Mutations in the Cytosolic DnaJ Homologue, *YDJ1*, Delay and Compromise the Efficient Translation of Heterologous Proteins in Yeast†

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ABSTRACT: The *Saccharomyces cerevisiae YDJ1* gene encodes a yeast homologue of DnaJ, an *Escherichia coli* molecular chaperone and regulator of Hsp70 function. We examined the function of Ydj1p in vivo by analyzing the activity and production of firefly luciferase (FFLux) and green fluorescent protein (GFP) after inducible expression in yeast strains containing a wild type or a mutant *YDJ1* gene. Although FFLux and GFP mRNA levels were similar in the wild type and mutant strains, the FFLux protein was translated about half as efficiently in the *ydj1-151* mutant compared to the wild type strain; the lower FFLux level was not the result of increased FFLux turnover in the mutant. In contrast, GFP translation was significantly delayed in the *ydj1-151* mutant compared to the wild type strain. Surprisingly, we observed that FFLux and GFP mRNA bound efficiently to polysomes in the *ydj1-151* mutant. Analysis of polysome profiles also revealed a modest increase in the amount of 60S ribosomal subunits in the *ydj1-151* strain, consistent with a translation defect in the mutant, although the Ydj1 protein was not found to be associated with polysomes. To determine whether the inducible expression of an endogenous yeast protein was also less efficient in the *ydj1-151* strain, we examined the inducible synthesis of the yeast TATA-binding protein (TBP) but observed no translation defect. Statistical analysis of the FFLux, GFP, and TBP encoding genes suggests that Ydj1p facilitates the expression of proteins that are poorly translated because both FFLux and GFP contain an abundance of codons that are rarely used in yeast.

Molecular chaperones are required for a diverse spectrum of cellular processes, including protein folding, assembly, transport, degradation, and solubilization of protein aggregates and activation of enzyme-catalyzed reactions. The DnaJ family represents one class of molecular chaperones and is defined as a group by the "J" domain, an ~70-amino acid motif that is highly conserved among all family members (*1*, *2*). Bacterial DnaJ and many of its eukaryotic homologues also possess a glycine/phenylalanine-rich domain, and a zinc-binding, cysteine-rich domain. These domains may help specify substrate binding and may mediate activities unique to individual DnaJ homologues.

Although many DnaJ homologues have been shown stimulate the ATP hydrolytic activity of another class of molecular chaperones, the DnaK family of 70 kDa heat shock proteins (hsp70s), DnaJ molecular chaperones are not simply regulators of another chaperone's activity. The characterization of DnaJ family members using defined biochemical assays indicates that J domain proteins can bind directly to unfolded protein substrates and prevent protein aggregation, and interact with protein substrates whose activity is subsequently modulated by chaperone complexes (*3*−*7*). Thus, it is not surprising that defects in the function of the DnaJ homologues lead to pleiotropic effects in vivo because the assembly, folding, and modulation of enzymes and structural proteins may represent vital regulatory steps that control many pathways.

The *YDJ1* gene encodes a cytoplasmic DnaJ homologue in the yeast *Saccharomyces cerevisiae* (*8*, *9*). Yeast strains with mutant forms of *YDJ1* are defective for ubiquitin-dependent and abnormal protein degradation (*10*), for the activity of *v-src* in yeast (*11*), for facilitating Cdc28p phosphorylation and degradation (*12*), for proficient steroid hormone receptor signaling in yeast (*13*, *14*), and for supporting the translocation of a mitochondrial and an ER-targeted precursor protein (*9*, *15*). In vitro analyses of Ydj1p function have demonstrated that it stimulates the ATPase activity of a yeast cytosolic hsp70, Ssa1p (*16*), can trigger the release of an unfolded polypeptide from Ssa1p in the presence of ATP, and can engineer the polymerization of Ssa1p (*16*, *17*). Furthermore, Ydj1p facilitates the refolding of denatured luciferase (*18*), and prevents the aggregation of rhodanase when diluted from denaturant (*7*). The *YDJ1* and *SSA1* genes have also been shown to interact (*19*). Thus, like many DnaJ family members, Ydj1p functions in a large number of diverse cellular pathways and may act as a chaperone both on its own and in conjunction with specific hsp70 proteins.

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Because molecular chaperones may be required at many stages of protein biogenesis, these factors would be expected to aid in the expression of heterologous proteins. High levels of protein expression are critical in biotechnology, in which genetically tractable organisms serve to produce many commercial protein products, and in common research environments, in which expressed proteins may serve as an antigen or may be "tagged" with a convenient ligand binding motif and function as an affinity matrix. However, few reports exist in which the contributions of chaperones in high-level protein expression systems were examined; in these reports, the results have sometimes been ambiguous (e.g., see ref *20*). In one study (*21*), it was shown that lowering the level of yeast BiP, the ER lumenal hsp70, profoundly decreased the secreted amounts of three heterologous proteins, while BiP overproduction had little effect. These data suggested that BiP acts positively to facilitate protein maturation in the ER, but does not retain improperly expressed (i.e., heterologous) proteins. It is also possible that saturating levels of BiP may already exist in the ER.

In this report, we show that Ydj1p is required for the efficient translation of two proteins heterologously expressed in yeast from a regulated promoter. When the transcription of both firefly luciferase (FFLux) and the green fluorescent protein (GFP) are induced in the *ydj1-151* mutant strain, either there is a significant delay between the time at which the mRNA is present and when the active protein is produced, as is the case for GFP expression, or the level of protein obtained is lower, as observed for FFLux synthesis. The compromised level of induction is neither the result of rapid protein turnover nor the consequence of profound changes in the relative numbers of ribosomal subunits, ribosomes, or polysomes, as has been described for strains carrying mutations in the *SIS1* gene (*22*), another DnaJ family member in yeast. The mRNAs for FFLux and GFP were polysome-associated at time points at which the levels of the corresponding proteins were low, indicating that mRNA processing and nuclear export were not compromised in the *ydj1-151* mutant. Because an endogenous yeast protein driven by an inducible promoter, the TATA-binding protein (TBP), was efficiently expressed in the *ydj1-151* mutant, the translation of every induced message is not affected in this strain. Since FFLux and GFP mRNAs contain a number of rarely used codons in yeast, we speculate that Ydj1p may be required to facilitate the efficient translation of mRNAs containing rare codons, a hypothesis that suggests a previously unknown role for molecular chaperones.

EXPERIMENTAL PROCEDURES

Yeast Strains and Molecular Methods. The yeast strains used in this study were ACY17b (*MAT*α *ade2-1 can1-100 his3-11,15 leu2-2,112 trp1-1 ura3-1 ydj1-2::HIS3 LEU2::ydj1-151*) and W3031b (*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*) and have been described previously (*15*). Cells were grown either in complete medium (YPD; 1% yeast extract, 2% bactopeptone, and 2% dextrose) for polysome profiles or in synthetic complete medium lacking uracil (SC-ura), but containing either glucose or galactose, when the maintenance of a *URA*-marked plasmid was desired.

The gene encoding firefly luciferase was excised from plasmid pGEM-luc (Promega) with *Bam*HI and *Sac*I, and the fragment was inserted into the *Bam*HI−*Sac*I sites of a modified pRS316 CEN/ARS vector containing the *GAL1-10* promoter, inserted at the *Eco*RI−*Bam*HI restriction sites. Plasmids containing the coding sequence of GFP behind a galactose-regulated promoter were generously supplied by both P. Silver (Dana-Farber Cancer Center, Boston, MA) and T. Stearns (Stanford University, Stanford, CA). Plasmids were introduced into host strains using lithium acetate-mediated transformation, and transformants were selected by growth on Sc-ura medium (*23*).

For construction of FFLux(287−294)-Spt15p, the *SPT15* gene was amplified from yeast genomic DNA by the PCR using Pfu-Turbo (Stratagene) and an oligonucleotide primer that inserted the eight-codon sequence GTACCTACAG-TACTTGCGTTTCTT immediately after the ATG translational start signal; the inserted sequence, corresponding to codons 287−294 of FFLux, includes six codons in poorly translated yeast. The amplified DNA contained flanking *Bam*HI and *Eco*RI restriction sites which allowed cloning into the corresponding sites of the galactose-regulated expression vector, pYES2 (Invitrogen). The resulting plasmid was amplified in a bacterial host and transformed into the wild type and *ydj1-151* strains as described above.

Preparation of Cell Extracts for Immunoblots, Northern Analysis, and Luciferase Assays. Yeast containing plasmids with the galactose-regulated genes was grown at 22 °C for 16−18 h to an optical density at 600 nm (OD) of 1−3 in SC-ura supplemented with 2% glucose. The cells were harvested, washed twice with SC-ura, and then resuspended in the same medium containing 2% galactose at an initial OD of ∼0.2. Cells were grown while being shaken at 22 °C unless otherwise noted.

At the indicated time points, ∼50 mL of the culture was harvested (12.5 ODs of cells) and washed once with water, and the resulting cell pellets were resuspended in 300 μL of water, split into thirds, and quick-frozen in liquid nitrogen. RNA was prepared from one aliquot of thawed cells, and Northern blots were prepared as previously described using ^{32}P-labeled, randomly primed probes (*24*, *25*). The probes used to detect the FFLux, TBP, and GFP messages corresponded to the full-length genes, while the actin probe consisted of the *Bam*HI−*Hin*dIII fragment from the yeast actin gene (*26*).

To prepare protein extracts for immunoblot analyses, the thawed cells were again pelleted and then resuspended in 20 μL of Laemmli sample buffer (*27*) containing 1 mM PMSF, 1 μg/mL leupeptin, and 0.5 μg/mL pepstatin. After incubation for 10 min at 65 °C, glass beads were added to the meniscus, and the cells were disrupted by agitation on a Vortex mixer at the maximum setting for 1 min. A 60 μL aliquot of sample buffer was added, and the extract was incubated for another 10 min at 65 °C. Samples were loaded onto 10% SDS−polyacrylamide gels, and after electrophoresis, the proteins were transferred to nitrocellulose membranes (Schleicher and Schuell, pore diameter of 0.2 μm). The antibodies used to detect specific proteins in the extracts were prepared against Ydj1p (*8*), the F1β subunit of the mitochondrial H^+-ATPase (*8*), firefly luciferase (Cortex Biochem, Inc.), Hsp104 (provided by S. Lindquist, University of Chicago, Chicago, IL), yeast BiP (*28*), GFP (provided by S.

Subramani, University of California, San Diego, CA), and yeast ribosomal protein L1 (provided by J. Woolford, Carnegie Mellon University, Pittsburgh, PA). Goat anti-rabbit horseradish peroxidase-conjugated second antibody (Amersham Life Sciences) was used to detect the primary antibody, and the complex was visualized using the enhanced chemiluminescence detection kit (Pierce). Where indicated, the resulting immunoblots were quantified using the NIH Image software.

For luciferase assays, one aliquot of the frozen cells was thawed, and the cells were again harvested by centrifugation and resuspended in 100 μL of water. An OD^{600} of 5 μL of this solution diluted in 1 mL of water was measured to ensure that identical numbers of cells were used for each data point in the luciferase assay. Between 10 and 25 μL of the washed cells was then assayed for light production upon mixing with luciferin in an Analytical Luminescence Laboratory (Ann Arbor, MI) Monolight 2010 Luminometer according to the manufacturer's specifications. All assays were conducted in triplicate, and the background amount of light produced from cells containing the FFLux gene but grown on glucose was typically <5% of that produced from cells grown on galactose.

Pulse and Pulse-Chase Analysis. Yeast was grown at 26 °C to log phase ($OD^{600} \sim 0.5$) for 6 h in SC-ura supplemented with 2% galactose to induce expression of the desired protein, harvested, washed, and then incubated in the same media at a final concentration of 4 OD^{600}/mL for 45 min before ^{35}S Easy Tag (NEN) was added to a final concentration of 100 μCi/mL for 10 min. Further ^{35}S incorporation was arrested by the addition of cycloheximide, and aliquots were removed at the desired times. Cell extracts were prepared as described previously (*29*) and incubated with specific antibodies for 16 h at 4 °C before Protein A-Sepharose (Pharmacia) was added and the incubation continued for another 1−2 h. Immunoprecipitates were washed (*29*), and the proteins were resolved by 10% SDS−PAGE. Gels were then dried and exposed to a PhosphorImager Screen for visualization and quantitation.

Polysome Profile Analysis. Crude extracts for polysome profiles were prepared from wild type and *ydj1-151* cells and centrifuged on 35 mL 7 to 47% sucrose gradients for 4 h at 100000*g* (*30*). The gradients were fractionated on a model 640 ISCO density gradient fractionator and observed using an ISCO UA5 UV monitor and chart recorder, and 1.2 mL aliquots were collected. Aliquots of these fractions were used directly for immunoblot analyses. When the mRNA profile of the fractions was desired (Figure 8), the samples were extracted twice with an equal volume of phenol/chloroform and ethanol precipitated (*25*).

DNA Sequence Analysis. For calculations of RNA folding energy, we employed the FOLD program of the GCG software package (*31*). Folding energies were calculated for several sets of RNA sequences: (a) sliding 40, 50, and 60 nucleotide (nt) windows ranging from the transcription start site through 100 nt past the translation start site; (b) windows of 40, 50, and 60 nt centered on the translation start site; and (c) the entire 5′ end of the mRNA, including the first 20, 50, and 100 nt beyond the translation start site. Codon usage biases were calculated as (a) a simple χ^2 of codon usage for each encoded amino weighted by the relative abundance of the amino acid (expected values were based on the nucleotide composition of each codon position) and (b) the Codon Adaptation Index (CAI; *32*), which measures the congruence of codon usage patterns to preferred codon usage of an organism (*S. cerevisiae* table from ref *33*). Rare codons are those with NSCU (normalized synonymous codon usage) values below 0.004, indicating that these codons were used 0.4% as frequently as preferred codons in highly expressed genes in *S. cerevisiae*. Codon usage in *S. cerevisiae* has been shown to be directly correlated with the abundance of its cognate tRNA (*34*, *35*).

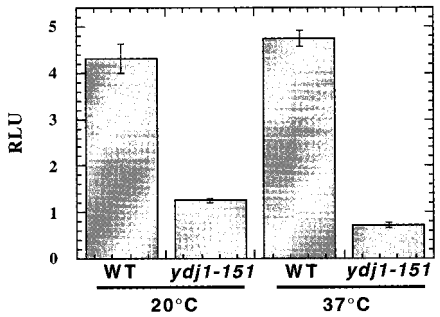

FIGURE 1: The *ydj1-151* mutation diminishes the activity of FFLux in yeast. Wild type (WT) or *ydj1-151* mutant (*ydj1-151*) cells containing a galactose-inducible FFLux expression plasmid were grown in medium containing 2% galactose for 5 h at 20 °C, and either left at 25 °C or kept at 37 °C for another 2 h before luciferase activity was assayed as described in Experimental Procedures. Values are the means of three independent determinations ± SD (standard deviation) and are expressed as relative light units (RLU) $\times 10^{-4}$.

RESULTS

These studies were initiated to determine whether cytosolic chaperones in yeast are required to fold a cellular enzyme. For this purpose, we chose to express firefly luciferase (FFLux) in yeast because it was shown previously that bacterial strains containing mutations in either the *dnaK*, *dnaJ*, or *grpE* genes were defective for refolding heat-inactivated FFLux (*36*). To this end, the gene encoding firefly luciferase was inserted behind a galactose-regulated yeast promoter and transformed into both the *ydj1-151* strain and an isogenic wild type strain (see Experimental Procedures). The *ydj1-151* mutant strain is temperature sensitive for growth and is unable to perform efficiently a variety of cellular processes (see the introductory section). When the expression of firefly luciferase (FFLux) was monitored in the wild type and *ydj1-151* yeast strains 7 h after the carbon source in the medium had been switched from glucose to galactose, we observed that 3.4-fold less light was produced in the *ydj1-151* strain (Figure 1). If the cells were shifted during the last 2 h of the incubation to 37 °C, no change in light production was evident for the wild type strain and a modest (∼40%) decrease was apparent in the *ydj1-151* mutant strain, suggesting that the primary *ydj1-151* defect was temperature-independent. From these data, we concluded that luciferase activity was compromised by a defect in Ydj1p function.

To determine whether the luciferase produced in the *ydj1-151* mutant strain was less stable than in the wild type strain, cells were grown in galactose to induce luciferase production, cycloheximide was added to prevent further protein synthesis, and the cells were incubated at 42 °C. We chose a more

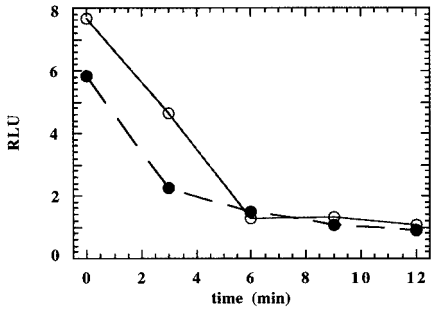

FIGURE 2: Ydj1p does not protect FFLux from thermal inactivation. Wild type (○) or *ydj1-151* (●) cells expressing FFLux were grown overnight in 2% galactose before they were shifted to 42 °C. Luciferase activity was measured at the indicated times as described in Experimental Procedures. Values are the means of three independent determinations.

restrictive temperature for these experiments because FFLux was shown to be inactivated at 42 °C when expressed in *Escherichia coli* (*36*). We reasoned that if Ydj1p directly stabilized the enzyme, a significant difference between its activity in the wild type and mutant strain should be apparent. For example, Wickner et al. (*37*) demonstrated that the ClpA chaperone protects luciferase from thermal inactivation in *E. coli*. As shown in Figure 2, there was a rapid loss in the rate of luciferase-produced light in both the wild type and *ydj1-151* mutant strains. Therefore, Ydj1p, like bacterial DnaJ (*36*), does not protect FFlux from thermal inactivation.

A decreased level of light production by FFLux in the *ydj1-151* strain could have been due to an enhanced rate of FFLux degradation. To investigate this possibility, the wild type and *ydj1-151* strains containing the FFLux-inducible gene were grown in medium supplemented with galactose for 7 h at 26 °C and pulse-chase analyses were performed at both permissive (26 °C) and restrictive temperatures (37 °C). In this experiment, equal amounts of radioactivity were used for each immunoprecipitation, minimizing the effect of any defects in protein translation. As shown in Figure 3, the levels of FFLux remained constant throughout a 45 min chase. We also observed that the levels of both Ydj1p and Ydj1-151p were constant (Figure 3). Therefore, the decreased level of luciferase activity in *ydj1-151* was not the result of enhanced FFLux degradation.

Because mutations in another DnaJ-related protein in yeast affect translation initiation (*22*), we next examined whether the translation of FFLux in the *ydj1-151* strain was compromised. FFLux expression was induced in galactose-containing medium at 25 °C in the wild type and *ydj1-151* strains, and newly synthesized proteins were radiolabeled with [^{35}S]methionine. We chose to perform these experiments at 25 °C because the major defect in FFLux activity in the *ydj1-151* strain was temperature-independent (Figure 1). As shown in Figure 4, ~2.5-fold more FFLux was present in wild type cells than in the *ydj1-151* strain. By comparison, the expression of a constitutively synthesized protein, the β subunit of the F1 mitochondrial ATPase (F1β), was indistinguishable between the wild type and *ydj1-151* strains. From these results, we concluded that less FFLux was translated in the *ydj1-151* temperature sensitive mutant strain, even at the permissive temperature.

To examine the FFLux translation defect in more detail, we analyzed a time course of FFLux induction in wild type and *ydj1-151* cells grown in galactose at 26 °C. Cell extracts were prepared at 2 h intervals for Northern and immunoblot analyses after the medium had been switched to galactose. After this switch, FFLux mRNA was apparent in both strains after 4−6 h, and the amount of message increased over time (Figure 5A). When immunoblots were examined using a polyclonal antibody directed against FFLux, the protein was apparent at much greater levels in the wild type strain than in the *ydj1-151* mutant strain at early times points (i.e., 6 h), and increased significantly over time (Figure 5B). FFLux levels in the *ydj1-151* strain, however, rose modestly (Figure 5B). In addition, hsp104 has been shown to facilitate the resolubilization of aggregated bacterial luciferase in yeast (*38*), but we observed that the amount of hsp104 in each fraction remained constant. Although the time course of FFLux mRNA induction was somewhat variable, and we and others have observed that the FFLux message is quite

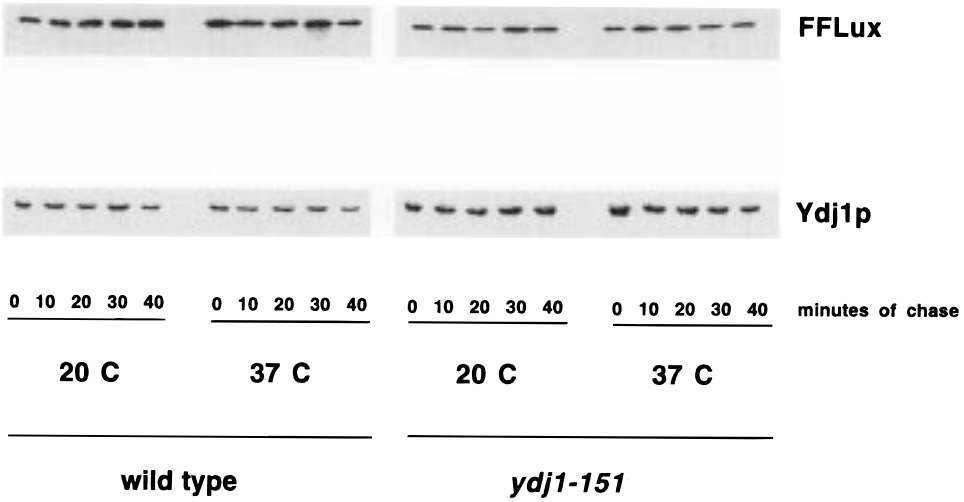

FIGURE 3: FFLux is stable in the *ydj1-151* strain at both permissive and nonpermissive temperatures. Wild type and *ydj1-151* mutant strains expressing FFLux were incubated in [^{35}S]methionine for 10 min at 20 °C; protein synthesis was halted by the addition of cycloheximide, and the cells were incubated at either 20 or 37 °C. Aliquots were removed at the indicated time points, and FFLux and Ydj1p were immunoprecipitated as described in Experimental Procedures. The proteins were resolved by SDS−PAGE and visualized by phosphorimager analysis.

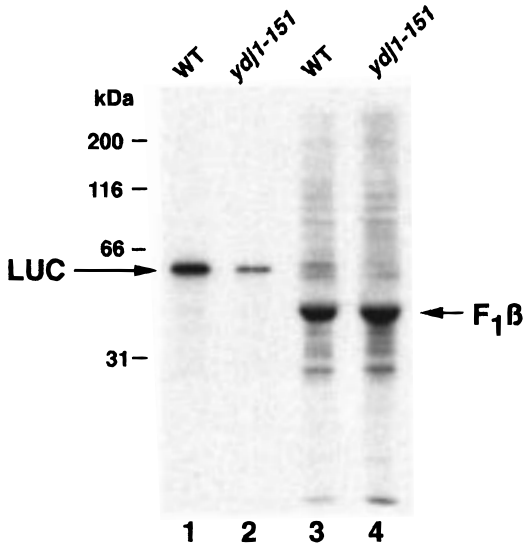

FIGURE 4: FFLux is inefficiently translated in the *ydj1-151* strain. Wild type and *ydj1-151* cells expressing FFLux were incubated in [^{35}S]methionine for 10 min at 20 °C, and cell extracts were prepared. FFLux (lanes 1 and 2) and F1β (lanes 3 and 4) were immunoprecipitated, resolved by SDS−PAGE, and visualized by phosphorimager analysis.

unstable (A. J. Caplan and J. L. Brodsky, data not shown), these results demonstrate that the presence of FFLux mRNA does not correlate with FFLux protein levels in the *ydj1-151* strain.

The difference between FFLux mRNA and protein levels in the wild type and *ydj1-151* mutant strains was also apparent when light production was assayed in cells taken at each time point. Figure 5C shows that FFLux activity [measured in relative light units (RLU)] in the wild type strain was evident at 4 h and increased dramatically, while FFLux activity in the *ydj1-151* mutant was barely detected at 6 h, even though FFLux message was present (Figure 5A). Because a lag was not apparent when the amount of FFLux protein was measured (Figure 5B), we suggest that the *ydj1-151* mutation not only affects the strength of protein expression but also compromises the activity of the protein that is expressed. One possibility is that Ydj1p directly aids in the folding of nascent FFLux, a hypothesis supported by previous observations (*6*, *7*, *18*). Another possibility is that Ydj1p is required for intracellular targeting of FFLux (see Discussion).

To verify that the different levels of FFLux present in Figure 5 did not arise from enhanced protein turnover in the *ydj1-151* mutant strain, we induced FFLux with galactose in the wild type and *ydj1-151* strains for 8 h, prepared mRNA for Northern blots, and performed both a pulse analysis to detect the levels of FFlux protein (as in Figure 4) and a pulse-chase analysis to monitor FFLux degradation (as in Figure 3). When the amounts of FFLux message in the wild type and *ydj1-151* strains after 8 h were normalized to the quantity of actin message, the values were 5.5 and 5.0, respectively. However, as shown in Figure 6A, the amount of FFLux protein in the wild type strain at this time point was ~11-fold greater than in the *ydj1-151* strain as determined from a 10 min [^{35}S]methionine pulse. When a pulse-chase experiment was performed in which the amount of counts in the immunoprecipitation was normalized so that the relative signals would be comparable, we found that the levels of

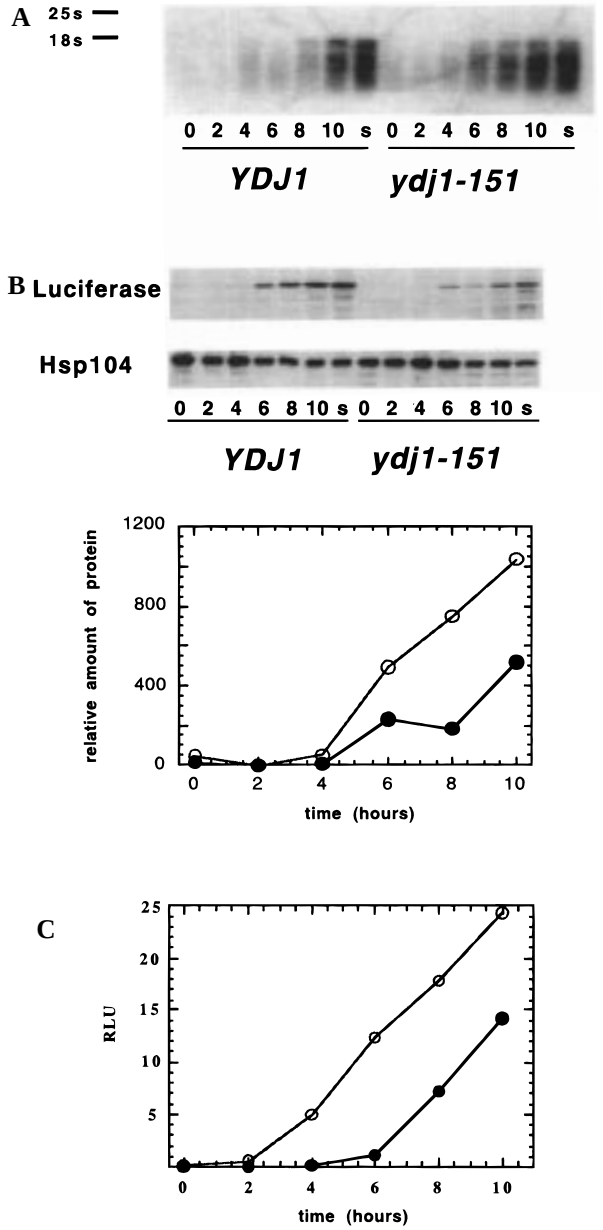

FIGURE 5: Time courses of FFLux mRNA and protein levels and activity after galactose induction in wild type and *ydj1-151* cells. Cells containing the galactose-inducible FFLux expression plasmid were incubated in 2% galactose for the indicated times (in hours), and (A) FFLux mRNA and (B) protein levels and (C) activity were assayed as described in Experimental Procedures. (A) Northern blot analysis of FFLux mRNA. Each lane contained 15 μg of total RNA, and equal loading of every fraction was observed by ethidium bromide staining of the gels (data not shown). The positions of the 25S and 18S rRNAs are indicated. (B) Western blot analysis using anti-FFLux and anti-Hsp104 antibodies. s denotes cells that had been incubated at 37 °C for the final 2 h before assays were conducted, demonstrating that FFLux protein and mRNA levels were unaffected by incubation at higher temperatures. Quantitation was performed as described in Experimental Procedures for wild type (○) and *ydj1-151* cells (●). (C) FFLux activity is shown in relative light units (RLU) ($\times 10^{-4}$) for wild type (○) and *ydj1-151* cells (●).

FFLux after 30 min in the wild type and *ydj1-151* strains were 85 and 76% of the initial amounts of the protein, respectively (Figure 6B). This result suggests that enhanced protein degradation did not give rise to the different levels of FFLux in the two strains.

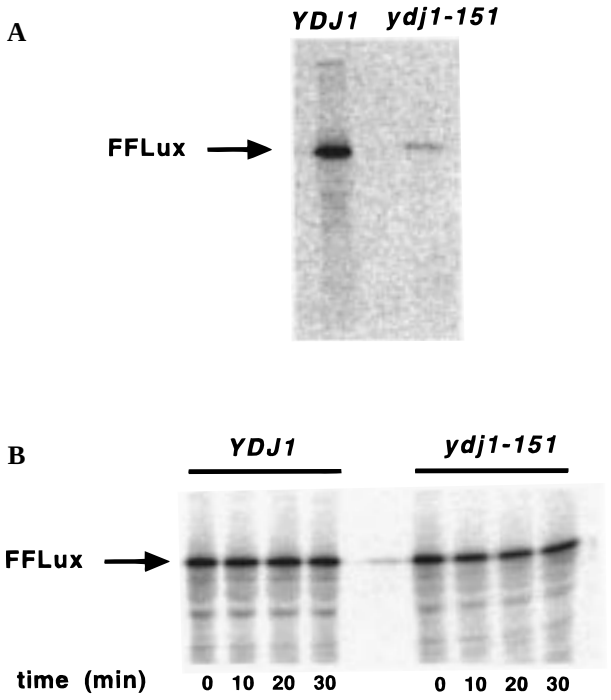

FIGURE 6: Lower FFLux protein levels do not arise from enhanced protein turnover in the *ydj1-151* mutant strain. FFLux was induced as in Figure 3 in the wild type and *ydj1-151* strains. mRNA was prepared for Northern Blot analysis, and a pulse (A) and pulse-chase analysis (B) for FFLux protein levels were performed as described in Experimental Procedures.

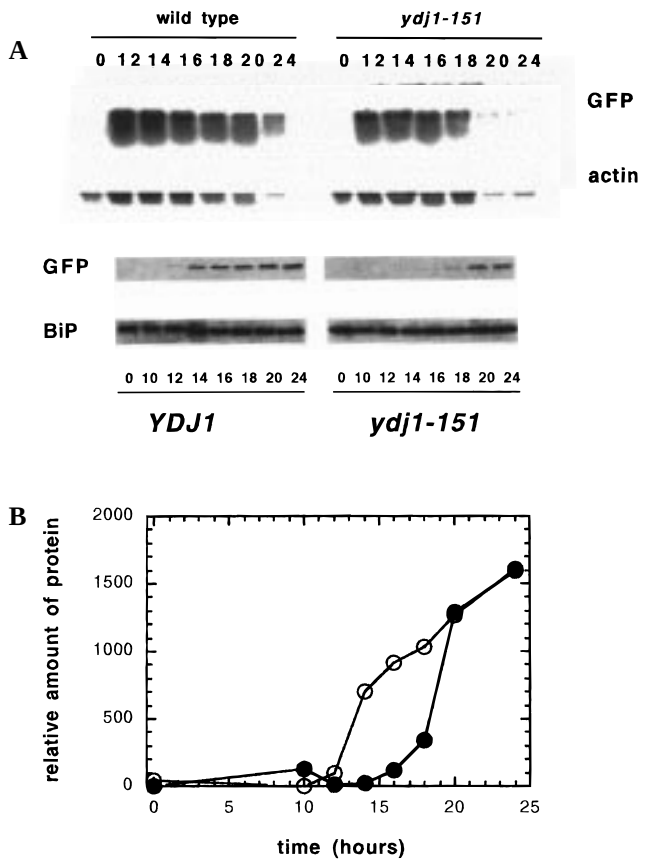

FIGURE 7: Time courses of GFP mRNA and protein production after galactose induction in wild type and *ydj1-151* cells. Cells containing the galactose-inducible GFP expression plasmid were incubated in 2% galactose for the indicated times (in hours), and (A) GFP mRNA and (B) protein levels were assayed as described in Experimental Procedures. (A) Northern blot of GFP and actin mRNA. Each lane contained 8 μg of total RNA. (B) Western blot using anti-GFP and anti-BiP antibodies. The immunoblot of BiP serves as a loading control. Quantitation of protein levels was carried out as described in Experimental Procedures for GFP levels in wild type (○) and *ydj1-151* mutant (●) cells.

To determine whether the observed translation defect was specific for FFLux, a galactose-inducible expression plasmid containing GFP was transformed into the wild type and *ydj1-151* strains. When a time course of GFP expression was analyzed in the two cell types, we observed that the synthesis of mRNA preceded the appearance of protein in the *ydj1-151* mutant strain. There was abundant GFP message present in both the wild type and *ydj1-151* strains 12 h after induction by growth in galactose-containing medium, and GFP became apparent at this time in the wild type strain; GFP in the *ydj1-151* strain was not visible until 16 h after induction (Figure 7A,B). Thus, there was a significant lag between the time that GFP mRNA and protein appeared in the *ydj1-151* mutant. Why a significantly longer time was required to express GFP than FFLux is unknown (14 h for GFP production compared to 6 h for FFLux expression in the wild type yeast strains), but could result from the different composition of the DNA sequence located between the *GAL1,10* promoter and the transcription start site in the two expression plasmids (also see Figure 10). The loss of GFP and actin mRNA over time may reflect the fact that the cells were approaching stationary phase and had begun to degrade the messages. Nevertheless, we conclude from these data that *ydj1-151* affects the initiation of GFP expression, while it affects the amplitude of FFLux expression and the activity of the expressed protein.

Yeast containing a temperature sensitive mutation in another cytosolic DnaJ homologue, *SIS1*, exhibits aberrant polysome profiles and defective translation initiation when incubated at the nonpermissive temperature (*22*). In addition, Sis1p associates with the 40S ribosome, and mutations in specific ribosomal subunits can suppress both the *sis1* temperature sensitive and translation defects (*22*). To determine whether Ydj1p similarly associates with specific ribosomal subunits and whether the *ydj1-151* temperature sensitive strain displays defects in polysome formation, wild type and *ydj1-151* mutant cells were grown at 22 °C, the cultures were divided, and each half was incubated for another 45 min at 37 °C before extracts were prepared for polysome profiles as described previously (*30*). The cells were grown in complete media to ensure that a slower growth phenotype would not affect the amounts of translating ribosomes and ribosomal subunits. As shown in Figure 8, wild type cells grown at 22 °C possessed an abundance of polysomes relative to 80S ribosomes (Figure 8A). When fractions across the gradient were subjected to immunoblot analysis, we found that Ydj1p was present at the top of the gradient, a region lacking polysomes and ribosomal subunits, but observed that some protein spread nonspecifically into fractions containing polysomes. However, overexposure of this gel failed to reveal a peak of immunoreactive protein in any other fraction (not shown). As a positive control, each fraction was also immunoblotted with an antibody against the yeast L1 protein, a component of the large ribosomal subunit. As expected, L1 was found exclusively in fractions containing this subunit (i.e., 60S, 80S, and polysome-

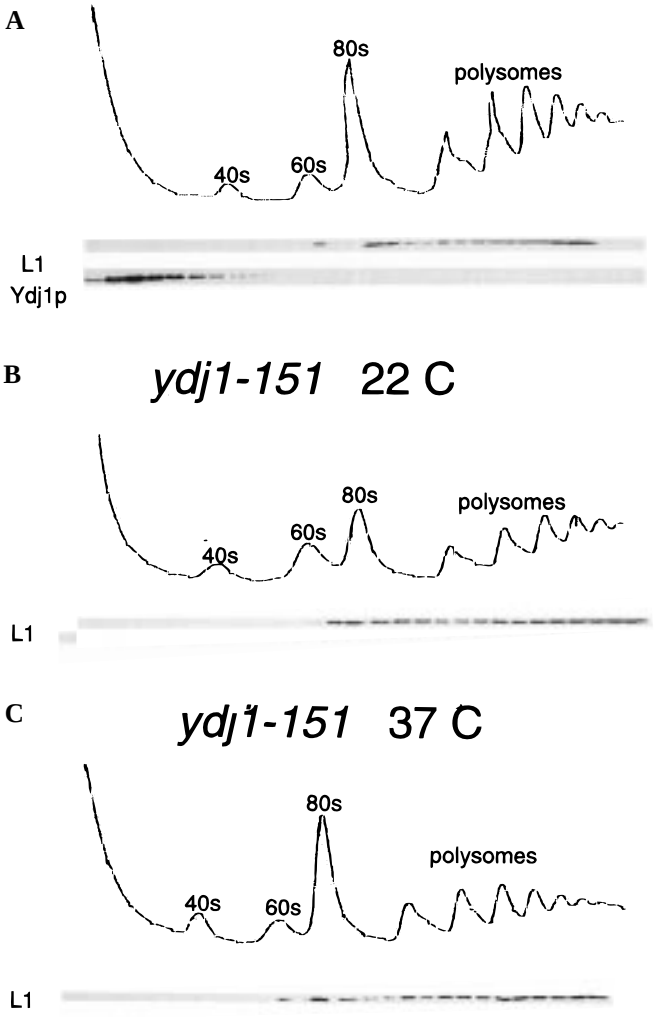

FIGURE 8: Polysome profiles of (A) wild type and (B) *ydj1-151* cells grown at 22 °C and (C) *ydj1-151* cells grown at 22 °C and then shifted to 37 °C for 45 min. Cells were grown and polysome profiles were performed as described in Experimental Procedures. Line tracings indicate the relative UV absorbances across the gradient measured at 260 nm. Where indicated, fractions from the gradients were collected and the presence of Ydj1p and/or L1 was assayed by immunoblot analysis. The positions of 40S, 60S, and 80S ribosomes and ribosomal subunits and polysomes are indicated.

containing fractions; Figure 8A). Wild type cells incubated at 37 °C for 45 min yielded identical polysome and immunoblot profiles as those shown in Figure 8A (data not shown). These results indicate that Ydj1p does not tightly associate with ribosomes or ribosomal subunits, unlike Sis1p (*22*).

When cell extracts from the *ydj1-151* strain grown either exclusively at 22 °C or shifted for 45 min to 37 °C were examined as described in the legend of Figure 8A, several noticeable differences in the polysome gradients were evident. First, at 22 °C (Figure 8B), there was an increase in the amount of 60S subunit relative to the 80S subunit in the *ydj1-151* cells, an effect we observed reproducibly for this strain. Second, the *ydj1-151* mutant cells shifted to 37 °C had elevated levels of the 80S subunit relative to polysomes (Figure 8C). This result mimics that observed with the *sis1* temperature sensitive mutant strain grown at the nonpermissive temperature (*22*), but the extent of the defect observed here is not nearly as dramatic. We concluded that although Ydj1p balances the composition of ribosomes and

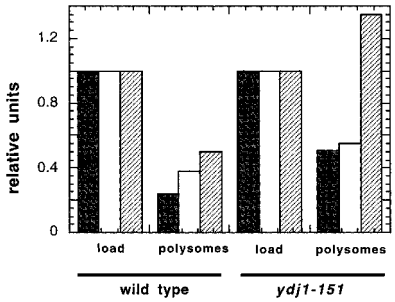

FIGURE 9: FFLux, GFP, and actin mRNAs cofractionate with polysomes. Wild type and *ydj1-151* cells were incubated in 2% galactose at 22 °C for 6 h (to transcribe FFLux mRNA and to detect actin message) or for 16 h (to transcribe GFP mRNA) before cell extracts were prepared and polysome profiles were determined. The "load" and "polysome" fractions from the gradients were collected; RNA was isolated, and Northern blotting was carried out as described in Experimental Procedures. mRNA levels were standardized to the amounts present in the load fractions: (filled bar) actin mRNA, (clear bar) FFLux mRNA, and (striped bar) GFP mRNA.

ribosomal subunits, gross abnormalities in the translation machinery in *ydj1-151* cells are not evident. The fractionation pattern of ydj1-151p at either 22 or 37 °C was identical to that obtained for Ydj1p (data not shown).

The observed translation defect in the *ydj1-151* cells may arise either from an inability of the specific mRNA to associate with translation-competent polysomes or because the mRNA is docked at polysomes but cannot be translated. To distinguish between these possibilities, we collected polysomes from FFLux-expressing wild type and *ydj1-151* cells 6 h after induction, and from GFP-expressing wild type and *ydj1-151* cells 16 h after induction. These fractions, along with aliquots of the material loaded onto the gradient, were analyzed for the presence or absence of FFLux mRNA by Northern blot analysis. Quantitation of the resulting Northern blot revealed that FFLux mRNA was plentiful on the polysomes from both strains 6 h after induction, at which time the FFLux protein was present in the *ydj1-151* cells at ~47% the level in wild type cells (Figure 9, clear bars; compare with Figure 5). We also examined whether GFP mRNA was bound to polysomes 16 h after galactose induction, a time point at which GFP is barely detected in the *ydj1-151* strain (Figure 7B). As shown in Figure 9 (striped bars), GFP mRNA was also present in the load and polysome fractions. Thus, the *ydj1-151* strain does not exhibit a defect in FFLux or GFP mRNA export from the nucleus or prevent these mRNAs from docking at the ribosome, but instead precludes the efficient translation of the mRNAs once the mRNAs interact with the ribosome. Of note, the levels of polysome-associated mRNAs were consistently higher in the *ydj1-151* mutant strain than in the wild type strain, suggesting that they accumulate on the polysomes due to translational arrest. As a positive control for these Northern blots, we observed that actin mRNA was present on the polysomes in both the wild type and mutant strains (Figure 9, filled bars).

To determine whether every galactose-inducible message was inefficiently translated in the *ydj1-151* mutant, we transformed this strain and the isogenic wild type strain with a plasmid encoding the yeast TATA-binding protein (TBP) driven by the *GAL* promoter; overexpression of TBP does not affect cell growth, viability, or transcription (*39*). When the transformed cells were switched from glucose- to

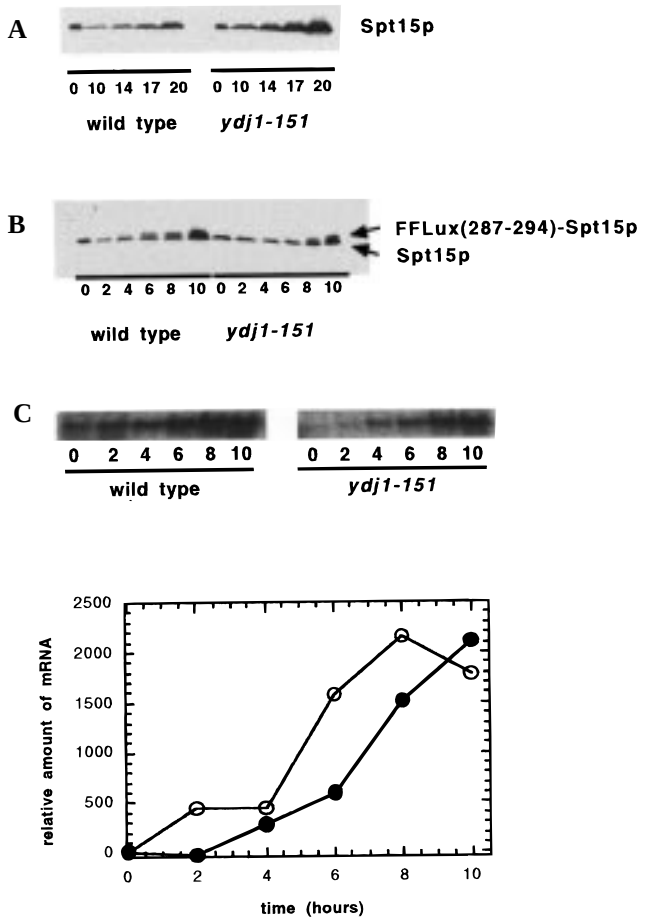

FIGURE 10: Galactose-induced expression of yeast TBP and FFLux-(287−294)-Spt15p in wild type and *ydj1-151* cells. Cells containing either (A) the galactose-inducible TBP expression plasmid or (B) the FFLux(287−294)-Spt15p expression plasmid were incubated in 2% galactose for the indicated times (in hours) before aliquots were removed and cell extracts were prepared. (A and B) TBP and FFLux(287−294)-Spt15p were detected by immunoblot analysis as described in Experimental Procedures. (C) mRNA corresponding to FFLux(287−294)-Spt15p was observed by Northern blot analysis in wild type and *ydj1-151* cells. The amount of mRNA was quantified after the signal at 0 h was subtracted (due to aberrant transcription of mRNA from the *GAL* promoter while cells were still in glucose-containing medium) for wild type (○) and *ydj1-151* (●) cells.

galactose-containing medium and assayed over time for the induction of TBP, we found that the rate of TBP production was nearly equal in the two strains, and the overall levels of protein produced were actually higher in the *ydj1-151* strain (Figure 10A). This result indicated that some inherent difference between the TBP, GFP, and FFLux messages may lead to a *ydj1-151* translation defect that is selective for GFP and FFLux.

What determines whether the *ydj1-151* mutation will affect the translation of a specific mRNA? To answer this question, we examined the TBP, GFP, and FFLux mRNAs for (1) their abilities to form secondary structure and (2) the presence of rarely used codons in yeast. Both of these factors are known to affect protein translation. First, we failed to detect any thermodynamically stable RNA secondary structures in the GFP, FFLux, or TBP mRNAs (data not shown; see Experimental Procedures). Second, to investigate codon usage in the three mRNAs, we calculated the codon adaptation index (CAI) of each mRNA when translated in yeast (see Experimental Procedures). The CAI measures the overall usage of codons preferred by an organism. The CAI values for the FFLux and GFP mRNAs were not significantly lower than those of the CAI of the TBP message (data not shown), indicating that overall codon usage bias is similar among the three genes. However, there are many consecutive rarely used codons [normalized synonymous codon usage (NSCU) < 0.004; see Experimental Procedures] in the GFP and FFLux mRNAs, but not in the TBP mRNA, and the percentage of rarely used yeast codons in GFP and FFLux is 2-fold higher than that in TBP (Figure 11). In addition, we noted that both GFP and FFLux contain four rarely used codons with NSCUs of less than 0.004 in the first 15 codons, while TBP contains none.

To examine whether the presence of multiple, rarely used codons within the first 15 amino acids would affect the translation of an mRNA in the *ydj1-151* strain, we inserted the codons corresponding to amino acids 287−294 from FFLux (see Figure 11) into *SPT15* immediately after the initiating methionine; codons 287−294 include six codons with low NSCU values, four of which are adjacent. The gene encoding the resulting fusion protein ["FFLux(287−294)-Spt15p"] was introduced into the galactose-regulatable yeast expression vector pYES2 and transformed into the wild type and *ydj1-151* mutant strains. After the transformed cells were grown in galactose, we noted that there was an ∼2 h delay in the translation of FFLux(287−294)-Spt15p in *ydj1-151* cells compared to that in isogenic wild type cells [Figure 10B; compare FFLux(287−294)-Spt15p in the wild type and *ydj1-151* cells at 6−8 h]. Because the fusion protein is ∼1 kDa larger than wild type TBP, the resulting immunoblot also showed that the endogenous levels of TBP were unaffected by galactose (Figure 10B). The shorter time required to induce the expression of FFLux(287−294)-Spt15p compared to TBP from their respective *GAL* promoters (compare the time courses in panels A and B of Figure 10) probably reflects the fact that FFLux(287−294)-Spt15p was cloned immediately after the promoter while TBP was present ∼110 bases downstream from the promoter. Surprisingly, Northern blot analysis indicated that there was also an ∼2 h delay in the appearance of FFLux(287−294)-SPT15 mRNA in the *ydj1-151* mutant strain (Figure 10C). These final results indicate that the *ydj1-151* mutation may also affect the amount of mRNA produced from an inducible promoter, arising either from enhanced mRNA degradation or from a reduced transcription level. These results also indicate that this string of rare codons at the extreme amino terminus of a protein does not alter its translation in the *ydj1-151* strain.

DISCUSSION

We demonstrate in this report that mutations in Ydj1p, the cytosolic DnaJ homologue in yeast, prevent the efficient translation of two heterologously synthesized proteins. We find that the amount of FFLux synthesized is reduced and that the initiation of GFP translation is delayed when the expression of the messages for these proteins is driven by an inducible promoter. Because the untranslated message is polysome-associated, we conclude that the translation defects occur after the message is exported from the nucleus. Since pre-mRNA processing is generally recognized as a prereq-

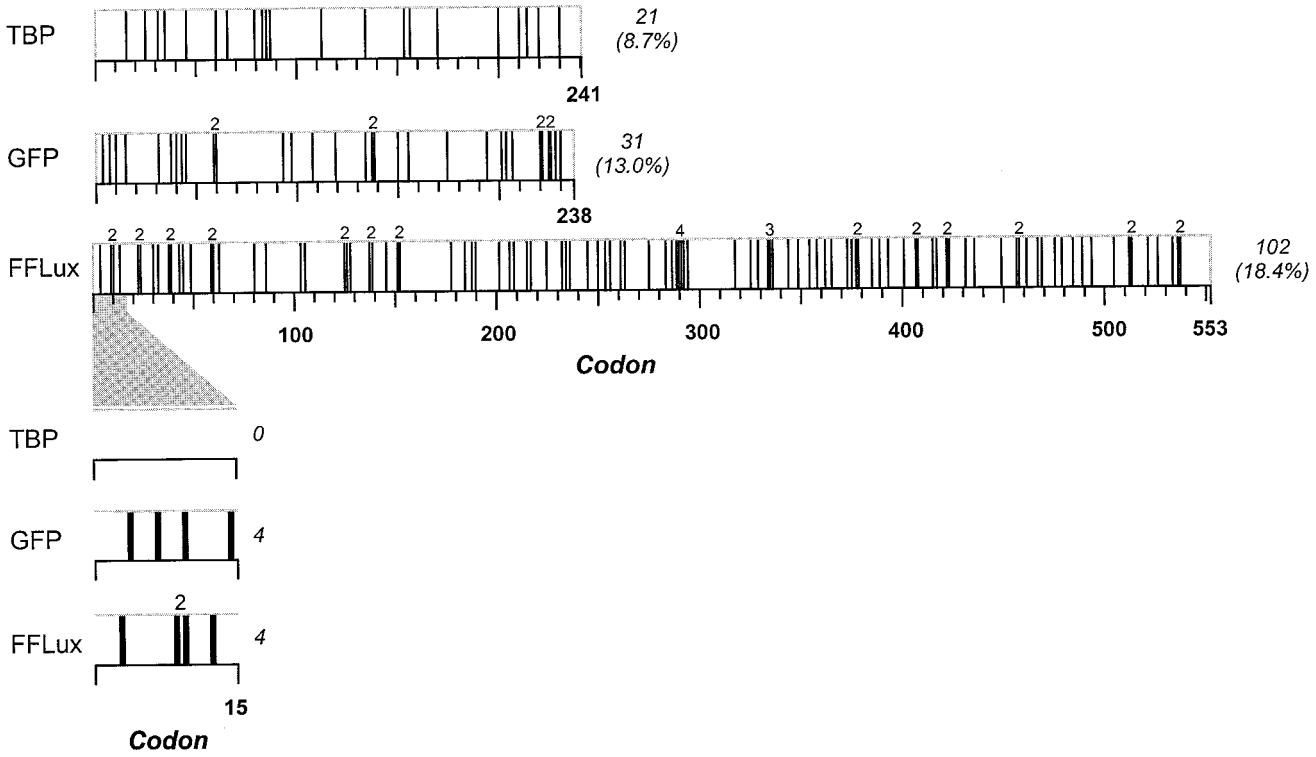

FIGURE 11: Distribution of rarely used yeast codons in the coding regions of TBP, GFP, and FFLux. (Top) The TBP, GFP, and FFLux coding regions are shown, and the codon positions are indicated in boldface. Vertical bars represent codons with an NSCU of <0.004 (see Experimental Procedures), and values above the bars indicate the numbers of consecutive codons with an NSCU of <0.004. Note that TBP contains no such consecutive codons. Values to the right indicate the numbers and percentage of codons in the genes with an NSCU of <0.004. (Bottom) An enlargement of the first 15 codons depicted in the top half of the figure. Values to the right indicate the number of codons with a CAI of <0.004.

uisite for nuclear export, we suggest that the *ydj1-151* defect does not affect the processing of nascent messages. In addition, we observe that the level of one endogenous yeast protein (F1β) is unaffected in the *ydj1-151* strain, as is the inducible expression of another protein, TBP. These results indicate that defects in the Ydj1p molecular chaperone affect the translation of only a subset of mRNAs in yeast (see below). We also suggest that Ydj1p is not required to activate a component required for general protein synthesis, and thus does not globally affect protein translation. Consistent with this conclusion, we fail to observe profound effects on the distribution of ribosomal subunits and polysomes in the *ydj1-151* mutant (Figure 8), and we and others have found that the growth rate of the *ydj1-151* strain is comparable to that of the wild type strain at the permissive temperature on either glucose or galactose-containing medium (*10*; data not shown). We also could not rescue the temperature sensitivity of the *ydj1-151* strain using a cycloheximide plate assay, which indicates that the gene may not be required for a specific step in protein translation or translocation (*40*).

On the basis of the data presented in Figures 10 and 11, we suggest that Ydj1p is required to translate efficiently genes containing multiple, rarely used codons. It is well established that translation is slowed when rare codons, and especially clusters of infrequently used codons, are encountered (*41*, *42*). If, for example, Ydj1p is required to charge poorly used tRNAs, to chaperone the activity of or fold specific tRNA synthetases, to catalyze the binding of charged tRNAs to the ribosome, or to facilitate peptidyl translocation, then defects in its activity may lead to significant delays in translation initiation (e.g., GFP) or to decreases in the efficiency of translation (e.g., FFLux). In essence, the *ydj1-151* mutation may compound translation defects inherent to messages containing rarely used codons, resulting in halted or reduced levels of translation. Since the FFLux and GFP mRNAs contain multiple regions rich in rarely used codons (Figure 11), it will be difficult to address whether correcting any one subset of rarely used codons will alleviate a translation defect specific to either GFP or FFLux.

Alternatively, Ydj1p, like other DnaJ homologues (*43*), may associate with nascent polypeptides emerging from the translating ribosome and promote their folding; a role for Ydj1p in post-translational protein folding is now established (see below; *7*, *18*). While we cannot exclude this hypothesis, our inability to identify a population of Ydj1p that binds specifically to polysomes suggests that Ydj1p does not chaperone nascent proteins from the ribosome, or that it associates transiently and thus cannot be detected using polysome fractionation procedures. Consistent with our results, however, Eggers et al. (*44*) failed to observe cytosolic DnaJ homologues associated with nascent polypeptides emerging from the ribosome.

Ydj1p may also be required for protein translation because it interacts transiently with a specific hsp70; hsp70s and DnaJ homologues form active chaperone complexes (*1*, *2*) and members of the hsp70 family of molecular chaperones have been shown to interact with polysomes in both mammalian and yeast cells (*45*−*47*). The yeast Ssb1 and Ssb2 proteins associate with polysomes, and mutations in the corresponding genes lead to defects in cell growth, decrease the number of translating ribosomes, and render cells hypersensitive to protein synthesis inhibitors (*46*). However, Ydj1p does not

stimulate the ATPase activity of Ssb1/2p (*48*) but interacts instead with Ssa1p, the major hsp70 in the yeast cytosol (*15*, *16*, *19*). Whether Ssa1p or another hsp70 partner for Ydj1p is also required for protein translation is currently unknown.

There is also a delay between the time that FFLux is expressed in yeast and the time at which luciferase activity is evident (Figure 5). We suggest that Ydj1p may be required to activate or fold FFLux after it has been translated. In support of this proposition, Ydj1p prevents protein aggregation in vitro (*7*) and with the cytosolic hsp70 in yeast, Ssa1p, helps refold denatured luciferase (*18*). DnaJ homologues in other cell types also aid in protein folding (*2*). Alternatively, Ydj1p may be required for the proper localization of FFLux after it has been translated. FFLux expressed in yeast, as in other cells, has been shown to target the peroxisome (*49*); however, these experiments were performed when cells were grown in the presence of oleic acid, a condition that supports peroxisome biogenesis. In contrast, FFLux is located in the yeast cytoplasm when oleic acid is absent from the medium (J. Höhfeld and F. U. Hartl, personal communication), conditions under which few peroxisomes are evident. Because the experiments reported here were also performed using medium that was not supplemented with oleic acid, we can exclude this second hypothesis.

Our discovery that mutations in *YDJ1* arrest translation once the mRNA is bound to the polysomes (Figure 9) is not unique. The unfolded protein response (UPR) in yeast offers another example in which an mRNA is polysome-associated but untranslated (*50*, *51*). The *HAC1* message, a transcription factor whose synthesis is induced by the UPR, contains an intron that inhibits translation elongation (*51*). Regulated splicing of the intron removes the translation block, and it has been shown that the intron is necessary and sufficient to attenuate translation. Lee and Goldberg (*52*) observed that Ydj1p was induced when proteasome inhibitors were introduced into yeast. Because the proteasome degrades misfolded proteins that induce the UPR and are subsequently exported from the ER (*53*), Ydj1p may be coupled to the UPR and help chaperone the proteolysis of aberrant proteins. Thus, it would be worthwhile to examine if Ydj1p is also required to regulate the translation of the *HAC1* message.

Another result from our studies was that the amount of FFLux(287−294)-Spt15p mRNA present after growth in galactose was reduced in the *ydj1-151* strain compared to the wild type strain (Figure 10C). We suggest three scenarios by which this effect arises. First, Ydj1p may also be required for the transcription of some genes. Second, Ydj1p may be required to stabilize some messages, and when mutated, the half-lives of these messages are shortened. And third, the lag in FFLux(287−294)-Spt15p mRNA production actually reflects a translation delay; in this case, as compared to that for FFLux (Figure 5) or GFP (Figure 7), the message is rapidly degraded if it cannot be efficiently translated. Differentiating between these possibilities will further enlighten us on the function of this versatile chaperone (see the introductory section). It should be noted that altering the growth characteristics of the *ydj1-151* strain in medium containing raffinose can alter the abundance of some messages, including FFLux and *v-src*, in these yeast (*11*; A. J. Caplan, unpublished observations).

In summary, many cellular processes are adversely affected in the *ydj1-151* mutant strain, and it will now be important to determine whether these pleiotropic effects may be related to defects in the translation of specific proteins; the absence or decreased level of such a specific factor could have secondary consequences on cellular physiology. Thus, the previously noted *ydj1* effects may be either indirect or direct consequences of the mutation. To address this possibility, the identification and analysis of genes that interact with *YDJ1*, in addition to *SSA1* (*19*), may prove to be valuable and is the topic of current investigations in our laboratories.

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