Posttranscriptional Regulation and Assembly into Ribosomes of a *Saccharomyces cerevisiae* Ribosomal Protein- β -Galactosidase Fusion

LINDA GRITZ, NADJA ABOVICH, JOHN L. TEEM,[†] AND MICHAEL ROSBASH*

Department of Biology, Brandeis University, Waltham, Massachusetts 02254

Received 10 July 1985/Accepted 16 September 1985

To study the regulation of ribosomal protein genes, we constructed a '*lacZ* fusion of the *Saccharomyces cerevisiae* *RP51A* gene, containing the first 64 codons of *RP51A*. In a strain lacking an intact *RP51A* gene (cells are viable due to the presence of an active *RP51B* gene), β -galactosidase activity is 10-fold greater than in a strain containing *RP51A*. *RP51A-lacZ* mRNA levels are equal in the two strains, indicating that regulation is posttranscriptional. In the absence of the *RP51A* gene, the fusion protein is predominantly cytoplasmic and associated with polysomes, whereas in the presence of *RP51A*, the fusion protein is predominantly nuclear, and none is associated with polysomes. Deletions were made in the *RP51A*-coding portion of the fusion gene. The most extensively deleted gene, containing only the first seven *RP51A* codons fused to *lacZ*, produced a high level of β -galactosidase activity in both the presence and the absence of the *RP51A* gene. In both cases, little or none of this shorter fusion protein was found associated with polysomes. Thus, a regulatory site (or sites) lies in the protein-coding region of *RP51A*. We suggest that posttranscriptional regulation of the *rp51* fusion protein is related to assembly of the protein into ribosomes.

The coordinate production of ribosomal proteins (r-proteins) poses an interesting problem in intracellular regulation. More than 70 proteins are coordinately synthesized with respect to each other, in relation to the synthesis of several species of rRNA, and in response to the varying demand for ribosomes under different environmental conditions. In *Escherichia coli*, coordinate synthesis is achieved, at least in part, by the arrangement of r-protein genes in polycistronic operons and regulation by autogenous control at the translational (9) and possibly transcriptional (18) levels. In eucaryotes, r-protein gene expression is probably more complex. Most r-protein genes are unlinked (6, 8, 10, 32), exist in multiple copies (6, 10, 20, 32), and contain introns (4, 10, 24). In addition, transcription, translation, and ribosome assembly occur in different compartments of the eucaryotic cell.

Many *Saccharomyces* r-protein genes have been cloned (3, 10, 32) and are being used to study r-protein expression and regulation. When extra copies of the cloned *tcml* gene encoding *rpl* are introduced into yeast on a multicopy plasmid, the steady-state level of mRNA increases three- to fourfold, but *tcml* protein synthesis is only slightly increased. Thus, dosage compensation of the *tcml* gene has been proposed to occur at the translational level (23).

To facilitate the analysis of *rp51* gene expression, the *Saccharomyces cerevisiae* *RP51A* gene has been fused to the *E. coli lacZ* gene. We have analyzed a number of integrated and episomal copies of *RP51A-lacZ* fusion genes in various strains designed to express different levels of *rp51*. The data indicate that fusion protein levels are modulated by the level of expression of the *RP51A* gene. We propose a regulatory mechanism involving ribosomal assembly.

MATERIALS AND METHODS

Strains and media. The haploid *S. cerevisiae* DBY745 (α *adel-101 leu2-3 leu2-112 ura3-52*) was constructed as described previously (5). PB12 is a derivative of DBY745 containing the *LEU2* gene in place of the *RP51A* gene (2). HR125-5D (a *leu2-3 leu2-112 ura3-52 trp1 his3 his4*) was constructed as described previously (R. Jensen, Ph.D. dissertation, University of Oregon, Eugene, 1983).

Yeast cells were grown in synthetic complete medium containing either 2% glucose or 2% galactose as described previously (27). Medium containing 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside was prepared as described previously (25).

Plasmid and strain constructions. Plasmids pHZ18 (30) and pZA64 (previously designated pZA50; J. L. Teem, Ph.D. dissertation, Brandeis University, Waltham, Mass., 1983) were constructed as described previously. pZA64 is identical to pHZ18 except for the 5' end of the '*lacI-Z* fusion gene; pZA64 contains the promoter and transcriptional and translational initiation sites of *RP51A*, whereas pHZ18 contains the *GAL10* UAS and *CYC1* transcriptional and translational initiation sites. YEp24 (5) and pLGSD5 (14) were as described previously. pPP-Z was a generous gift from H. Fried. pYE51B-1 is a derivative of pYE51B (2) containing the *LEU2* gene.

DNA cleavage, ligation, and transformation were performed as described previously (11, 12). Plasmids pHZ18 Δ 2 μ m and pZA64 Δ 2 μ m were constructed by partial *EcoRI* digestion and religation to remove the 2 μ m DNA portions of pHZ18 and pZA64. pHZ18 Δ 2 μ m and pZA64 Δ 2 μ m were cleaved with *SalI* which, upon transformation of DBY745, directed integration (22) of these plasmids at the homologous *SalI* site in the intron of the genomic copy of *RP51A*, creating strains HZint and ZAint, respectively (Fig. 1). The predicted structures of the *RP51A* locus in these strains were confirmed by Southern hybridization (data not shown). pZA7 was constructed by partial BAL 31 digestion of the

* Corresponding author.

[†] Present address: Whitehead Institute, Cambridge, MA 02139.

FIG. 1. Construction of a galactose-inducible, glucose-repressible, *CYC1-RP51A* fusion gene. The 2 μ portion of pHZ18 was removed by partial *EcoRI* digestion and religation to generate pHZ18 Δ 2 μ . Strain DBY745 was then transformed with pHZ18 Δ 2 μ cleaved in the *RP51A* intron with *SalI* to direct integration of the plasmid at the homologous *SalI* site of the genomic *RP51A*. This integration event effected an exchange of 5' ends of the fusion and wild-type genes, resulting in an integrated *RP51A-lacZ* fusion gene with the genomic *RP51A* promoter adjacent to a *CYC1-RP51A* fusion gene with a GAL-CYC1 hybrid promoter. pZA64 was integrated in the same manner for comparison with the galactose-inducible gene. ~~~, chromosomal DNA; —, plasmid DNA; □, coding region.

RP51A coding region of pZA64 as follows: pZA64 was cleaved at the *RP51A-lacZ* junction with *Bam*HI and then treated with BAL 31 nuclease for various lengths of time to remove 10 to 350 base pairs of the fusion gene. The remaining plasmid ends were made blunt with the large fragment of DNA polymerase, followed by cleavage in the *lacZ* DNA with *Sst*I. To replace the *lacZ* portion of the plasmid removed by BAL 31, the *SmaI-SstI* fragment of pZ1 (a plasmid used to construct pZA64; Teem, Ph.D. dissertation) containing this region of pZA64 was ligated to the BAL 31-treated DNA. Yeast cells transformed with these plasmids were identified as blue colonies on plates lacking uracil and containing 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (7). The endpoints of the deletions were determined by DNA sequencing (26). pHZA7 was constructed by replacing the *SalI-SstI* fragment of pHZ18 with the *SalI-SstI* fragment of pZA7.

cDNA synthesis. Yeast RNA was prepared as described previously (15). An oligonucleotide (5'TTCACCAGCGAGACGGGC3') complementary to the proximal portion of the '*lacI*' fragment of pLG200 (13) was purchased from the Nucleic Acids Laboratory, University of Massachusetts Medical School, Worcester, Mass. cDNA was synthesized from 5 μ g of total yeast RNA as described previously (30) except that dideoxyTTP was used instead of dTTP to limit the synthesis to a 30-base cDNA product, analogous to the procedure previously described (2).

β -Galactosidase assay. Enzyme assays were performed as described previously (19) except that the yeast cells were lysed by vortexing with glass beads.

Immunoblot analysis. Exponentially growing cells were pelleted, suspended in sodium dodecyl sulfate sample buffer (17), lysed by vortexing with glass beads, and boiled for 3

min. An amount of the lysate (10 μ l), corresponding to approximately 1 ml of the original culture, was subjected to electrophoresis in a 7.5% acrylamide sodium dodecyl sulfate gel (17), and the protein was transferred to nitrocellulose (31). The nitrocellulose filter was treated with rabbit anti-serum raised against β -galactosidase and then with 125 I-labeled protein A (113 Ci/g; New England Nuclear Corp.) as described previously (29). Protein-antibody complexes were visualized by autoradiography.

Genetic analysis. Yeast crosses and tetrad analysis were accomplished by standard techniques (21).

Polysome preparation. Cells were grown in 50-ml cultures to an A_{600} of 0.6, then treated with 100 μ g of cycloheximide per ml, quickly chilled, and harvested. The pellets were suspended in diethylpyrocarbonate-treated 50 mM Tris hydrochloride-100 mM NaCl-30 mM MgCl₂-0.2% Triton X-100-1 mM β -mercaptoethanol (pH 7.5) and broken by vortexing with glass beads at 4°C. The lysate, cleared of cell debris by centrifugation at 10,000 rpm for 10 min in an SS34 rotor, was loaded on a 10 to 30% sucrose gradient in the above buffer plus 0.48 M NaCl and centrifuged at 40,000 rpm for 60 min in an SW40 rotor at 0°C. Gradients were fractionated through an ISCO UA-5 absorbance monitor at a wavelength of 260 nm to identify the fractions containing ribosomal subunits, monosomes, and polysomes.

Indirect immunofluorescence. Fixation and indirect immunofluorescence of yeast cells were performed as described previously (28) with monoclonal β -galactosidase antibody 9E3, derived by T. Mason, and fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Cells were treated with 1 μ g of 4,6-diamidino-2-phenylindole per ml to stain DNA.

TABLE 1. Effect of *RP51* on β -galactosidase activity^a

Plasmid or integrant	Fusion gene ^b	Activity of strain grown in carbon source:			
		DBY745(<i>RP51A</i>)		PB12(Δ <i>RP51A</i>)	
		Glucose	Galactose	Glucose	Galactose
Plasmid					
YEpl24	None	0		0	
pZA64	<i>RP51A-lacZ</i> (64)	30		343	
pPP-Z	<i>tcml-lacZ</i> (372)	51		58	
pZA7	<i>RP51A-lacZ</i> (7)	303		464	
pHZ18	<i>GAL-CYC1-RP51A-lacZ</i> (64)	<1	283	<1	277
pLGSD5	<i>GAL-CYC1-lacZ</i> (0)		1,993		2,211
pHA7	<i>GAL-CYC1-RP51A-lacZ</i> (7)		1,639		1,829
Integrand					
ZAint	<i>RP51A-lacZ</i>	15	18		
HZint	<i>GAL-CYC1-RP51A</i> and <i>RP51A-lacZ</i>	70	22		
HZint(pYE51B-1)	<i>GAL-CYC1-RP51A</i> and <i>RP51A-lacZ</i>	20	20		

^a Strains DBY745, containing *RP51A*, and PB12, a derivative of DBY745 lacking *RP51A*, were transformed with plasmids containing various *lacZ* fusion genes. pHZ18 and pZA64 were integrated in DBY745 to generate ZAint and HZint as described in the legend to Fig. 1. HZint was transformed with pYE51B-1 containing *RP51B* to generate HZint(pYE51B-1). β -Galactosidase activity was assayed, and units were determined as described in Materials and Methods. Error is $\leq 20\%$.

^b The presence of a fusion gene altered the growth rate of the host strain by $\leq 25\%$. Values in parentheses are the number of ribosomal protein gene codons in fusion gene.

RESULTS

The effect of *RP51A* on *RP51A-lacZ* expression. To study the expression of *RP51A-lacZ* fusion genes under various levels of *RP51A* gene expression, a plasmid (pZA64) was constructed which contained 750 base pairs of the *RP51A* 5' noncoding DNA and 612 base pairs of the *RP51A* coding DNA, including the intron, fused to the *E. coli lacZ* gene. Two strains were transformed with pZA64: DBY745, containing the wild-type *RP51A* gene, and PB12, a derivative of DBY745 lacking the *RP51A* gene. PB12 still contains *RP51B*, the second gene encoding rp51. The *RP51B* gene product is nearly identical to the *RP51A* product, differing only in the C-terminal amino acid. In wild-type cells, *RP51A* provides 60 to 70% of the total rp51 mRNA, and *RP51B* provides 30 to 40%. PB12 grows more slowly than DBY745 because it is growth rate limited by the amount of rp51 mRNA (2). Thus,

RP51A-lacZ gene expression could be studied in the presence or absence of sufficient *RP51* gene product.

The amount of β -galactosidase activity derived from pZA64 in these strains is shown in Table 1. In the absence of the *RP51A* gene, there is approximately 10-fold more β -galactosidase activity than in cells containing the *RP51A* gene. The presence or absence of *RP51A* does not affect the expression of other fusion genes, including a fusion of the *tcml* ribosomal protein gene to *lacZ* (*tcml-lacZ*, Table 1). Thus, the effect of *RP51A* on β -galactosidase activity appears to be specific for the *RP51A-lacZ* gene.

To confirm that the differences in the amount of β -galactosidase observed between DBY745(pZA64) and PB12(pZA64) were due to the presence or absence of *RP51A*, PB12(pZA64) was mated with HR125-5D, a *leu2* strain of opposite mating type containing the wild-type *RP51A* gene. The resulting diploid strain was sporulated, and asci were dissected. β -Galactosidase levels were measured in four tetrads (Table 2). All the germinated spores contained *URA3* and had β -galactosidase activity, consistent with each spore containing pZA64. In each tetrad, two spores had high β -galactosidase levels, equal to that in PB12(pZA64), and two spores had low β -galactosidase activity at the level of DBY745(pZA64). In each case, the cells with high activity contained the *LEU2* gene, which replaced *RP51A* in PB12. The cells with low activity did not grow without leucine and therefore contained the wild-type *RP51A* gene. Thus, the high *RP51A-lacZ* expression cosegregated with the deletion of *RP51A*, confirming that the presence of *RP51A* was responsible for the low expression of the *RP51A-lacZ* gene.

Another approach was used to vary the level of *RP51* gene expression without altering the *RP51A* gene copy number. A *CYC1-RP51A-lacZ* fusion gene, under the control of a hybrid galactose upstream activation sequence and *CYC1* promoter (14), had previously been created in plasmid pHZ18 (30). Cleavage within the *RP51A* intron of pHZ18 Δ 2 μ m and directed integration (22) of the plasmid into the intron of the genomic copy of *RP51A* effected the exchange of the 5' end of the pHZ18 fusion gene with the 5' end of the genomic *RP51A*, all integrated at the *RP51A* locus (HZint; Fig. 1). This integrated, galactose-inducible *RP51A* gene, designated *GAL-CYC1-RP51A*, codes for a slightly altered rp51 protein

TABLE 2. Cosegregation of high *RP51A-LACZ* expression and *RP51A::LEU2*^a

Tetrad	β -Galactosidase activity (units)	<i>LEU2</i>
1A	433	+
1B	390	+
1C	31	-
1D	36	-
2A	319	+
2B	337	+
2C	45	-
2D	73	-
3A	432	+
3B	44	-
3C	477	+
3D	37	-
4A	57	-
4B	562	+
4C	37	-
4D	309	+

^a Tetrads were dissected from asci derived from a cross between PB12(pZA64) and HR125-5D. β -Galactosidase activity was assayed in the progeny, and growth on synthetic complete medium lacking leucine was scored. +, Growth; -, no growth.

FIG. 2. Primer extension of fusion RNA. An 18-base synthetic oligonucleotide complementary to the *lacI* portion of the fusion gene was used to prime reverse transcription of fusion RNA extracted from different strains (grown in glucose unless otherwise noted), resulting in synthesis of a 30-base product terminated with dideoxyTTP as described in Materials and Methods. Lanes: 1, DBY745(YEp24); 2, DBY745(pZA64); 3, PB12(pZA64); 4, DBY745(pZA7); 5, PB12(pZA7); 6, HZint grown in galactose; 7, HZint; 8, HZint(pYE51B-1). Variation among lanes 2 to 5 and 6 to 8 was within limits of experimental error.

due to the *CYC1* and linker sequences at the 5' end of the coding region. In parallel, the directed integration of pZA64 Δ 2 μ m in DBY745 resulted in the construction of an *RP51A-lacZ* fusion gene adjacent to the wild-type *RP51A* gene (ZAint, Fig. 1). In HZint, the level of *RP51A* gene expression can be modulated by growth in glucose or galactose; ZAint serves as a control. β -Galactosidase activity increased threefold when *GAL-CYC1-RP51A* transcription was repressed by glucose in HZint, but amounts of β -galactosidase in ZAint were the same in glucose and galactose (Table 1). Again, the expression of *RP51A-lacZ* was modulated by the expression of *RP51A*.

To determine whether *RP51B* gene expression had any effect on *RP51A-lacZ* expression, HZint was transformed with pYE51B-1 containing *RP51B* and the *LEU2* gene on a 2 μ m plasmid. In the presence of additional copies of *RP51B* in HZint(pYE51B-1) cells, repression of *RP51A* gene expression in glucose did not result in elevated levels of β -galactosidase (Table 1). Thus, *RP51A-lacZ* expression was lowered by extra *RP51B* gene expression as well as by *RP51A* expression.

Fusion RNA levels. To determine whether the observed differences in β -galactosidase activity are also manifest at the RNA level, RNA was prepared from strains DBY745 (pZA64) and PB12(pZA64). To measure fusion RNA levels, a primer of 18 nucleotides (which hybridizes to the *lacI* portion of the fusion RNA) was used to synthesize DNA complementary to this RNA. To eliminate the complex quantitation of multiple 5' ends (30), a partial primer extension of the RNA was accomplished with reverse transcriptase with dideoxyTTP instead of dTTP, limiting the cDNA synthesis to a 30-base cDNA product. The cDNA products are shown in Fig. 2: the level of fusion RNA was not affected by the absence of *RP51A*. Full-length extensions with this primer and with a primer complementary to the 3' exon of *RP51A* (just upstream of the *RP51A-lacI* junction) gave similar results; the level of the fusion precursor RNAs and the mature RNAs were the same in both strains (data not shown). The same was true of strain HZint containing an integrated fusion gene and a galactose-inducible, glucose-repressible *RP51A* gene: growth in glucose or galactose or extra copies of *RP51B* had no effect on fusion RNA levels (Fig. 2). Thus, regulation of the fusion gene is posttranscriptional.

Association of the fusion protein with ribosomes. pZA64 contains the first 64 (of the 135) codons of *RP51A* fused to the *lacZ* gene. To determine whether this region of rp51 is sufficient for assembly into ribosomes, polysomes and ribosomal subunits were fractionated by sucrose gradient centrifugation and analyzed with respect to β -galactosidase activity (Fig. 3). In strains containing wild-type *RP51A*, no β -galactosidase activity was found in polysomes. However, in the absence of *RP51A*, at least 30% of the total cellular β -galactosidase activity was found in polysome-containing fractions. To distinguish whether this activity was associated with polysomes or cosedimented in another particle, cell extracts were treated with RNase to reduce polysomes to monosomes. In this case, the β -galactosidase activity that previously cosedimented with polysome fractions now cosedimented with the monosome peak (Fig. 3A). Thus, in the absence of wild-type *RP51A* but not in wild-type strains, a fraction of the rp51- β -galactosidase fusion protein is associated with polysomes.

To determine the cellular localization of the fusion protein in the presence or absence of wild-type *RP51A*, indirect immunofluorescence was performed (Fig. 4). In the presence of *RP51A*, the level of fusion protein appears low and the protein is localized to the nucleus (Fig. 4A). In the absence of *RP51A*, the fluorescence is much greater and is located primarily in the cytoplasm (Fig. 4B). These results are consistent with the differences in protein levels observed between the two strains and with the fusion protein being found on polysomes in the absence of *RP51A*.

These observations led to the idea that the fusion protein itself may be the target site of regulation. To test this hypothesis, deletions were made in the *RP51A* coding region of pZA64. The most extensively deleted fusion gene, containing only the first seven codons of *RP51A*, was constructed in plasmid pZA7. Strains transformed with pZA7 had high levels of β -galactosidase activity in the presence or absence of *RP51A* (Table 1). pZA7 fusion transcript levels were the same as in pZA64 either in the presence or absence of *RP51A* (Fig. 2). Indirect immunofluorescence of strains containing pZA7 revealed no differences in localization of this fusion protein (nuclear and cytoplasmic) in the presence or absence of *RP51A* (Fig. 4C; data not shown). These data indicate that a site required for the posttranscriptional regulation observed in pZA64 lies within the protein-coding region of *RP51A*. In addition, little or none of the shorter fusion protein encoded by pZA7, with only seven codons of *RP51A*, was found in polysomes (Fig. 3B).

A similar strategy was applied to the pHZ18-encoded gene. This fusion protein contains the same 63 rp51 amino acids as pZA64, but they are preceded by 14 amino acids encoded by linker and 5' leader sequences used in the construction of pHZ18 (30). Unlike pZA64, this protein has the same β -galactosidase activity in DBY745 and PB12 (Table 1). The activity is significantly reduced relative to the activity of the parent gene in pLGSD5. Like pZA64, however, a deletion of the coding region (removing precisely the same region which generated pZA7 from pZA64) resulted in a substantial increase in β -galactosidase activity; indeed, the deletion derivative pHZA7 has nearly the same activity as the parent plasmid pLGSD5 (Table 1). The data are consistent with the notion that, like pZA64, the pHZ18-encoded fusion is negatively regulated at the posttranscriptional level and a significant target for that regulation lies within the rp51-coding sequence.

The different levels of β -galactosidase activity observed among the different fusions and strains were reflected in the

FIG. 3. β -Galactosidase assays of polysome profiles of fusion-containing strains. Ribosomes and polysomes were sedimented in a sucrose gradient as described in Materials and Methods. β -Galactosidase activity in each fraction was plotted, with the positions of 60S subunits, monosomes, and polysomes indicated above. (A) Symbols: X, DBY745(pZA64); O, PB12(pZA64); Δ , PB12(pZA64) treated with RNase (note reduced scale). (B) Symbols: X, DBY745(pZA7); O, PB12(pZA7).

amounts of β -galactosidase protein as determined by immunoblot analysis (Fig. 5). Thus, the 10-fold difference in enzymatic activity of pZA64 in the presence or absence of *RP51A* is due to a real difference in fusion protein levels and not to other factors altering the apparent activity of the protein. Similarly, the increased activity in DBY745(pZA7) reflects increased levels of protein and not other factors, such as increased enzyme activity of a shorter fusion protein.

DISCUSSION

In this study, fusion of a yeast r-protein gene to the *E. coli lacZ* gene has facilitated analysis of the regulation of r-protein synthesis. In addition, the use of a strain lacking the *RP51A* gene (2) and the construction of a galactose-inducible, glucose-repressible *RP51A* gene has allowed modulation of *RP51* gene expression.

The data presented indicate that expression of an *RP51A-lacZ* gene, containing the first 64 codons of *RP51A*, is affected by the level of wild-type *RP51A* expression. In the absence of a wild-type *RP51A* gene, β -galactosidase activity derived from the fusion protein is 10-fold higher than in the presence of the *RP51A* gene. A similar, but less dramatic, effect is observed when *RP51A* gene expression is repressed by glucose in strain HZint; fusion protein levels are threefold higher than when *RP51A* is induced by galactose. Differences in protein levels are not observed at the RNA level.

Also, several other '*lacZ*' fusions, including a fusion to *tcml*, another r-protein gene, are unaffected by the absence of *RP51A* (Table 1). Therefore, the regulation appears to be gene specific and occurs at the posttranscriptional level.

When rp51 is rate limiting for ribosome assembly (due to the absence of the *RP51A* gene), a significant fraction of the pZA64 fusion protein is associated with ribosomes (Fig. 3). The fact that the fusion-protein-containing ribosomes are in polysomes suggests that they are functional and that the first 63 amino acids of rp51 are sufficient for nuclear transport, ribosome assembly, and transport of the fusion-protein-containing ribosomes to the cytoplasm. Experiments with indirect immunofluorescence with anti- β -galactosidase antibodies are consistent with this interpretation (Fig. 4A and B). The data suggest that pZA64-encoded fusion protein is transported to the nucleus and, in the absence of an adequate synthesis of rp51, is assembled into ribosomes and retransported back to the cytoplasm. Interestingly, the first six amino acids of rp51, Gly-Arg-Val-Arg-Thr-Lys (30), appear to provide some nuclear transport information to the fusion protein (Fig. 4C); a fusion protein lacking these six amino acids (14) is entirely cytoplasmic in location (data not shown).

pZA64 does not rescue the growth-rate deficiency of strain PB12 (data not shown), suggesting that relatively few ribosomes contain the rp51 fusion or that these rp51-fusion-containing ribosomes are somewhat defective. In this con-

FIG. 4. Cellular location of rp51- β -galactosidase fusion proteins. Cells were treated with β -galactosidase antibody, followed by fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G and 4,6-diamidino-2-phenylindole as described in Materials and Methods. (A) DBY745(pZA64); (B) PB12(pZA64); (C) DBY745(pZA7), strain PB12(pZA7) is identical in appearance (not shown). FITC, Fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G; DAPI, 4,6-diamidino-2-phenylindole. DNA stain same field as FITC.

text, it is perplexing that there is active β -galactosidase activity in polysome fractions (Fig. 3), as active β -galactosidase is a tetramer (34). Perhaps the β -galactosidase moiety is external and available to form a tetramer with other external β -galactosidase subunits. Based on these observations, we suggest that the level of fusion protein is related to the assembly of newly synthesized fusion protein into nascent ribosomes. Ribosome or preribosomal assembly may influence, directly or indirectly, the translation of the fusion protein; alternatively, rp51- β -galactosidase which is not assembled into a ribosome or preribosomal particle may be specifically degraded.

Autogenous control of translation regulates the synthesis of at least some *E. coli* r-proteins, and the target site for the feedback regulation is at the 5' end of the mRNA (16, 33). If a similar mode of feedback regulation is occurring in *RP51A*, the mechanism differs from that of *E. coli* in that a significant target site lies within the protein-coding portion of the gene, as pZA7, containing the leader and first 21 nucleotides of *RP51A*, is only slightly regulated as compared with pZA64 (Table 1). The alternative regulatory scheme implies that the fusion protein and rp51 compete for a positive factor or for assembly into ribosomes or both. In a wild-type background in which ample rp51 is synthesized, the fusion protein (which contains only the first 63 of the 135 amino acids of rp51 and a long C-terminal fusion to β -galactosidase) competes poorly and is largely degraded. In the absence of an adequate

synthesis of rp51 (as in strain PB12), fusion protein is assembled into ribosomes and is relatively stable (Fig. 3). This explanation is supported by the results of the accompanying manuscript (1) which demonstrate over synthesis but very rapid degradation of excess rp51 when the *RP51A* gene is introduced into wild-type cells on a multicopy plasmid. Taken together with the results presented in this report, the data suggest that there may be a site on rp51, between amino acids 1 and 63, accessible to a protease if the protein (or fusion) is not assembled into ribosomes.

The pHZ18-encoded fusion protein contains the same 63 amino acids as pZA64 but has 14 additional amino acids at its N terminus (30). This protein has the same β -galactosidase activity in strains containing or lacking *RP51A* (Table 1). In addition, the pHZ18-encoded fusion protein, although well targeted to the nucleus, is not associated with polysomes (data not shown). In contrast, indirect evidence suggests strongly that the *GAL-CYC1-RP51A* protein of HZint, containing the same abnormal N terminus as the pHZ18-encoded fusion protein but the entire rp51 protein and a normal rp51 C terminus, is assembled into polysomes. When grown in galactose, strains containing this gene rescue the growth defect of deletions of wild-type *RP51A* genes (L. Gritz, unpublished observations). Taken together, all of these data suggest that a normal rp51 N terminus (63 amino acids) or the entire rp51 protein (with an abnormal 14 amino acid addition on the N terminus) is sufficient for ribosome assembly in the absence of an adequate supply of wild-type rp51 protein. If both ends of the protein are aberrant (pHZ18), ribosome assembly does not take place.

When either the *RP51A* or the *RP51B* gene is deleted, cell growth rate and ribosome assembly are limited by the amount of rp51 (1, 2). Synthesis from one gene is not sufficient and cannot completely compensate for the absence of the other. However, a comparison of RNA levels and growth rates suggests that some posttranscriptional compensation takes place; cells with deletion of a single *RP51* gene grow somewhat better than expected, on the basis of the

FIG. 5. Immunoblot of various plasmid-containing strains with antisera to β -galactosidase. Immunoblot of cell extracts fractionated by gel electrophoresis was carried out as described in Materials and Methods. Lanes: 1, DBY745(YEp24); 2, DBY745(pZA64); 3, PB12(pZA64); 4, DBY745(pZA7); 5, PB12(pZA7). Lane 2 is faint in this exposure; in longer exposures, lane 2 displays a pattern of bands identical to that of lane 3, whereas lane 1 remains blank.

remaining rp51 mRNA (2). The results presented here offer an explanation for this observation and suggest that, under normal growth conditions, there is a small excess of rp51 protein synthesis under posttranscriptional regulation. In the absence of a single *RP51* gene, this excess compensates partially for the missing mRNA. Experiments to identify definitively the nature of the posttranscriptional regulation and to verify the role of ribosome assembly are currently in progress.

ACKNOWLEDGMENTS

We thank our colleagues in this laboratory for stimulating discussions, especially H. Colot, T. James, C. Pikielny, and B. Rymond for constructive comments, and T. Tishman for typing the manuscript.

L.G. was supported by a Damon Runyon-Walter Winchell Cancer Fund Fellowship, DRG-747. This work was supported by Public Health Service grant GM23549 from the National Institutes of Health.

LITERATURE CITED

1. Abovich, N., L. Gritz, L. Tung, and M. Rosbash. 1985. Effect of *RP51* gene dosage alterations on ribosome synthesis in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 5:3429-3435.
2. Abovich, N., and M. Rosbash. 1984. Two genes for ribosomal protein 51 of *Saccharomyces cerevisiae* complement and contribute to the ribosomes. *Mol. Cell. Biol.* 4:1871-1879.
3. Bollen, G. H. P. M., L. H. Cohen, W. H. Mager, A. W. Klaassen, and R. J. Planta. 1981. Isolation of cloned ribosomal protein genes from the yeast *Saccharomyces carlsbergensis*. *Gene* 14:279-287.
4. Bollen, G. H. P. M., C. M. T. Molenaar, L. H. Cohen, M. M. C. van Raamsdonk-Duin, W. H. Mager, and R. J. Planta. 1982. Ribosomal protein genes of yeast contain intervening sequences. *Gene* 18:29-37.
5. Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis. 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. *Gene* 8:17-24.
6. Bozzoni, I., E. Beccari, Z. X. Luo, and F. Amaldi. 1981. *Xenopus laevis* ribosomal protein genes: isolation of recombinant cDNA clones and study of the genomic organization. *Nucleic Acids Res.* 9:1069-1086.
7. Davies, J., and F. Jacob. 1968. Genetic mapping of the regulator and operator genes of the *lac* operon. *J. Mol. Biol.* 36:413-417.
8. D'Eustachio, P., O. Meyuhas, F. Ruddle, and R. P. Perry. 1981. Chromosomal distribution of ribosomal protein genes in the mouse. *Cell* 24:307-312.
9. Fallon, A. M., C. S. Jinks, G. D. Strycharz, and M. Nomura. 1979. Regulation of ribosomal protein synthesis in *Escherichia coli* by selective mRNA inactivation. *Proc. Natl. Acad. Sci. USA* 76:3411-3415.
10. Fried, H. M., N. J. Pearson, C. H. Kim, and J. R. Warner. 1981. The genes for fifteen ribosomal proteins of *Saccharomyces cerevisiae*. *J. Biol. Chem.* 256:10176-10183.
11. Gray, M. R., H. V. Colot, L. Guarente, and M. Rosbash. 1982. Open reading frame cloning: identification, cloning, and expression of open reading frame DNA. *Proc. Natl. Acad. Sci. USA* 79:6598-6602.
12. Gritz, L., and J. Davies. 1983. Plasmid-encoded hygromycin B resistance: the sequence of hygromycin B phosphotransferase gene and its expression in *Escherichia coli* and *Saccharomyces cerevisiae*. *Gene* 25:179-188.
13. Guarente, L., G. Lauer, T. M. Roberts, and M. Ptashne. 1980. Improved methods for maximizing expression of a cloned gene: a bacterium that synthesizes rabbit beta-globin. *Cell* 20:543-553.
14. Guarente, L., R. R. Yocum, and P. Gifford. 1982. A *GAL10-CYC1* hybrid yeast promoter identifies the *GAL4* regulatory region as an upstream site. *Proc. Natl. Acad. Sci. USA* 79:7410-7414.
15. Hereford, L. M., and M. Rosbash. 1977. Number and distribution of polyadenylated RNA sequences in yeast. *Cell* 10:453-462.
16. Johnsen, M., T. Christensen, P. P. Dennis, and N. P. Fiil. 1982. Autogenous control: ribosomal protein L10-L12 complex binds to the leader sequence of its mRNA. *EMBO J.* 1:999-1004.
17. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
18. Lindahl, L., R. Archer, and J. M. Zengel. 1983. Transcription of the S10 ribosomal protein operon is regulated by an attenuator in the leader. *Cell* 33:241-248.
19. Miller, J. H. 1972. Experiments in molecular genetics, p. 353-355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. Monk, R. J., O. Meyuhas, and R. P. Perry. 1981. Mammals have multiple genes for individual ribosomal proteins. *Cell* 24:301-306.
21. Mortimer, R. K., and D. Hawthorne. 1966. Yeast genetics. *Annu. Rev. Microbiol.* 20:151-168.
22. Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein. 1981. Yeast transformation: a model system for the study of recombination. *Proc. Natl. Acad. Sci. USA* 78:6354-6358.
23. Pearson, N. J., H. M. Fried, and J. R. Warner. 1982. Yeast use translational control to compensate for extra copies of a ribosomal protein gene. *Cell* 29:347-355.
24. Rosbash, M., P. K. W. Harris, J. L. Woolford, and J. L. Teem. 1981. The effect of temperature-sensitive RNA mutants on the transcription products from cloned ribosomal protein genes of yeast. *Cell* 24:679-686.
25. Rose, M., M. J. Casadaban, and D. Botstein. 1981. Yeast genes fused to beta-galactosidase in *Escherichia coli* can be expressed normally in yeast. *Proc. Natl. Acad. Sci. USA* 78:2460-2464.
26. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74:5463-5467.
27. Sherman, F., G. R. Fink, and J. B. Hicks. 1982. Methods in yeast genetics: a laboratory manual, p. 62. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. Silver, P. M., L. P. Keegan, and M. Ptashne. 1984. Amino terminus of the yeast *GAL4* gene product is sufficient for nuclear localization. *Proc. Natl. Acad. Sci. USA* 81:5951-5955.
29. Stahl, H. D., R. L. Coppel, G. V. Brown, R. Saint, K. Lingelbach, A. F. Cowman, R. F. Anders, and D. J. Kemp. 1984. Differential antibody screening of cloned *Plasmodium falciparum* sequences expressed in *Escherichia coli*: procedure for isolation of defined antigens and analysis of human antisera. *Proc. Natl. Acad. Sci. USA* 81:2456-2460.
30. Teem, J. L., and M. Rosbash. 1983. The expression of a beta-galactosidase gene containing the ribosomal protein 51 intron is sensitive to the *rna2* mutation of yeast. *Proc. Natl. Acad. Sci. USA* 80:4403-4407.
31. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76:4350-4354.
32. Woolford, J. L., L. M. Hereford, and M. Rosbash. 1979. Isolation of cloned DNA sequences containing ribosomal protein genes from *Saccharomyces cerevisiae*. *Cell* 18:1247-1259.
33. Yates, J. L., and M. Nomura. 1981. Feedback regulation of ribosomal protein synthesis in *E. coli*: localization of the mRNA target sites for repressor action of ribosomal protein L1. *Cell* 24:243-249.
34. Zabin, I. 1963. Proteins of the lactose system. Cold Spring Harbor Symp. Quant. Biol. 28:431-435.