not express increased β-galactosidase. Given that previous random spore analysis demonstrated linkage between the temperature sensitivity and the RNA defect, this firmly establishes *grs1-1* as the mutation in 2-1-1.

***GRS1* is an essential gene:** We also created a heterozygote containing a *grs1* null allele by inserting the kana-

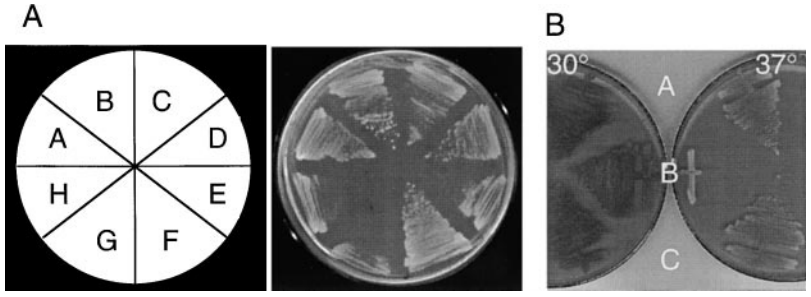

Figure 6.—Identification of *GRS1* as complementing clone. (A) Complementation of growth defect by *GRS1*. Transformed cells were grown at 37°. A, 2-1-1 plus p366/76; B, YPH499 plus p366; C and D, YCp111/GRS1; E, Ycplac111; F, p366/57ΔTFC1; G, p366/76ΔGRS1; and H, p366. (B) Growth phenotype of YCM101. Growth at 37° and 30° on rich media is shown. A, W303; B, YCM101 (*grs1-1* integrated and looped out); C, YCM102 (*grs1-1* integrated into *GRS1* locus of W303 but not looped out).

mycin resistance gene into the *GRS1* locus of a wild-type diploid (W303a/α). Upon sporulation and dissection of this diploid, we determined that *GRS1* is an essential gene in that, of the 20 viable spore products, only kanamycin-sensitive strains (*GRS1* gene present) were recovered. No spore product was recovered that was kanamycin resistant (*GRS1* gene knocked out).

Identification of the *grs1-1* mutation: Finally, sequence analysis of the *grs1-1* mutation revealed that nucleotides 1656 and 1657 were both C to T transitions that result in a single amino acid change of proline at position 552 to a phenylalanine (P552F). Two independently derived clones derived from the mutant strain (2-1-1) contained this mutant allele, whereas a clone derived from the wild-type strain (YPH499) did not harbor the transition mutation.

Examination of polyadenylation and cleavage: To determine if defects in 3′ RNA processing are evident in the *grs1-1* mutant strain, whole cell extracts were prepared and *in vitro* cleavage and polyadenylation examined. A radiolabeled *CYC1* 3′-end substrate was synthesized *in vitro* and incubated in the presence of yeast extracts made from wild-type or 2-1-1 cells. As the *grs1-1* allele is temperature sensitive, extracts were prepared from cells grown at 24°, but reactions were performed with extracts heat-treated at 37° for 5 min (Figure 7, lanes 5–8) or non-heat-treated extracts (Figure 7, lanes 1–4). In addition, a set of reactions was performed with dATP substituting for ATP to demonstrate accumulation of the upstream cleavage product (Figure 7, lanes 9–12). These results indicate that the primary defect in strain 2-1-1 and YCM101 does not affect polyadenylation or cleavage.

Transcription run-on: To assess the localization of active polymerases to the regions downstream of normal transcription termination sites in the mutant strain YCM101, transcriptional run-on experiments were completed. We anticipated polymerases would localize to regions downstream from the normal transcription termination site. Data from a representative result from the transcription run-on experiment are shown in Figure 8B, and a graphic representation is shown in Figure 8C. Using the pGCYC plasmid as a template for the transcription run-on reaction (Figure 8A), we observed wild-type transcriptional patterns in the mutant strain at the permissive temperature (30°; Figure 8C, compare YCM101 at 30° to W303 at 30°). However, mutant cells shifted to the nonpermissive temperature (37°) demonstrated high polymerase density downstream from the polyadenylation site (Figure 8B). This pattern resembles the polymerase density observed in cells harboring a transcription template with a defective transcription termination site (pGcyc1-512; Figure 8C, compare *cyc1*-512 at 30° to *CYC1* 37°). Thus, increased levels of polymerase density were found in the regions downstream of the normal transcriptional termination site in the mutant strain YCM101, providing additional evidence of a defect in transcription termination in strains harboring the *grs1-1* allele.

DISCUSSION

RNA defects associated with the *grs1-1* mutation: The observation that an enzyme traditionally associated with the aminoacylation of tRNA affects 3′-end formation is unexpected. Because of the role of tRNA synthetases in translation, an indirect role for Grs1p may be predicted in that translation of a factor essential for 3′-end formation might be adversely affected in the absence of a pool of correctly aminoacylated $tRNA^{gly}$. Yet, we clearly observe defects in RNA processing and metabolism consistent with a defect in 3′-end formation using multiple assays. First, Northern analysis demonstrates a defect in transcription termination. We observe a decrease in the level of terminated transcripts as the temperature is increased in our mutant strains (2-1-1 and YCM101) compared to the wild-type strain. Examination of the expression of a control gene (*URA3*) indicates a correlated general decrease in transcription is not observed. Second, the plasmid loss assay demonstrates the mutant

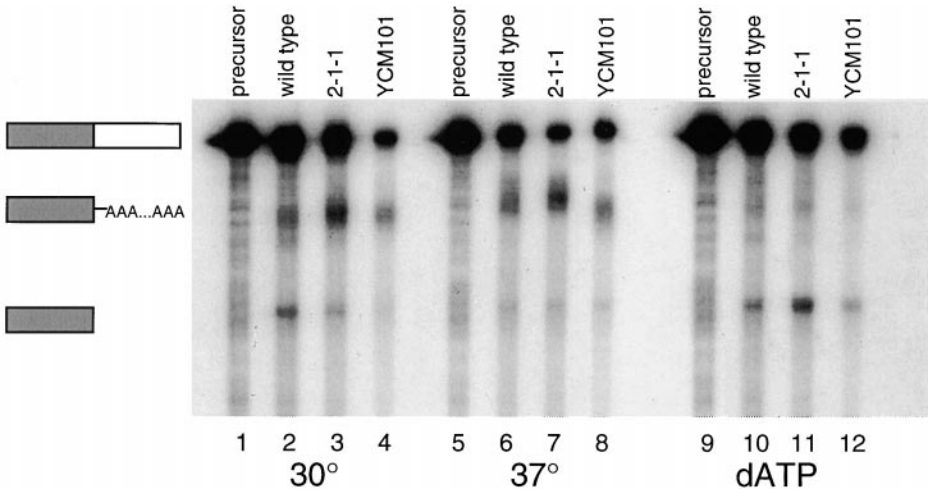

Figure 7.—Polyadenylation and cleavage. *In vitro* reactions using extracts from the wild-type strain W303 (lanes 2, 6, and 10), strain 2-1-1 (lanes 3, 7, and 11), and YCM101 (lanes 4, 8, and 12) were compared to reactions with no added extract (lanes 1, 5, and 9). Heat-treated (37°) extracts were used for the reactions in lanes 5–8, and dATP was included in the reactions in lanes 9–12. A radiolabeled pre-mRNA from the 3′-end of the CYC1 gene was used as the processing substrate.

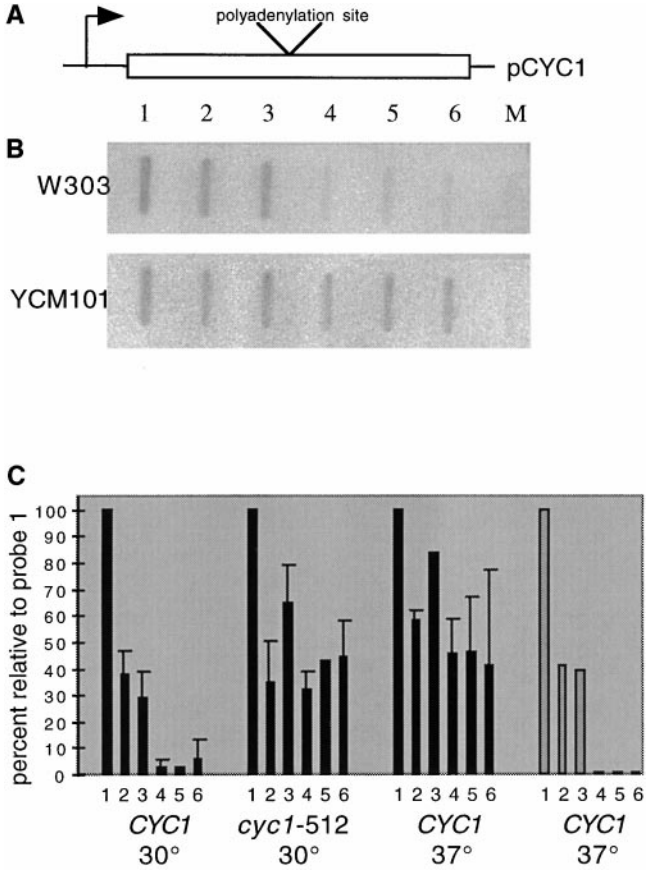

Figure 8.—Transcription run-on analysis of YCM101 *vs.* wild type (W303). (A) Plasmid template used for TRO. (B) Representative results. Shown is a typical result obtained in TRO analysis of YCM101 transformed with pGCYC *vs.* W303 transformed with pGCYC shifted to 37° for one generation (2 hr) in galactose-containing media. Total cellular RNA was hybridized to a slot blot containing M13 probes specific for the regions of the CYC1 construct shown in Figure 8A. (C) Graphic representation of the results of TRO analysis. The graph represents data from at least two independent experimental samples. The dark bars are from strain YCM101 and the light bars are from W303.

strain (2-1-1) produces increased levels of readthrough transcripts, relative to wild type. Third, *in vitro* analysis of the terminated RNA products shows a clear defect in accumulation of terminated products. Fourth, polyadenylation and cleavage are normal in the mutant strain. Fifth, transcription run-on results demonstrate increased polymerase density in regions downstream from a normal transcription termination site. The observed phenotypes are consistent with a general defect that affects the transcription termination reaction. A failure to demonstrate a role for GRS1 in polyadenylation is not surprising in that the polyadenylation reaction has been functionally reconstituted, and GRS1 is not a required component of the reaction (Keller and Minvielle-Sebastia 1997). Thus, these results represent the first example of a transcription termination factor in *S. cerevisiae* that affects transcription termination independent of 3′-end processing of mRNA.

Roles of tRNA synthetases independent from aminoacylation: Although tRNA synthetases are traditionally thought to be responsible for charging tRNA, several lines of evidence, including what is presented in this article, demonstrate that tRNA synthetases can be multifunctional proteins. Notable is the observation that tRNA synthetases are involved in a variety of cellular functions, unlinked to their roles in protein synthesis (First 1998). For example, the tyrosyl-tRNA synthetase binds a region of the group I intron catalytic core, which resembles a tRNA structure (Caprara *et al.* 1996) and is thought to stabilize the catalytically active intron structure, an activity generally reserved for RNA molecules (Mohr *et al.* 1994). In addition, a role for tRNA synthetases in splicing of group II introns is postulated (Lambowitz and Perlman 1990). Another paradigm is found in bacteria where tRNA synthetases have been linked to transcription termination. Prokaryotic tRNA synthetases are able to regulate termination of their own primary transcripts (Platt 1998; Yanofsky *et al.* 1996). Third, an RNA-binding capacity has been identified in mammalian cells, demonstrating that human glutaminyl-tRNA synthetase binds to a secondary structure present in the 3′-end of its own primary transcript (Schray and Knippers 1991). These observations on the roles of tRNA synthetases raise the strong possibility that the mutant phenotypes observed in our transcription termination screen are the consequence of a specific role for Grs1p in mRNA 3′-end formation in *S. cerevisiae*. Recent findings (Lund *et al.* 1998) have shown that tRNA synthetases are capable of aminoacylating tRNA in the nucleus, the site of transcription termination. These observations allow us to speculate that GRS1 is present in the nucleus of *S. cerevisiae* as part of the tRNA proofreading system, as well as 3′-end formation of mRNA.

Identification of tRNA-like structures in the 3′-ends of yeast genes: As part of the specificity of the aminoacylation reaction, synthetases generally recognize the anticodon region, the acceptor arm, and additional nucleotides within the cognate tRNA. The exact mechanisms used by synthetases to bind to tRNA vary among the different synthetases and no single region of the tRNA or single domain of the synthetases is exclusively responsible for this RNA:protein interaction (Freist *et al.* 1996). Clearly, the overall structure of tRNA is well conserved (Hinnesbusch and Liebman 1991; Mans *et al.* 1991; Hopper and Martin 1992; Nameki *et al.* 1997; Shiba *et al.* 1997; Saks *et al.* 1998). Thus, we considered whether yeast mRNAs might contain sequences that would allow tRNA synthetase recognition.

An examination of the 3′-ends of several yeast mRNAs revealed the presence of a sequence that appears conserved in the 3′-end of several RNA polymerase II transcripts. The semiconserved sequence (GUUCGANYC) corresponds to the TψC loop of tRNA, a critical structural feature of tRNAs (Figure 9A). Thus, the tRNA-like sequence element could potentially play a role in mediating the Grs1p interaction with 3′-ends of yeast

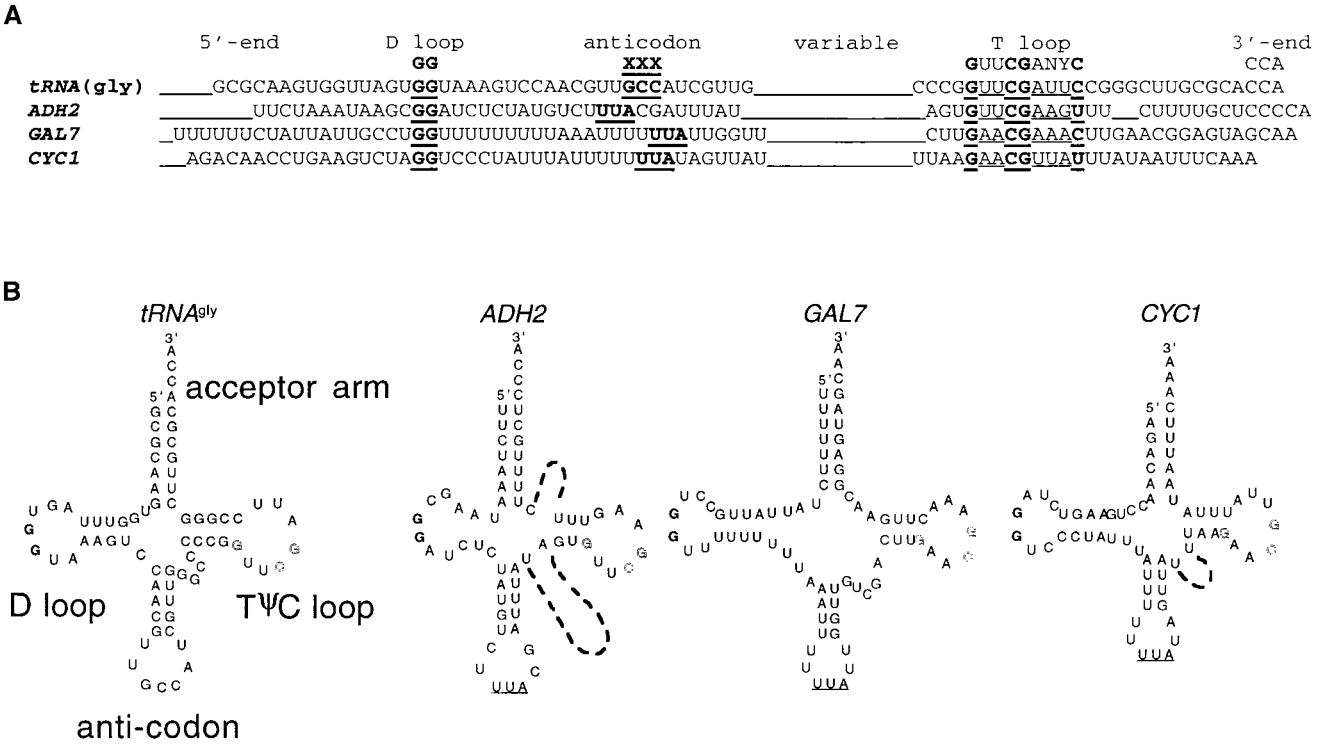

Figure 9.—Hypothetical tRNA-like structures. (A) Conservation of elements in 3′-ends. The alignment of the 3′-ends of *ADH2*, *GAL7*, and *CYC1*, as compared to $tRNA^{gly}$ and a model tRNA structure is shown. Bold elements emphasize the conserved nucleotides, and the— represents insertions of nucleotides from 4 to 35 nucleotides in length. (B) Diagrammatic representation of the hypothetical tRNA-like structures. The underlined nucleotides correspond to the potential anticodon, the shadowed nucleotides to the highly conserved elements of the potential TψC loop, and the bold nucleotides to the highly conserved dinucleotide sequence in the potential D-loop. Note that the site of polyadenylation is found in the acceptor arm for both the *ADH2* 3′-end and the *GAL7* 3′-end. Shown are $tRNA^{gly}$, the *ADH2* 3′-end, the *GAL7* 3′-end, and the *CYC1* 3′-end.

genes transcribed by RNA polymerase II and transcription termination.

Are there tRNA-like structures found at the 3′-ends of mRNA? A structure is traditionally defined as tRNA-like "on account of its ability to react efficiently with one or more tRNA-specific enzymes" (Mans *et al.* 1991). The ability to form the classic cloverleaf conformation is not a necessary prerequisite for being classified tRNA-like. As shown in Figure 9, A and B, the 3′-ends of *GAL7*, *CYC1*, and *ADH2*, three classically investigated RNA polymerase II transcriptional terminators, contain a potential tRNA-like structure that is defined by the following: an element that resembles the TψC loop; a GG sequence in a region that is a potential D-loop; an anticodon that would not charge any amino acid to the RNA (UUA); and, in two of three examples, an acceptor arm that contains the polyadenylation site (Russo and Sherman 1989; Hyman *et al.* 1991). The stems in the structures as drawn are not predicted to be of comparable stability as those found in a typical tRNA; however, the tertiary structure of some previously described tRNA-like molecules stabilizes comparable structures in other RNA molecules (Mans *et al.* 1991). In fact, tRNA tertiary structure is reported to be stabilized by interactions between the GG dinucleotide present in the D-loop and bases within the TψC loop (reviewed in Schimmel and Alexander 1998). Additionally, site-directed mutagenesis of a conserved nucleotide from the TψC-like element of the *ADH2* 3′-end did increase the level of readthrough in our reporter construct (data not shown).

The presence of a potential tRNA-like structure allows one to envision a role for tRNA processing components, such as tRNA synthetases, in the mRNA 3′-end formation reaction. Furthermore, the *grs1-1* mutation may affect some aspect of the synthetase involved in charging tRNA may be a mutation that is exclusively involved in 3′-end formation, demonstrating a role for the protein distinct from its role in protein synthesis. As noted, tRNA synthetases recognize tRNA as a part of the aminoacylation reaction; yet, some have been observed to bind to or interact with other RNAs, including rRNA and mRNA (Labouesse 1990; Schray and Knippers 1991; Mohr *et al.* 1994; Caprara *et al.* 1996).

DNA sequencing of the *grs1-1* gene predicts a single amino acid change from proline to phenylalanine at position 552. Importantly, the crystal structure of the glycyl-tRNA synthetase from *Thermus thermophilus* has been solved and the C-terminal domain that contains the mutation in Grs1p is thought to constitute the domain that recognizes and binds to the tRNA at the anticodon loop (Logan *et al.* 1995). A P to F mutation,

such as the one found in *grs1-1*, is likely to adversely affect this domain and suggests that RNA recognition may be modified in the mutant protein. Importantly the yeast enzyme is conserved in this domain with the *T. thermophilus* sequence (data not shown). Thus, an inability of the glycl-tRNA synthetase mutant to recognize a structure in the mRNA 3′-end may account for the decreased levels of termination observed in a *grs1-1* mutant.

Other 3′-end formation effectors with roles in tRNA metabolism: Grs1p is not the only *trans*-acting factor implicated in 3′-end formation with roles in tRNA metabolism. *PTA1* has been described both as an effector of pre-tRNA processing (O'Connor and Peebles 1992) and as component of both PFI and CFII, factors important for the polyadenylation (Preker *et al.* 1997) and cleavage of mRNA (C. Moore, personal communication). Additionally, an examination of an RNA pseudoknot in the 3′-untranslated region of tobacco mosaic virus indicates the sequence forms a structure similar to a tRNA. This structure is recognized by tRNA synthetases and can serve as the 3′-terminus of mRNA to functionally substitute for a poly(A) tail (Gallie and Walbot 1990). Also, a defect in *RET1*, the gene for the second largest subunit of RNA polymerase III, has been demonstrated to suppress a lethal mutation of poly(A)-polymerase, and an association of RNA polymerase III with the 3′-end formation components has been suggested (Briggs and Butler 1996). Finally, RNAs that can function as both tRNA and mRNA, termed tmRNA, have been identified in bacterial systems (Muto *et al.* 1998). These observations suggest that RNA metabolism, recognition, and function is not as nonoverlapping and polymerase-specific as traditionally envisioned.

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