A Mutation in the tRNA Nucleotidyltransferase Gene Promotes Stabilization of mRNAs in *Saccharomyces cerevisiae*

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To identify *trans*-acting factors involved in mRNA decay in the yeast *Saccharomyces cerevisiae*, we have begun to characterize conditional lethal mutants that affect mRNA steady-state levels. A screen of a collection of temperature-sensitive mutants identified ts352, a mutant that accumulated moderately stable and unstable mRNAs after a shift from 23 to 37°C (M. Aebi, G. Kirchner, J.-Y. Chen, U. Vijayraghavan, A. Jacobson, N. C. Martin, and J. Abelson, *J. Biol. Chem.* 265:16216-16220, 1990). ts352 has a defect in the *CCA1* gene, which codes for tRNA nucleotidyltransferase, the enzyme that adds 3' CCA termini to tRNAs (Aebi et al., *J. Biol. Chem.*, 1990). In a shift to the nonpermissive temperature, ts352 (*cca1-1*) cells rapidly cease protein synthesis, reduce the rates of degradation of the *CDC4*, *TCM1*, and *PAB1* mRNAs three- to fivefold, and increase the relative number of ribosomes associated with mRNAs and the overall size of polysomes. These results were analogous to those observed for cycloheximide-treated cells and are generally consistent with models that invoke a role for translational elongation in the process of mRNA turnover.

To a first approximation, changes in the expression of specific genes are manifested by changes in the steady-state levels of individual mRNAs. Although such changes are generally assumed to result primarily from differential transcription or RNA processing activities, differences in the decay rates of individual mRNAs can also have profound effects on the overall levels of expression of specific genes. While the potential importance of mRNA stability as a mechanism for regulating gene expression has been recognized (reviewed in references 2, 6, 13, 27, 31, and 33), the structures and mechanisms involved in the determination of individual mRNA decay rates have yet to be elucidated.

We have begun an investigation of mRNA decay in the yeast *Saccharomyces cerevisiae*, an organism amenable to both biochemical and genetic manipulation (11). The principal hypothesis which guided our initial studies was that a comparison of the structures and properties of mRNAs that decayed rapidly versus those that decayed slowly would provide information about the basic rules of mRNA turnover (15, 26). Hence, our first objective was the identification of both stable and unstable mRNAs that were encoded by yeast genes that had already been well characterized. To accomplish this, we developed simple and reliable assays in which mRNA decay was analyzed after thermal or chemical inactivation of RNA polymerase II (16, 25). In addition to characterizing mRNA structural features that affect their decay rates, we have also been involved in determining the protein factors involved in mRNA turnover (20, 31). To identify *trans*-acting factors involved in the decay of inherently unstable mRNAs, we have begun to characterize conditional lethal mutants that affect the accumulation of such RNAs. A screen of a collection of temperature-sensi-

tive mutants identified ts352, a mutant that accumulated moderately stable and unstable mRNAs after a shift from 23 to 37°C (1). Complementation of ts352 with a yeast genomic DNA library led to the identification of the gene responsible for the temperature-sensitive lesion (1). ts352 has a defect in the *CCA1* gene, which codes for tRNA nucleotidyltransferase, the enzyme that adds 3' CCA termini to tRNAs (1, 4). At the nonpermissive temperature, ts352 (*cca1-1*) cells accumulate tRNAs which are shorter than mature tRNAs because CCA termini are absent (1). In the experiments described in this report, we sought an explanation for the increases in mRNA steady-state levels observed in temperature-shifted *cca1-1* cells. We report that reduction in the pool of functional tRNAs leads to a rapid decrease in the ability of *cca1-1* cells to synthesize protein, a stabilization of transcripts, and a shift of mRNAs from lighter to heavier polysomes. These results were analogous to those observed in cycloheximide-treated cells and are generally consistent with models that invoke a role for translational elongation in the process of mRNA turnover.

MATERIALS AND METHODS

Yeast strains and media. ts352 was originally identified in a collection of temperature-sensitive mutants (1). To isolate the mutation of interest (recently named *cca1-1* [4]), ts352 (*MAT α cca1-1 ade2-101 his3 Δ 200 ura3-52 tyr1*) was backcrossed by Aebi et al. (1) to the congenic strain SS328 (*MAT α ade2-101 his3 Δ 200 ura3-52 lys2*). Temperature sensitivity of ts352 was attributable to a single mutation because all 49 tetrads showed 2:2 segregation of the temperature-sensitive phenotype (1). Moreover, temperature sensitivity and increases in mRNA accumulation cosegregated in each of five tetrads examined (1). The segregants of one of these five tetrads, tetrad 1 (kindly provided by Nancy Martin), have the following genotypes: *MAT α cca1-1 ade2-101 his3 Δ 200 ura3-52 lys2* (352-1A), *MAT α cca1-1 ade2-101 his3 Δ 200 ura3-52* (352-1B), *MAT α ade2-101 his3 Δ 200 ura3-52*

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lys2 tyr1 (352-1C), and *MAT α ade2-101 his3 Δ 200 ura3-52 tyr1* (352-1D) (1). Segregants 352-1A and 352-1C, harboring the mutant (*cca1-1*) and wild-type (*CCA1*) tRNA nucleotidyltransferases, respectively, were used in the studies described in this report and are hereafter referred to as 1A and 1C. Cells were grown in yeast extract-peptone-dextrose or synthetic complete medium lacking methionine (SC-Met) prepared as described elsewhere (35).

Measurement of translation rates. Cells were grown in SC-Met at 24°C to an optical density at 600 nm (OD_{600}) of 0.5 to 0.7. Cell cultures were shifted to 36°C by the addition of an equal volume of SC-Met preheated to 50°C and incubated thereafter at 36°C. Aliquots of the culture (1 ml) were removed to 1 μ Ci of [³⁵S]methionine (15 mCi/ml, 38 TBq/mmol; Amersham) and incubated for 4 min at 36°C. Incorporation of labeled methionine was monitored by trichloroacetic acid (TCA) precipitation; 0.2 ml of cold (4°C) 50% TCA was added to each aliquot, after which the aliquots were incubated on ice for 10 min, heated to 70°C for 20 min, and subsequently filtered through GF/C filters. The filters were washed with 10 ml of 5% TCA (4°C) and 10 ml of 95% ethanol and then dried and counted in a scintillation counter.

Measurement of mRNA decay rates. As described previously (16, 25), decay rates of individual mRNAs were determined by RNA blotting analyses of RNA isolated at different times after inhibition of transcription with the antifungal agent thiolutin (18, 38) (generously provided by Nathan Belcher, Pfizer Central Research, Pfizer, Inc., Groton, Conn.). Cells were grown to an OD_{600} of approximately 0.70 (at temperatures indicated in the text), and thiolutin was added to 3 μ g/ml. For temperature shift experiments, cells were grown at 24°C to an OD_{600} of 0.70, centrifuged, resuspended in 20 ml of SC-Met, equilibrated to 24°C, and then shifted to 36°C rapidly by the addition of 20 ml of medium prewarmed to 50°C. For RNA isolation, 4-ml aliquots were removed at the indicated times.

RNA extraction, RNA blotting, hybridization, and data analysis. RNA was isolated, and blots were prepared as described previously, using 20 μ g of RNA per lane (16). mRNA half-lives ($t_{1/2}$ s) were determined by hybridizing RNA blots with probes specific for the genes of interest. DNA probes used in these experiments included the following: *PAB1* (*SalI-EcoRI* fragment from pYPA [16]), *TCM1* (*HindIII-EcoRI* fragment from rTCM1 [16]), *HIS4* (*SphI-SacI* fragment from pUC18-HIS4 [16]), *TRT1* (*HindIII-EcoRI* fragment from pDH1 [16]), *CDC4* (*SalI* fragment from p514100; kindly supplied by Stephen Johnson), and *URA5* (*KpnI-EcoRI* fragment from pURA5; kindly supplied by Francois Lacroute). Decay rates, expressed as mRNA half-lives, were determined by direct counting of RNA blots with a Betagen blot analyzer, normalization of the data such that time zero (t_0) was equal to 100%, and plotting of the data with respect to time on semilog axes (16, 25).

Polysome analysis. Cytoplasmic extracts, prepared as described by Baim et al. (3), were fractionated on 15 to 50% sucrose gradients buffered with 50 mM Tris-acetate (pH 7.4)–50 mM NH_4Cl –12 mM $MgCl_2$ –1 mM dithiothreitol. Gradients were centrifuged in an SW41 rotor at 40,000 rpm for 135 min at 4°C and analyzed by continuous monitoring of A_{254} (24). No significant differences in recovery of ribosomes from mutant or wild-type cells were observed. To facilitate comparisons between different gradients, polysome-to-monomosome ratios were determined by cutting and weighing the polysome and monosome regions of the respective gradient profiles. Unless stated otherwise, each experiment was repeated an average of five times.

FIG. 1. Translation rates of wild-type and *cca1-1* cells at 36°C. Wild-type (segregant 352-1C; ●) and *cca1-1* (segregant 352-1A; ○) cells were grown at 24°C and shifted abruptly to 36°C; at various times after the temperature shift, translation rates were determined by pulse-labeling with [³⁵S]methionine. Data are presented as the percentage of methionine incorporation at a given time point compared with t_0 (for the same cells).

RESULTS

Protein synthesis is impaired at 36°C in *cca1-1* cells. The effect of the ts352 mutation (also known as *cca1-1* [4]) on protein synthesis was monitored by assaying the incorporation of radioactive methionine into protein during brief labeling periods. Cells harboring either the wild-type (*CCA1*) or mutant tRNA nucleotidyltransferase gene were shifted to 36°C, and at various times after the temperature shift, incorporation of [³⁵S]methionine during a 4-min pulse was determined. When wild-type cells were shifted to 36°C, their translation rate decreased transiently and then returned to initial rates by 40 to 60 min after the shift (Fig. 1). The translation rate of temperature-shifted *cca1-1* cells displayed a different profile. When these cells were shifted to 36°C, there was a rapid and continuous decrease in the translation rate (Fig. 1); by 50 min after the shift, less than 10% of the original translation activity remained. Consistent with the observed reduction in protein synthesis rates was a severe reduction in the growth rate of *cca1-1* cells at temperatures above 32°C (Fig. 2).

mRNA decay rates in *cca1-1* cells are reduced at the nonpermissive temperature. The results shown in Fig. 1 demonstrate that *cca1-1* cells cease protein synthesis rapidly at 36°C. Since a considerable body of evidence suggests that mRNA translation and mRNA turnover are intimately linked (see reference 31 for a review), we sought to determine whether the elevated mRNA levels observed at the nonpermissive temperature in cells harboring the *cca1-1* allele (1) might be attributable to reductions in mRNA decay rates. Using the drug thiolutin to inhibit transcription (16), we determined half-lives of several mRNAs at both the permissive (24°C) and nonpermissive (36°C) temperatures. At various times after thiolutin addition, cell aliquots were harvested, RNAs were isolated, and the decay of different transcripts was monitored by Northern (RNA) blotting, using probes for mRNAs that had been shown previously to have half-lives ranging from 3 to 18 min at 36°C (16, 31). The results of these experiments demonstrate that at 24°C, (i) decay rates of the *CDC4*, *PAB1*, *TCM1*, *HIS4*, *URA5*, and

FIG. 2. Growth rates of wild-type and *cca1-1* cells at different temperatures. Cultures of wild-type (segregant 352-1C; A) and *cca1-1* (segregant 352-1A; B) cells were grown overnight at 24°C in medium lacking methionine. Aliquots of these cultures were diluted in the same medium and placed in separate flasks, and growth of the different cultures was monitored by measuring the OD₆₀₀ for 7 h at 24°C (Δ), 27°C (○), 30°C (▲), 32°C (●), 34°C (▽), and 36°C (◇).

TRT1 mRNAs in *cca1-1* cells were comparable to those observed in wild-type cells (Table 1) and (ii) in both mutant and wild-type cells, all mRNAs examined had slower decay rates at 24°C than had previously been measured at 36°C (16,

TABLE 1. mRNA decay rates in mutant and wild-type cells at 24°C^a

mRNA	$t_{1/2}$ (min)	
	Wild type	<i>cca1-1</i> allele
<i>PAB1</i>	31	26
<i>CDC4</i>	60	45
<i>TCM1</i>	27.5	25.5
<i>HIS4</i>	60	60
<i>URA5</i>	25	25
<i>TRT1</i>	40	40

^a Cultures of *CCA1* (segregant 352-1C) or *cca1-1* (segregant 352-1A) cells were grown at 24°C in SC-Met medium. Thiolutin was added to inhibit transcription, and relative levels of the *CDC4*, *PAB1*, *TCM1*, *HIS4*, *URA5*, and *TRT1* transcripts were measured by RNA blotting. mRNA half-lives were calculated as described previously (25).

FIG. 3. mRNA decay in wild-type and *cca1-1* cells after a shift to 36°C. Cultures (100 ml) of either segregant 352-1A (wild type) or segregant 352-1C (*cca1-1*) were grown in SC-Met medium to an OD₆₀₀ of 0.70. The cells were centrifuged and resuspended in 20 ml of the same medium, equilibrated for 10 min at 24°C, and then shifted to 36°C by the addition of an equal volume of medium preheated to 50°C. At 5, 20, 40, and 60 min after the temperature shift, the decay rates of the *CDC4*, *PAB1*, and *TCM1* transcripts at 36°C were measured by removing aliquots of cells, inhibiting transcription with thiolutin, and monitoring transcript levels by RNA blot analysis. Control lanes contained RNA isolated from cells prior to the temperature shift.

31). Temperature-dependent changes in yeast mRNA decay rates have been reported previously (16).

To determine the effect of the *cca1-1* mutation on mRNA degradation at the nonpermissive temperature, cell cultures were shifted to 36°C, and at 5, 20, 40, and 60 min after the temperature shift, decay of the *PAB1*, *CDC4*, and *TCM1* mRNAs was analyzed by thiolutin inhibition of transcription and subsequent RNA blotting assays. The results from these experiments demonstrate that at 36°C, mRNA degradation was three- to fivefold slower in *cca1-1* cells than in cells containing the wild-type tRNA nucleotidyltransferase gene (Fig. 3; Table 2). The onset of mRNA stabilization in *cca1-1* cells was rapid, occurring within 20 min of the temperature shift, a time when the translation rate was inhibited by approximately 80% (Fig. 1 and 4; Table 2). At both 40 and 60 min after the temperature shift, when translation rates in *cca1-1* cells were reduced to 10% of their t_0 value, all transcripts examined were stable. In contrast, in cells harboring the wild-type tRNA nucleotidyltransferase gene, decay of the same transcripts was not stabilized at 36°C. Rather, degradation of each of the transcripts examined in *CCA1*⁺ cells was accelerated at the elevated temperature (Tables 1 and 2).

TABLE 2. mRNA decay rates in mutant and wild-type cells shifted to 36°C for different lengths of time^a

mRNA	$t_{1/2}$ (min)							
	5 min ^b		20 min		40 min		60 min	
	WT	ts	WT	ts	WT	ts	WT	ts
<i>PAB1</i>	7	9.5	13	41	17	>60	13	>60
<i>TCM1</i>	13	10.5	11	31	13	>60	12	>60
<i>CDC4</i>	12	10	9	50	10	50	14	>60

^a Decay rates were calculated as described previously (25), using the data presented in Fig. 3. WT, wild-type cells; ts, *ccal-1* cells.

^b Time at 36°C.

Comparison with mRNA decay rates in cells treated with cycloheximide. Cycloheximide-induced increases in mRNA abundance and decreases in mRNA decay rates have been observed previously for a large number of mRNAs in different experimental systems (8, 16, 19, 37, 39; reviewed in reference 27). Since inhibiting translation by mutation in a gene whose product is essential in protein synthesis also stabilizes mRNAs (Fig. 1 and 4), it is likely that cycloheximide effects on mRNA decay are not drug-related artifacts but rather may be indicative of a requirement for translational elongation in the decay of mRNA. To compare directly the effects of cycloheximide with the results obtained with *ccal-1*, the half-lives of six transcripts from *CCA1*⁺ cells were determined in the presence or absence of cycloheximide. A culture of wild-type cells was divided in half; one culture was treated with cycloheximide for 40 min, while the second culture was left untreated. Subsequently, transcription was inhibited by the addition of thiolutin, and mRNA decay rates were determined. The results demonstrate that the half-lives of all six transcripts were increased in the presence of cycloheximide (Fig. 4; Table 3) and that the extent of mRNA stabilization was comparable to that observed in *ccal-1* cells shifted to the nonpermissive temperature for at least 40 to 60 min (Fig. 3; Table 2).

FIG. 4. mRNA decay at 36°C in the presence and absence of cycloheximide. Wild-type cells (200 ml; segregant 352-1C) were grown at 24°C to an OD₆₀₀ of 0.7, centrifuged, resuspended in 36 ml of SC-Met medium, and divided into two equal aliquots. Cultures were shifted to 36°C by the addition of 18 ml of the same medium preheated to 50°C and either containing or lacking cycloheximide. The final cycloheximide concentration was 125 µg/ml. Forty minutes after addition of medium, the relative levels of the *CDC4*, *PAB1*, *TCM1*, *HIS4*, *URA5*, and *TRT1* transcripts were measured by inhibiting transcription with thiolutin and monitoring transcript levels by RNA blot analysis. Control lanes contained RNA isolated from cells prior to the temperature shift.

TABLE 3. mRNA decay rates in the presence and absence of cycloheximide^a

mRNA	$t_{1/2}$ (min)	
	- Cycloheximide	+ Cycloheximide
<i>PAB1</i>	14	40
<i>CDC4</i>	18	>60
<i>TCM1</i>	18.5	>60
<i>HIS4</i>	40	>60
<i>URA5</i>	18	>60
<i>TRT1</i>	22	>60

^a Decay rates were calculated as described previously (25), using the data presented in Fig. 4.

Inhibiting translation by either cycloheximide treatment or the *ccal-1* mutation causes polysomes to accumulate. The results shown in Fig. 3 and 4 indicate that inhibition of protein synthesis, either by cycloheximide addition or by a mutation in the tRNA nucleotidyltransferase gene, leads to

FIG. 5. Polysome profiles of wild-type and mutant cells. Wild-type (segregant 352-1C) or mutant (segregant 352-1A) cells were grown at different temperatures in the presence or absence of cycloheximide. Cells were harvested, and cytoplasmic extracts were prepared and fractionated on sucrose gradients as described in Materials and Methods. (A) Wild-type cells were grown at 24°C in SC-Met medium (500 ml) to an OD₆₀₀ of 0.7, centrifuged, and resuspended in 300 ml of the same medium. Aliquots (100 ml) were placed into separate flasks and equilibrated at 24°C for 10 min. An equal volume of preheated medium was added to each culture so that the final temperatures in these cultures were 24, 30, and 36°C, respectively. Incubation at these temperatures was continued for an additional 40 min prior to collection of cells for polysome analysis. Shown here are the results from the 24 and 36°C cultures; results from all three cultures are summarized in Table 4. (B) Mutant cells were analyzed as described above for wild-type cells. (C) Wild-type cells were grown at 24°C in SC-Met medium (340 ml) to an OD₆₀₀ of 0.7, centrifuged, resuspended in 200 ml of the same medium, and then divided into two 100-ml aliquots. Cycloheximide (cyclo) was added to one aliquot at a final concentration of 125 µg/ml, while the other culture was untreated. The cells were incubated for 40 min, and polysomes were then prepared and analyzed.

TABLE 4. Polysome/monosome ratios in mutant and wild-type cells^a

Temp (°C)	Polysome/monosome ratio		
	Wild type	ts352	Wild type + cycloheximide
24	1.26 ± 0.27	0.80 ± 0.22	ND
30	1.19 ± 0.23	0.88 ± 0.18	6.01 ± 0.40
36	1.19 ± 0.25	2.39 ± 0.31	ND

^a Polysomes were prepared and analyzed on 15 to 50% sucrose gradients as described in the legend to Fig. 5. The ratios of polysomes to monosomes were calculated as described in Materials and Methods. Each analysis was repeated five times, with the exception of the cycloheximide experiments, which were repeated twice. Data are averages of all experiments. ND, not determined.

stabilization of mRNAs. Previous studies have shown that cycloheximide-mediated reductions in translational elongation rates lead to increases in the relative amounts of heavy polysomes (7, 21). To compare further the mechanisms of mRNA stabilization in cycloheximide-treated cells versus temperature-shifted *ccal-1* cells, polysome profiles of cells grown under (or shifted to) three different conditions were analyzed. (i) Wild-type cells were grown at 24, 30, and 36°C, (ii) *ccal-1* cells were grown at 24, 30, and 36°C, and (iii) wild-type cells were grown in the presence or absence of cycloheximide at 30°C. Polysomes from these cultures were prepared and analyzed on sucrose gradients; the results are summarized in Fig. 5 and Table 4. The results of these experiments indicate that when wild-type cells are grown at different temperatures, there is a slight increase in the amount of heavy polysomes at 36°C, but the ratio of polysomes to monosomes does not vary significantly as a function of temperature (Table 4). In *ccal-1* cells, however, the polysome-to-monomosome ratio in cells shifted to 36°C increased threefold relative to that observed in the same cells grown at 24 or 30°C (Table 4). For comparison, the polysome profiles of wild-type cells grown at 30°C in the presence or absence of cycloheximide were also analyzed. The results of these experiments demonstrate that cycloheximide increases the polysome-to-monomosome ratio approximately sixfold (Fig. 5; Table 4). Collectively, the results summarized in Table 4 indicate that when protein synthesis was inhibited, either by shifting *ccal-1* cells to the nonpermissive temperature or by the addition of cycloheximide to the cell cultures, the overall number of ribosomes per transcript increased.

DISCUSSION

We report here that following a shift to 36°C, cells harboring a temperature-sensitive mutation (*ccal-1*) in the yeast tRNA nucleotidyltransferase gene rapidly cease protein synthesis, reduce their rates of mRNA degradation, and increase the relative number of ribosomes associated with mRNAs. These observations lead us to draw the following conclusions about tRNA and mRNA metabolism in *S. cerevisiae*.

The rapid decrease of protein synthesis at 36°C in strains harboring the *ccal-1* allele indicates that there is most likely a rapid equilibrium between tRNAs containing or lacking 3' CCA termini. This conclusion is supported by experiments reported previously (1) in which it was shown that (i) temperature shifts of *ccal-1* cells lead to the accumulation of tRNAs that are shorter than normal, (ii) such shorter tRNAs are better substrates for terminal CCA addition in vitro than are wild-type tRNAs (i.e., the shorter tRNAs must be

generally lacking CCA termini), and (iii) such tRNA shortening begins immediately after the shift to the nonpermissive temperature. A comparison of the polysome profiles of temperature-shifted *ccal-1* and wild-type cells suggests that the reduction in the pool of functional tRNAs has its primary effect on translational elongation. After a shift to 36°C, *ccal-1* cells increase the overall number of ribosomes per mRNA, as evidenced by a threefold increase in the ratio of polysomes to monosomes and an overall shift to larger polysomes. This effect is comparable to that observed in cycloheximide-treated cells (Fig. 5; Table 4) and generally considered to be diagnostic of a reduction in translational elongation rates (7, 21). It should be noted, however, that the magnitude of the effect on polysome profiles in cycloheximide-treated cells was greater; cells treated with cycloheximide prior to the preparation of polysomes increased their polysome/monosome ratio sixfold (Table 4). Although the reason for this difference is not clear at present, we consider it possible that the *ccal-1* mutation also interferes with translational initiation. If the CCA termini of all (or most) tRNAs are comparably affected in temperature-shifted *ccal-1* cells, then the initiator methionine tRNA should not be efficiently charged and translational initiation rates may be reduced. Cycloheximide, on the other hand, appears to affect only translational elongation (7, 21, 32).

The reduction in mRNA decay rates that paralleled the reduction in protein synthesis activity suggests that ongoing translation is essential for the decay of most mRNAs. The latter conclusion is supported by the reduced mRNA decay rates observed in cycloheximide-treated cells (Fig. 4; Table 3) (8, 19, 37, 39; reviewed in reference 27) and provides an explanation for the increased steady-state levels of the *STE3*, *PAB1*, and *TCM1* transcripts observed in the initial screen that resulted in the identification of the *ccal-1* mutant (1). Moreover, since inhibiting translation by mutation is an event distinctly different from treating cells with a drug, these results substantiate the significance of previous studies in which cycloheximide stabilized mRNAs (16, 19, 37, 39) and minimize the possibility that the stabilization of cellular transcripts by cycloheximide is due to secondary effects, e.g., cycloheximide effects on protein phosphorylation (22, 23).

The biochemical basis for mRNA stabilization as a consequence of protein synthesis inhibition remains unknown. The results presented here do not differentiate between (i) the existence of labile mRNA turnover factors that are degraded when protein synthesis is inhibited and (ii) an active role for translocating ribosomes in the actual mechanics of mRNA decay. Our own bias is that the act of translation is the important event in this process. This conclusion is drawn from observations that the mRNA-stabilizing effects of cycloheximide occur very rapidly (16, 30a), and additional experiments with yeast cells which show that (i) mRNA instability elements have been localized to coding regions (12, 14, 15, 17, 26, 31), (ii) the function of the coding region instability element from the *MATα1* mRNA is dependent on ribosome progression up to or through the element (26), and (iii) destabilization of mRNAs by premature translational termination requires downstream sequences that appear to act as translational reinitiation sites (30, 31). In addition to these observations for yeast cells, experiments with mammalian cells demonstrate that (i) the nucleases capable of degrading mammalian mRNAs in vitro are associated with polysomes (29, 34), (ii) the exonuclease activity that degrades histone mRNA in a cell-free mRNA decay system is not affected by treating the cells with

cycloheximide (28), (iii) sequences in the coding regions of the β -tubulin, *c-myc*, and *c-fos* genes are important for rapid or regulated turnover of their transcripts (5, 36, 39), and (iv) the regulation of tubulin, histone, and *c-myc* mRNA turnover requires ongoing protein synthesis (9, 10, 36). Collectively, these results suggest an important role for the ribosome (or one of its subunits) in the decay process. This role may simply be passive, i.e., translation of a sufficient number of codons to expose a nascent peptide critical to the turnover of the respective mRNA (5), or active, e.g., as a component of the site recognition machinery (27) or as the agent which delivers or activates a specific nuclease (10, 26, 31).

The mRNA-stabilizing effects of the *ccal-1* mutation, and all of the other experimental evidence linking mRNA translation and turnover, suggest that genetic schemes to identify mutations in mRNA decay pathways must be approached cautiously. Mutations that alter protein synthesis may, indirectly, alter mRNA decay rates; i.e., it may often be difficult to determine whether a mutation that alters turnover rates is directly or indirectly involved in the decay process.

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