Depletion of Yeast Ribosomal Proteins L16 or rp59 Disrupts Ribosome Assembly

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Abstract. Two strains of *Saccharomyces cerevisiae* were constructed that are conditional for synthesis of the 60S ribosomal subunit protein, L16, or the 40S ribosomal subunit protein, rp59. These strains were used to determine the effects of depriving cells of either of these ribosomal proteins on ribosome assembly and on the synthesis and stability of other ribosomal proteins and ribosomal RNAs. Termination of synthesis of either protein leads to diminished accumulation of the subunit into which it normally assembles. Depletion of L16 or rp59 has no effect on synthesis of most other ribosomal proteins or ribosomal RNAs. However, most ribosomal proteins and ribosomal RNAs that are components of the same subunit as L16 or rp59 are rapidly degraded upon depletion of L16 or rp59, presumably resulting from abortive assembly of the subunit. Depletion of L16 has no effect on the stability of most components of the 40S subunit. Conversely, termination of synthesis of rp59 has no effect on the stability of most 60S subunit components. The implications of these findings for control of ribosome assembly and the order of assembly of ribosomal proteins into the ribosome are discussed.

EUKARYOTIC ribosome biogenesis requires the coordinate assembly of 70–80 different ribosomal proteins (r-proteins)[1] with four species of ribosomal RNA (rRNA) to yield the mature 40 and 60S ribosomal subunits. r-proteins, rRNAs and the two ribosomal subunits normally accumulate in roughly equimolar amounts. The assembly process is complicated not only by the large number of molecules involved, but also by its compartmentalization: unlinked r-protein genes are transcribed in the nucleus, the mRNA is transported to the cytoplasm for translation, and newly synthesized r-proteins then enter the nucleus where they associate in the nucleolus with nascent rRNA transcripts. Mature ribosomal subunits are then transported from the nucleolus into the cytoplasm where assembly is completed before these subunits function in translation (reviewed in Hadjiolov, 1985; Warner, 1989).

Successful reconstitution of active *Escherichia coli* ribosomes led to the elucidation of an assembly pathway for each subunit (reviewed in Nomura and Held, 1974; Röhl and Nierhaus, 1982). A variety of biophysical and biochemical studies provide a low-to-moderate resolution model for the positions or functions of individual r-proteins and rRNAs within each ribosomal subunit of *E. coli* (reviewed in Wittmann, 1986; Stern et al., 1989). However, only limited data exist regarding the role of individual r-proteins in the biogenesis, structure, or function of eukaryotic ribosomes.

Studies of ribosome assembly in vivo and of the function of some r-proteins in this process have been performed in prokaryotes by depriving the cells of certain ribosomal components. When synthesis of rRNA is terminated in bacteria with rifampicin, many r-proteins are synthesized but degraded (Dennis, 1974). Various results have been obtained when cells are deprived of r-proteins: in some cases, mutants carrying a null or temperature-sensitive allele of an r-protein gene display a shortage of the subunit of which the protein is a part (Dabbs, 1986; Marvaldi et al., 1979; Nashimoto and Nomura, 1970). In other cases, the synthesis of both subunits is slowed or stopped (Nashimoto and Nomura, 1970; Pichon et al., 1979). Surprisingly, mutants lacking certain r-proteins exhibit neither aberration in subunit accumulation nor impairment of growth (Dabbs, 1986).

As in prokaryotes, inhibition of yeast or mammalian rRNA synthesis or processing results in degradation of newly synthesized r-proteins (Gorenstein and Warner, 1977; Warner, 1977; Tsurugi and Ogata, 1979; Yamagishi and Nomura, 1988). Terminating synthesis of two 60S subunit r-proteins, L3 and L29, in yeast (Nam and Fried, 1986) results in a lack of accumulation of many other r-proteins of the 60S subunit. Diminished accumulation of 25S rRNA, which is a component of the 60S subunit, was also observed. Components of the 40S subunit were not affected. Although these authors found that 60S subunit accumulation was diminished upon termination of L29 or L3 synthesis, in most cases they were

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1. *Abbreviations used in this paper*: r-protein, ribosomal protein; rRNA, ribosomal RNA.

unable to conclude whether this effect was mediated at the level of synthesis or degradation of r-proteins (Nam and Fried, 1986).

To begin to characterize the function in ribosome assembly of the *Saccharomyces cerevisiae* 40S ribosomal subunit protein, rp59, and the 60S ribosomal subunit protein, L16, we constructed strains that are conditional for synthesis of either protein. We describe the effect of terminating synthesis of these r-proteins on ribosome assembly. The work presented in this paper demonstrates that synthesis rates of most 40 or 60S subunit proteins and RNAs are not affected by terminating synthesis of an individual r-protein. However, most proteins and RNAs that are members of the same subunit as the protein whose synthesis is terminated are degraded rapidly in the absence of assembly into ribosomes. The stability of protein and RNA components of the opposite subunit is not affected.

Materials and Methods

Strains, Plasmids and Culture Conditions

Strains used in this study are described in Table I. Plasmids pBM126 and p*HIS3CEN3ARS2* were gifts of M. Johnston (Washington University Medical School) (Johnston and Davis, 1984) and B. Futcher (Cold Spring Harbor Laboratory) (Futcher and Carbon, 1986), respectively. Yeast strain JWY146 was derived from SC252 by replacing the wild-type *RPL16B* locus with the *rpl16b::LEU2* deletion-insertion allele (Rotenberg et al., 1988) using the one-step gene replacement method of Rothstein (1983). JWY1413 was generated from JWY146 by replacing the wild-type *RPL16A* locus with an *RPL16A* allele that is under the control of the *GAL1* promoter. This allele was constructed from plasmid pBM1-35 containing a *GAL1-RPL16A* promoter fusion (Tsay et al., 1988). A 1.1-kb Bam HI–Bgl II fragment from the 5′ nontranscribed region of wild type *RPL16A* was placed 5′ to the *GAL1* promoter in pBM1-35 by making the ends of the fragment blunt using the Klenow fragment of DNA polymerase I (a gift of Dr. William E. Brown, Carnegie Mellon University), ligating Eco RI linkers to the blunt ends and inserting the resulting fragment into the Eco RI site at the 3′ end of the *GAL10* promoter in pBM1-35. The resulting plasmid was used as a source of *RPL16A-5′*-nontranscribed-region-*GAL1-RPL16A* fragment for transformation of JWY146 to achieve a replacement of the wild-type *RPL16A* allele (Rothstein, 1983). The proper integration of this construct was confirmed by Southern hybridization analysis of genomic DNA and by galactose-dependent growth of the strain.

The conditional null allele of *CRY1* was constructed as follows. A 409-bp Hind III-Hinf I fragment from the 5′ nontranscribed region of *CRY1* was removed from plasmid pBR*CRY1H′*, which contains a 2.2-kb *CRY1* Hind III fragment (Larkin, 1985). The Hind III and Hinf I restriction sites are at positions 454 and 46 bp, respectively, upstream of the *CRY1* translation initiation codon. The Hinf I site was converted to a Hind III site by repair of sticky ends and linker ligation before the deletion-bearing plasmid was ligated back together. The Klenow fragment of DNA polymerase I was used to make the ends of the resulting 1.8-kb *CRY1* Hind III fragment blunt. Bam HI linkers were then ligated to the blunt ends. The resulting Bam HI fragment was inserted into the Bam HI site of the plasmid, pBM126, such that the *CRY1* gene was placed under the control of the *GAL1* promoter. Plasmid pBM126 contains *CEN4*, *ARS1*, *URA3*, and the divergent *GAL1-10* promoters. Plasmid shuffling was used to generate strain JWY1884, in which the only functional *CRY* gene is the *GAL1-CRY1* allele on pBM126. First, strain JWY1873, bearing genomic deletions of *CRY1* and *CYR2* and carrying plasmid p*HIS3CEN3ARS2-CRY1*, was transformed with pBM126-*CRY1*. Strain JWY1884 was derived from this transformant by screening for cells that lost plasmid p*HIS3CEN3ARS2-CRY1* after nonselective growth.

Table I. Strains Used in this Study

Strain	Genotype	Source
SC252	*MATα ade1 leu2-3,112 ura3-52*	J. Hopper*
JWY146	*MATα ade1 leu2-3,112 ura3-52 rp116b::LEU2*	Rotenberg et al., 1988
JWY1413	*MATα ade1 leu2-3,112 ura3-52 rp116b::LEU2 GAL1::RPL16A*	This study
JWY1873	*MATα his3Δ200 trp1Δ101 leu2Δ2 ura3-267 cry1::TRP1 cry2::LEU2 + pHIS3CEN3ARS2-CRY1*	This study
JWY1884	*MATα his3Δ200 trp1Δ101 leu2Δ2 ura3-167 cry1::TRP1 cry2::LEU2 + pBM126-CRY1*	This study

* Hershey Medical Center.

Yeast were grown at 30°C in YEPD (1% yeast extract, 2% peptone, 2% dextrose), YEP-Gal (1% yeast extract, 2% peptone, 2% galactose), or synthetic media (Sherman et al., 1986) containing either glucose or galactose. Various supplements were left out of synthetic media, depending on the experiment, in order to select for plasmids or to increase efficiency of radioactive labeling. Bacteria were grown in LB medium (0.5% NaCl, 0.5% yeast extract, 1% tryptone) supplemented with ampicillin (100 μg/ml). YEPD and LB were purchased from Gibco Laboratories (Grand Island, NY). Yeast extract, peptone and tryptone were from Difco Laboratories (Detroit, MI). All restriction enzymes, linkers, and T4 DNA ligase were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN).

Growth Curves and Viability Tests

Growth of JWY1413 with or without depletion of L16 was assayed as follows: cells grown in galactose medium to a concentration of $\sim 2 \times 10^7$ cells/ml were pelleted, washed and resuspended in sterile dH_2O. Halves of this cell suspension were used to inoculate galactose or glucose medium at a concentration of $\sim 10^7$ cells/ml (50 Klett units). Klett readings were taken and samples were removed from each culture every hour to test for cell viability.

Viability was tested by sonicating the cells to disrupt clumps, counting them on a hemacytometer, and spread-plating ~100 cells on galactose medium. Cells were also plated on glucose medium to insure that no revertants or contaminants arose in the cultures. Numbers of colonies that grew on plates spread with cells from the glucose- vs. galactose-containing cultures were compared with estimate viability after shifting to glucose.

Growth of JWY1884 with or without depletion of rp59 was assayed similarly, except that the OD_{610} of the cultures was monitored instead of Klett units.

RNA Hybridization Analysis

Gel electrophoresis of RNAs, blotting to Nytran (Schleicher & Schuell, Keene, NH) and hybridization with radioactive DNA probes were performed as described previously (Rotenberg et al., 1988).

Polyribosome Characterization

Polyribosomes were purified and fractionated as described in Rotenberg et al. (1988).

Pulse–Chase Experiments

Pulse-chase experiments were performed to measure rates of synthesis and degradation of r-proteins as described previously (Tsay et al., 1988) with the following exceptions. JWY1413 or JWY1884 cells were pulse-labeled with 200 μCi/ml Tran^{35}S-Label (1,185 Ci/mmol; ICN Biomedicals, Inc., Irvine, CA), for 45 s and chased with unlabeled methionine (2 mg/ml culture) for 1 h during growth in synthetic, galactose-containing media (lacking methionine for JWY1413, lacking methionine and uracil for JWY1884) and 30 min and 2 h after shifting from galactose to glucose. To provide an internal control for differential loss or extraction of r-proteins, cells from the pulse or the chase were mixed with an equal volume of cells that had been labeled continuously for two generations with 125 μCi/ml L-[*methyl*-^{3}H]methionine (80 Ci/mmol; NEN Research Products, Wilmington, DE). Total protein was extracted from the cells as described previously (Tsay et al., 1988) and separated by two-dimensional PAGE (Gorenstein and Warner, 1976). Spots corresponding to r-proteins were excised from the gels, dissolved in H_2O_2, and radioactivity was counted, correcting for spill-

over and using ^{35}S and ^{3}H quench curves to correct for quenching, in a scintillation counter (LS5801; Beckman Instruments, Palo Alto, CA). Rates of synthesis of each protein were determined using the following equation (Gorenstein and Warner, 1976):

$$A_i = \frac{^{35}S/^{3}H \text{ of } i^{th} \text{ r-protein}}{\text{average } ^{35}S/^{3}H \text{ of total r-proteins}}$$

Pulse-labeling experiments were performed twice for each strain and the results were averaged.

25 and 18S rRNAs were labeled as described previously (Nam and Fried, 1986; Kief and Warner, 1981). Cells grown in galactose or shifted from galactose to glucose for 30 and 120 min were pulse-labeled with 0.1 mCi/ml L-[*methyl*-^{3}H]methionine (50 Ci/mmol) for 5 min. Half of the pulse-labeled culture was mixed with an equal volume of cells that had been labeled for two generations with 0.5 μCi/ml [2-^{14}C]uracil (50 mCi/mmol; NEN Research Products, Wilmington, DE). Cells were collected onto a Metricel-membrane filter, (Gelman Sciences, Ann Arbor, MI), and were rapidly frozen by placing the filter on a block of dry ice. The remaining half of the pulse-labeled cells was chased for 15 min by incubation with excess unlabeled methionine (100 μg/ml culture). The chased cells were cofiltered with ^{14}C-labeled cells and frozen. The frozen filters were broken into pieces and total RNA was extracted from the cells adhering to the filters (Last et al., 1984). RNA species were separated by electrophoresis on 2.75% polyacrylamide gels (Udem and Warner, 1972; Nam and Fried, 1986). The gels were slices and counted using corrections for spillover and quenching.

Synthesis and turnover of 5.8 and 5S rRNAs were analyzed as for 18 and 25S rRNAs except that they were labeled with 25 μCi/ml [5,6-^{3}H]uridine (50 Ci/mmol; NEN Research Products), and chased with a 1,000-fold molar excess of unlabeled uridine. The long-term label was [2-^{14}C]uracil (80 Ci/mmol); the cells were labeled with 0.5 μCi/ml for two generations. RNAs were separated by electrophoresis on 8% polyacrylamide gels (Udem and Warner, 1972). Pulse-labeling experiments of rRNAs were performed at least twice and nearly identical results were obtained each time.

Results

Construction of Strains Conditional for Synthesis of Ribosomal Proteins L16 or RP59

To study the effect on ribosome assembly when cells are

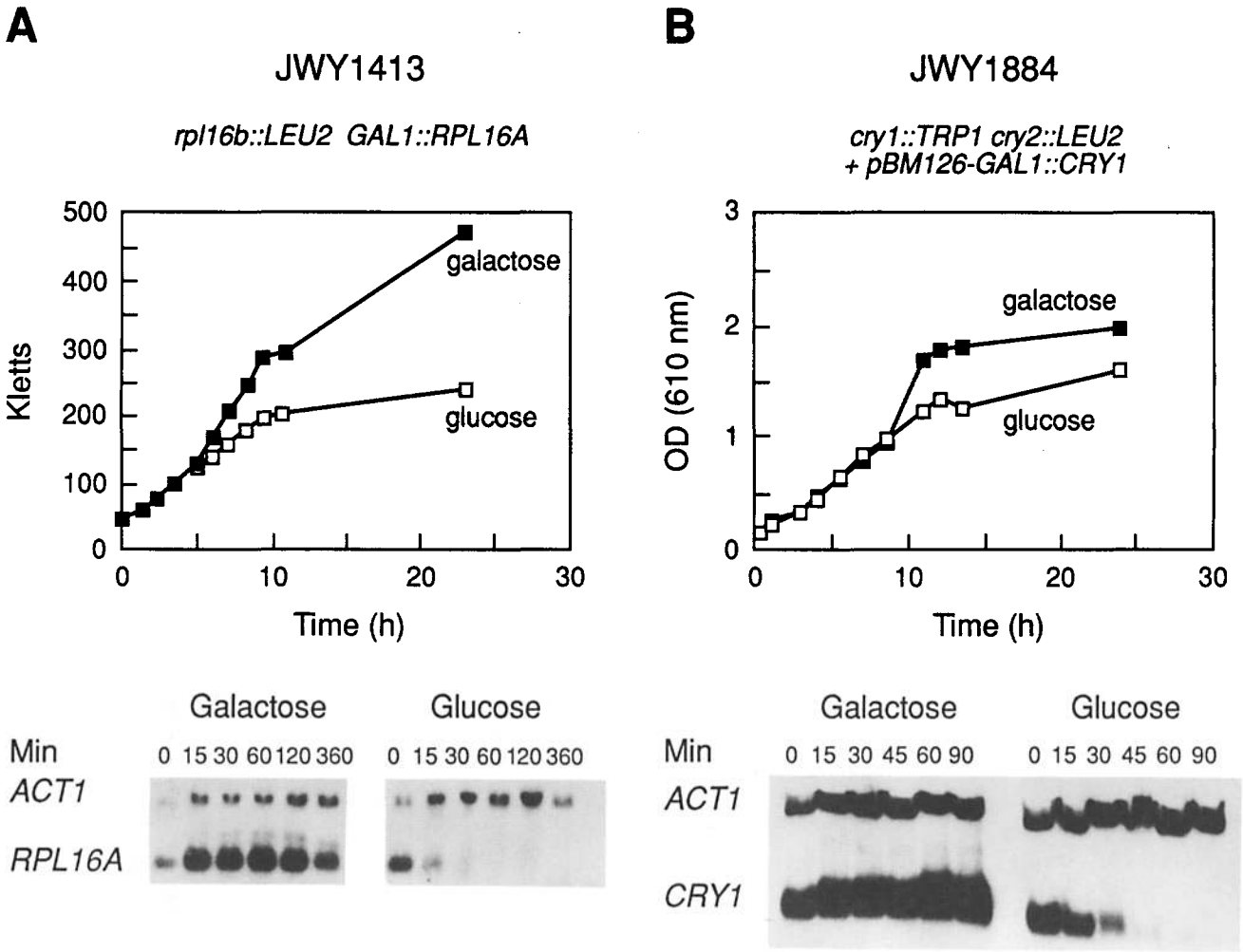

Figure 1. Growth of JWY1413 (*a*, *top*), and JWY1884 (*b*, *top*) continues in galactose, but terminates after two or three generations in glucose. Glucose was added at 0 h. (*a*, *bottom*) RNA hybridization analysis reveals that *RPL16A* mRNA is synthesized in JWY1413 grown in galactose. Synthesis of *RPL16A* mRNA is terminated upon shifting JWY1413 to glucose, and is not detectable after 30 min, although *ACT1* mRNA levels are normal. The faint band migrating between *ACT1* and *RPL16A* mRNA corresponds to mRNA encoded by *YPT1*, a gene that is present on the DNA fragment used as a probe that also contains *ACT1*. (*b*, *bottom*) RNA hybridization analysis shows that *CRY1* mRNA is synthesized when JWY1884 is grown in galactose. 45 min after shifting to glucose, *CRY1* mRNA is not detectable, although levels of *ACT1* mRNA are normal.

deprived of particular r-proteins we constructed haploid strains conditional for synthesis of the 60S subunit protein, L16, or the 40S subunit protein, rp59 (Fig. 1). Both of these r-proteins are essential for viability (Rotenberg et al., 1988; Paulovich, A. G., J. R. Thompson, J. C. Larkin, J. G. Anthony, and J. L. Woolford, Jr., manuscript in preparation). The only source of L16 in strain JWY1413 is an allele of *RPL16A* that has been placed under the control of the repressible *GAL1* promoter. Similarly, rp59 is only produced by a *GAL1-CRY1* allele in strain JWY1884. In galactose-containing media JWY1413 and JWY1884 double at approximately the same rate as their wild-type parent strains (data not shown). Therefore, functional L1b or rp59 are expressed by the respective *GAL1* promoter fusion constructs in galactose. When galactose is replaced by glucose, transcription of these *GAL1*-promoter fusions is repressed.

Levels of *RPL16A* or *CRY1* mRNA after shifting cells to glucose medium were examined by RNA hybridization analyses. *RPL16A* mRNA was no longer detectable in JWY1413 30 min after shifting to glucose (Fig. 1 *a*). Similarly, *CRY1* mRNA was greatly diminished in JWY1884 by 30 min post-shift and was undetectable by 90 min (Fig. 1 *b*). The diminution of *RPL16A* or *CRY1* mRNA resulted in decreased synthesis of L16 or rp59 proteins, which was confirmed by direct assay of r-protein synthesis (see below).

The growth of strains JWY1413 or JWY1884 was arrested about two or three cell doublings after shifting from galactose to glucose (Fig. 1). This phenotype was partially reversible. About 75% of the JWY1413 cells were viable up to 48 h after shifting to glucose if galactose was restored to them (data not shown). We did not test the viability of strain JWY1884 after shifting to glucose. A uniform terminal morphology, typical of cell division cycle mutants (Hartwell et al., 1973), was not observed in the arrested cultures; the cells appeared to be arrested at all stages of the cell cycle.

The ability of JWY1413 and JWY1884 cells to double approximately twice in glucose, and the prolonged viability of JWY1413 cells in this medium are similar to the observations of Li et al. (1990), who disturbed ribosome biogenesis by depleting cells of U14 small nucleolar RNA, which is necessary for production of 18S rRNA. After termination of U14 synthesis, cells were able to divide about twice, and they were viable for at least 12 h if returned to galactose. Like cells depleted of U14 (Li et al., 1990), cells whose synthesis of L16 or rp59 has been terminated will not form visible colonies on solid glucose-containing medium, although they will undergo a few divisions.

All subsequent experiments were performed when the cells were at the early to mid log phase of growth, and at least 2 h before the observed divergence of growth rate of glucose- vs. galactose-grown cultures.

L16 or RP59 Is Necessary for Accumulation of 60 or 40S Ribosomal Subunits, Respectively

The levels of 40 and 60S ribosomal subunits, 80S monosomes and polyribosomes from JWY1413 and JWY1884 were analyzed to determine whether ribosome assembly or function is perturbed upon depletion of L16 or rp59. Extracts of these cells were fractionated by sucrose velocity gradient centrifugation. A comparison of the gradient profile obtained from JWY1413 cells grown in galactose (Fig. 2 *a*) to the profiles for JWY1413 cells shifted to glucose for 30 min or 2 h (Fig. 2, *b* and *c*) shows that the cells accumulated fewer free 60S subunits after synthesis of L16 was terminated. A new peak that sedimented slightly farther in the gradient than 80S monosomes was evident. Such peaks are sometimes observed in association with each species of polyribosome as well (data not shown). These peaks represent "halfmer polyribosomes," which contain mRNAs associated with an integral number of ribosomes plus a 48S preinitiation complex. Presumably, the preinitiation complex is stalled on the mRNA due to a shortage of free 60S subunits with which to associate (Helser et al., 1981; Rotenberg et al., 1988).

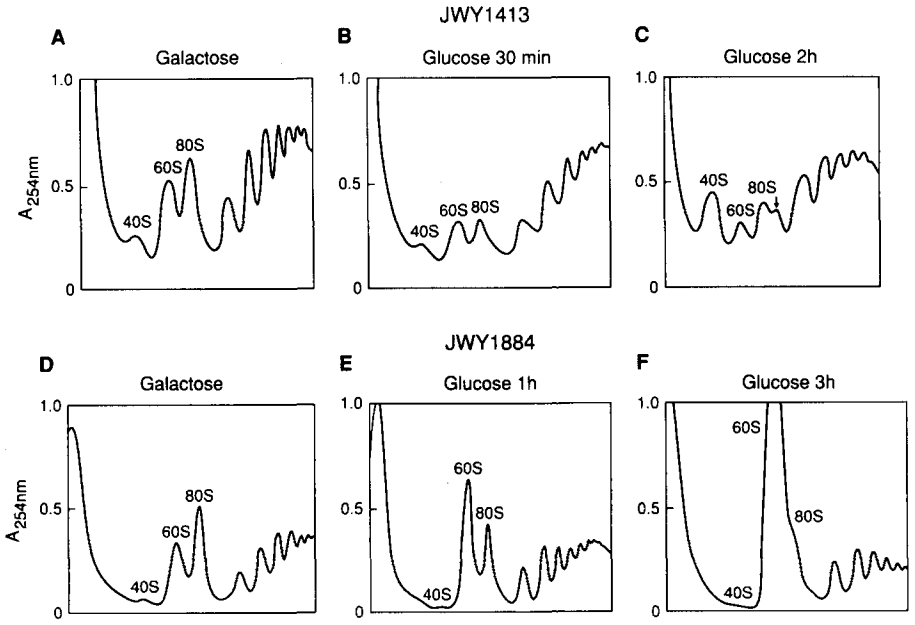

Figure 2. Polyribosome profiles of JWY1413 (*a–c*) and JWY1884 (*d–f*) grown in galactose (*a* and *d*) and shifted to glucose (*b*, *c*, *e*, and *f*). Polyribosomes were separated on 7–47% sucrose gradients. The bottom of each gradient is on the right. Peaks representing 40 and 60S subunits and 80S monosomes are labeled. The arrow in *c* represents "halfmer" polyribosomes.

Table II. Relative Synthesis Rates (A_i) of Ribosomal Proteins before and both 30 min and 2 h after, Terminating Synthesis of L16

r-protein	30-min galactose		30-min glucose		Stability index	2-h galactose		2-h glucose		Stability index
	Pulse	Chase	Pulse	Chase		Pulse	Chase	Pulse	Chase	
	A_i					A_i				
60S§										
L3*	0.77	1.07	0.64	0.62	43	1.55	0.75	0.49	0.35	−32
L2‡	0.67	0.89	0.63	0.74	13	0.40	0.90	0.59	0.65	104
L1 (YL3)	0.38	0.64	0.34	0.93	−38	0.95	0.81	0.61	1.35	−61
L4	1.24	1.20	1.24	1.45	−17	1.04	1.00	0.97	1.10	−15
L5‡*	1.24	0.78	1.43	0.59	52	1.14	0.73	1.48	0.39	143
rp10	1.09	1.11	1.05	1.39	−23	1.04	1.07	1.10	1.69	−33
L6	1.34	0.86	1.34	0.80	8	1.34	0.84	1.34	0.69	22
L10‡	0.97	1.57	0.91	1.17	26	1.04	1.77	1.03	0.99	77
L21‡	1.06	0.86	1.10	0.69	29	0.93	0.77	1.14	0.44	115
rp23‡	1.12	0.86	1.13	0.70	24	0.89	0.76	1.21	0.49	111
L8	0.93	0.96	0.75	1.14	−32	0.84	1.12	0.71	1.02	−7
rp27‡*	0.90	0.83	0.92	0.55	54	1.05	0.86	1.05	0.48	79
rp29‡	0.73	0.83	0.78	0.80	11	0.70	0.80	0.84	0.25	284
rp31/rp32	1.21	0.88	1.36	0.75	32	1.36	0.95	1.69	0.93	27
rp33‡*	1.03	0.92	1.04	0.61	52	0.93	0.96	1.04	0.23	367
rp38‡*	1.13	0.92	1.17	0.57	67	1.00	0.70	1.16	0.29	180
L16	0.81	0.76	0.11	0.35	−71	1.29	1.12	0.13	0.52	−78
L29*‡§	0.33	0.31	0.24	0.14	61	0.36	0.23	0.16	0.06	70
rp47‡*	1.22	0.99	1.29	0.69	52	1.13	0.98	1.24	0.56	92
rp62‡	1.21	0.72	1.15	0.53	29	1.11	0.77	1.16	0.33	144
rp64‡*	1.35	0.91	1.47	0.58	71	1.19	0.82	1.28	0.26	239
40S										
S7	0.93	1.22	0.95	1.42	−12	0.86	1.15	0.79	1.51	−30
S4	0.53	0.94	0.50	1.20	−26	0.57	0.99	0.46	1.20	−33
S3	0.83	0.90	0.87	1.10	−14	0.84	0.87	0.91	1.18	−20
S2	1.00	1.15	0.81	1.34	−30	1.00	1.23	1.04	1.51	−15
S12	0.69	1.15	0.83	1.43	−3	1.37	0.75	0.91	1.82	−73
S11	1.37	0.89	1.23	1.25	−36	1.15	0.88	1.11	1.56	−46
S13	1.14	1.10	1.22	1.13	4	1.03	1.00	1.06	1.04	−1
S18	1.21	1.70	1.29	2.00	−9	1.27	1.82	1.27	2.39	−24
S31	1.23	2.26	1.32	2.85	−15	1.27	2.42	0.99	3.13	−40
S24	1.06	1.05	1.19	1.33	−11	0.92	0.96	1.15	1.28	−6
rp51	0.86	0.86	0.78	1.28	−39	0.91	1.05	1.05	1.26	−4
S21	1.25	1.00	1.30	1.10	−5	1.17	0.82	1.28	1.14	−21
rp55	1.26	0.90	1.15	0.97	−15	1.10	0.87	1.00	0.95	−17
rp59	0.90	1.00	0.96	1.22	−13	0.98	1.22	1.08	1.42	−5
S27‡	1.17	0.87	1.13	0.63	33	1.33	0.78	1.23	0.45	60
rp63	0.54	0.33	0.60	0.46	−20	0.47	0.36	0.60	0.49	−6
Unknown										
rp46	1.25	3.14	1.33	2.39	40	1.72	2.75	1.27	2.91	−30
rp53	1.11	1.00	1.13	1.00	2	1.26	1.03	1.37	1.04	8
rp56‡	0.23	0.30	0.31	0.33	23	0.26	0.39	0.36	0.34	59
rp58‡	0.84	0.67	0.77	0.47	31	0.82	0.77	0.96	0.30	200
rp60	1.10	1.10	1.12	1.38	−19	1.06	1.14	1.05	1.50	−25

Relative rates of synthesis (A_i) of r-proteins in strain JWY1413 grown in galactose or shifted to glucose were determined as described in Materials and Methods. The stability index is defined in the text. Proteins were considered to be rapidly degraded if their Stability Index was ≥50%.
* Proteins rapidly degraded after 30 min in glucose.
‡ Proteins rapidly degraded after 2 h in glucose.
§ Proteins showing decreased synthesis rates.

These molecular phenotypes suggest that termination of L16 synthesis leads to a shortage of 60S subunits, but not 40S subunits. We observed these phenotypes previously in studies of mutants containing null alleles of either *RPL16A* or *RPL16B* (Rotenberg et al., 1988).

Termination of synthesis of the 40S subunit protein, rp59, led to the accumulation of fewer free 40S subunits and a large excess of free 60S subunits (Fig. 2, *d–f*). Note that the excess of 60S subunits in JWY1884 is much greater than the excess of free 40S subunits in JWY1413. We assume this difference is due to the presence of some excess 40S subunits in JWY1413 in halfmers. A shortage of 40S subunits should result in fewer translation initiation events, thereby shifting the distribution of polyribosomes to those associated with few-

er ribosomes. Indeed, the average polyribosome size was smaller in cells depleted of rp59 (Fig. 2, *d–f*). These phenotypes show that rp59 is necessary for accumulation of 40S ribosomal subunits, but not 60S subunits.

Termination of Synthesis of L16 Results in Degradation of 60S Ribosomal Subunit Proteins and RNAs, but Has No Effect on 40S Subunit Constituents

Since L16 is necessary for accumulation of 60S subunits, we determined whether the accumulation of fewer 60S subunits upon termination of synthesis of L16 is due to inhibition of synthesis of protein or RNA constituents of the 60S subunit, or results from aborted subunit assembly and subsequent degradation of the proteins and RNAs. The relative rates of synthesis and degradation of 42 different r-proteins in cells synthesizing L16 were compared with rates in cells in which L16 synthesis had been terminated. This was achieved by performing pulse–chase experiments after continuous growth of JWY1413 in galactose and after shifting to glucose for 30 min and for 2 h.

Table III. Relative Synthesis Rates (A_i) of Ribosomal Proteins before and both 30 min and 2 h after, Terminating Synthesis of rp59

r-protein	30-min galactose		30-min glucose		Stability index	2-h galactose		2-h glucose		Stability index
	Pulse	Chase	Pulse	Chase		Pulse	Chase	Pulse	Chase	
	A_i					A_i				
60S										
L3	0.56	0.78	0.65	0.94	−4	0.82	0.77	0.75	1.35	−48
L2	0.66	0.52	0.88	1.13	−39	0.66	0.99	0.82	1.34	−8
L1 (YL3)	0.54	0.90	0.63	1.19	−12	0.71	1.16	0.56	1.67	−45
L4	0.99	1.13	1.19	1.32	3	1.13	1.00	1.15	1.53	−33
L5	1.11	0.92	1.25	1.00	4	1.07	0.80	1.30	1.20	−19
rp10*‡	0.96	1.12	1.18	0.69	100	1.03	1.07	1.06	0.10	1001
L6	1.20	0.93	1.23	1.10	−13	1.16	0.79	1.40	1.13	−16
L10	0.89	1.25	1.06	1.56	−5	1.12	1.53	1.30	2.05	−13
L21	1.23	0.81	1.19	1.08	−27	1.07	0.88	1.08	1.25	−29
rp23	0.91	0.88	1.15	0.93	20	1.00	0.77	1.18	1.24	−27
L8	0.80	1.06	0.88	1.25	−7	0.89	0.91	0.87	1.32	−33
rp27‡	0.99	0.94	0.90	0.96	−11	0.33	0.90	1.11	1.17	159
rp29	1.43	1.12	0.95	1.17	−36	0.90	1.07	0.78	1.39	−33
rp31/rp32	1.04	0.64	1.12	0.91	−24	1.19	0.92	1.18	1.08	−16
rp33	0.90	1.06	1.06	1.05	19	0.96	0.93	1.10	1.28	−17
rp38	0.90	0.91	1.06	0.97	10	1.00	0.83	1.11	1.13	−18
L16	0.90	1.12	0.98	1.23	−1	1.03	0.96	1.14	1.39	−24
L29*	0.28	0.48	0.60	0.50	106	0.46	0.47	0.65	0.45	48
rp47	1.30	1.15	1.17	1.11	−7	1.24	1.05	1.39	1.59	−26
rp62	0.86	0.82	0.96	0.69	33	1.07	0.72	1.13	0.95	−20
rp64	1.19	0.90	1.36	1.18	−13	1.30	0.89	1.33	0.32	−31
40S										
S7*‡	0.69	1.16	0.83	0.88	59	0.76	0.97	0.94	0.48	150
S10‡	1.06	0.29	1.01	0.21	32	0.78	0.77	0.75	0.09	723
S4§‡	0.50	0.89	0.46	0.58	41	0.49	0.75	0.24	0.18	104
S3§	0.74	0.85	0.80	0.72	28	0.88	0.82	0.68	0.43	47
S2*‡	1.14	1.14	1.17	0.70	67	1.05	1.07	1.18	0.27	345
S12	0.65	0.67	0.78	1.29	−38	1.04	0.95	0.87	1.29	−38
S11	1.76	0.92	1.21	0.92	−31	1.54	0.89	1.24	0.83	−14
S13	1.02	1.25	1.16	1.20	18	1.18	1.06	0.97	1.08	−19
S18*‡	0.67	1.85	1.20	1.00	231	1.31	1.59	1.21	0.28	425
S31*	0.32	1.22	1.35	1.78	189	1.38	2.04	0.95	1.27	11
S24*‡	0.92	1.03	1.04	0.69	69	1.06	1.01	0.90	0.30	186
rp51*‡	0.96	1.12	0.99	0.63	83	1.13	1.07	1.10	0.17	513
S21*‡	1.09	0.97	1.28	0.66	73	1.14	0.79	1.26	0.13	572
rp55‡	1.17	0.63	1.18	0.60	6	1.08	0.75	1.18	0.16	412
rp59	2.33	1.09	0.51	0.62	−62	2.60	1.03	0.16	0.39	−84
S27	1.10	0.96	1.12	0.91	7	0.97	0.90	1.24	0.99	16
rp63‡	0.64	0.47	0.66	0.34	43	0.60	0.38	0.61	0.23	68
Unknown										
rp46	5.00	3.88	1.22	3.88	−76	1.90	3.83	1.16	4.86	−52
rp53	1.13	1.14	1.14	0.82	40	1.27	1.09	1.21	0.75	38
rp56	0.21	0.28	0.28	0.34	10	0.31	0.30	0.24	0.37	−37
rp58	0.71	0.73	0.84	0.74	17	0.80	0.51	0.89	0.87	−35
rp60	0.92	1.13	0.83	1.14	−11	1.16	1.03	0.84	1.05	−29

Relative rates of synthesis (A_i) of r-proteins in strain JWY1884 grown in galactose or shifted to glucose were determined as described in Materials and Methods.
* Proteins rapidly degraded after 30 min in glucose.
‡ Proteins rapidly degraded after 2 h in glucose.
§ Proteins showing decreased synthesis rates.

The relative synthesis rates of most r-proteins (A_is) measured by pulse-labeling JWY1413 cells grown continuously in galactose did not differ significantly from the A_is of cells shifted to glucose for 30 min or 2 h (Table II). Two exceptions were L3 and L29, whose synthesis rates appeared to decrease after the 2-h shift to glucose. Several other proteins (e.g., L8, L1, S12) also showed a decrease in pulse A_i after the shift to glucose, but we do not consider this biologically significant since these proteins' chase A_is are high, indicating that ultimately, relatively normal levels of these proteins accumulated. These exceptional proteins do not detract from the conclusion that the synthesis of most (34 out of 42) r-proteins was not affected by depleting L16.

Turnover of r-proteins was assessed by comparing A_i values after pulse-labeling cells to A_is after a pulse followed by a 1-h chase. A comparison of the change from pulse A_is to chase A_is for cultures maintained continuously in galactose versus cultures shifted from galactose to glucose gives information about the stability of each protein. Thus, a protein that becomes unstable after shifting to glucose would exhibit a larger difference between pulse A_i and chase A_i in glucose than it would in galactose. To quantitate these differences, we used the following formula to derive a "stability index" (Tables II and III):

$$\frac{\dfrac{\text{Glucose pulse } A_i}{\text{Glucose chase } A_i} - \dfrac{\text{Galactose pulse } A_i}{\text{Galactose chase } A_i}}{\dfrac{\text{Galactose pulse } A_i}{\text{Galactose chase } A_i}} \times 100$$

We define a stability index value of 50 or greater to indicate significant degradation of a protein after the cells were shifted to glucose.

The stability indices show that 7 of the 21 60S subunit r-proteins assayed were rapidly degraded after 30 min in glucose. After 2 h in glucose, 13 of the 21 60S r-proteins assayed were rapidly degraded. In contrast, all but one of the 16 40S r-proteins tested were as stable in glucose-shifted cells as they were in galactose-grown cells. These data are summarized in histograms in Fig. 3, where the number of r-proteins that fall within a certain range of stability indices are plotted versus that range. It is clear that after 30 min or 2 h in glucose, r-proteins of the 40S subunit were similarly stable, i.e., their stability indices cluster around zero (Fig. 3, *top*). On the other hand, r-proteins of the 60S subunit were mostly unstable; their stability indices are significantly greater than those of their 40S counterparts (Fig. 3, *bottom*). These differences became more exaggerated after the cells were shifted to glucose for 2 h (Fig. 3, compare *bottom*, *left* and *right*).

Some r-proteins from the 60S subunit are degraded earlier or to a greater extent than others (compare rp29 and rp64, Table II). There are still other 60S r-proteins whose rates of synthesis and degradation are not affected at all (e.g. rp10, L1, Table II, Fig. 3). Perhaps these differences in stability reflect the assembly pathway of the proteins into the ribosome. We return to this possibility in the Discussion.

The synthesis and stability of rRNAs upon depletion of L16 was also assayed. 60S subunits contain 25, 5.8, and 5S rRNA species, whereas only one species of rRNA, the 18S, is found in 40S subunits. The 25S, 5.8, and 18S rRNAs are derived from a 35S rRNA precursor that is processed in sev-

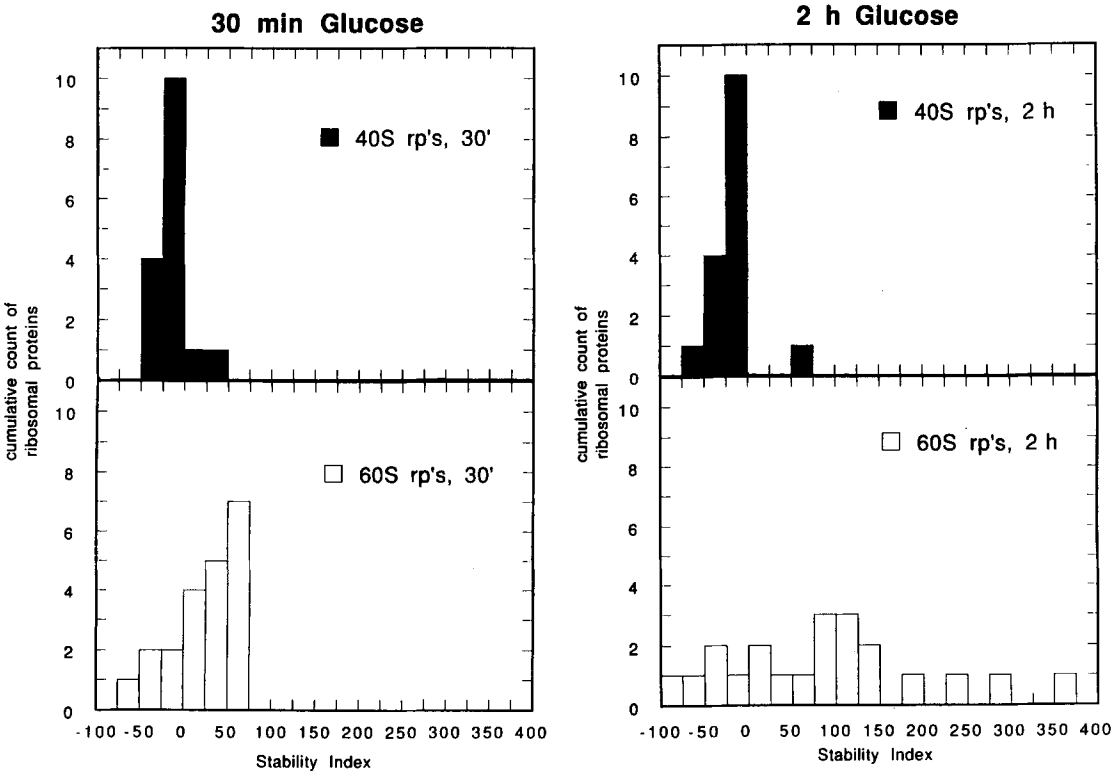

Figure 3. Most 60S subunit r-proteins become increasingly unstable with time (30 min and 2 h) after termination of L16 synthesis. Most 40S subunit r-proteins remain stable. The number of r-proteins (*rp's*) falling with each range of stability indices is plotted versus that range of indices. The stability index of each protein is shown in Table II. A stability index of ≥50% signifies an unstable protein (see text).

eral steps to generate the mature rRNA species (Udem and Warner, 1972). Processing intermediates of concern here include the 27S rRNA, which is a precursor to the 25S rRNA, and the 20S rRNA, which is a precursor to the 18S rRNA. The 5S rRNA is synthesized as a separate transcript (Udem and Warner, 1972).

To examine synthesis and degradation of 25 and 18S rRNAs, cells grown continuously in galactose or shifted to glucose for 30 min or 2 h were pulse-labeled for 5 min and chased for 15 min. A comparison of radioactivity profiles from the pulse-labeled samples resolved by gel electrophoresis demonstrates that depletion of L16 resulted in no significant change in amounts of 27S rRNA precursor or of 20S rRNA precursor (Fig. 4). The fact that the amounts of 27S and 20S rRNA precursors were approximately the same in glucose-shifted cells as they were in galactose-grown cells suggests that rRNA synthesis is not altered upon depletion of L16. In other experiments we observed that levels of 35S rRNA detected by pulse-labeling were approximately the same in galactose-grown or glucose-shifted cultures (data not shown). However, it is apparent from the chase experiments that accumulation of mature 25S rRNA was diminished after 30 min in glucose and greatly decreased by 2 h. In contrast, 18S rRNA levels were unaffected (Fig. 4).

The effect of terminating synthesis of L16 on the synthesis and turnover of 5.8 and 5S rRNA levels was determined in a separate labeling experiment. Although 5.8S rRNA was not detectable after a 5-min pulse-labeling of galactose- or glucose-grown cells, it accumulated in the galactose-grown cells by the end of the chase (Fig. 5). The inability to detect 5.8S rRNA after a 5-min pulse is consistent with the previous observation that processing of pre-rRNA to form 5.8S rRNA takes >5 min (Udem et al., 1971). In contrast, no 5.8S rRNA accumulated after the 15-min chase in glucose-shifted cells, even 30 min after repression of *GAL1-RPL16A*.

A comparison of the ratio of short-term to long-term label in the 5S rRNA peaks (Fig. 5, *a* and *c*, *pulse* and *chase*) shows that the synthesis and turnover of 5S rRNA was not affected to the same degree as 5.8S upon depletion of L16. Since 5.8 and 5S rRNAs are both components of the 60S subunit, one might expect that they would be equally unstable when there is a shortage of L16. However, newly synthesized 5S rRNA was more stable than the 5.8S rRNA.

We conclude that depletion of the 60S subunit r-protein, L16, does not affect synthesis of either 60S or 40S subunit components. However, most newly synthesized r-proteins, and 25S and 5.8S rRNAs, which are constituents of the 60S subunit, are degraded, whereas nearly all of the components of the 40S subunit are unaffected.

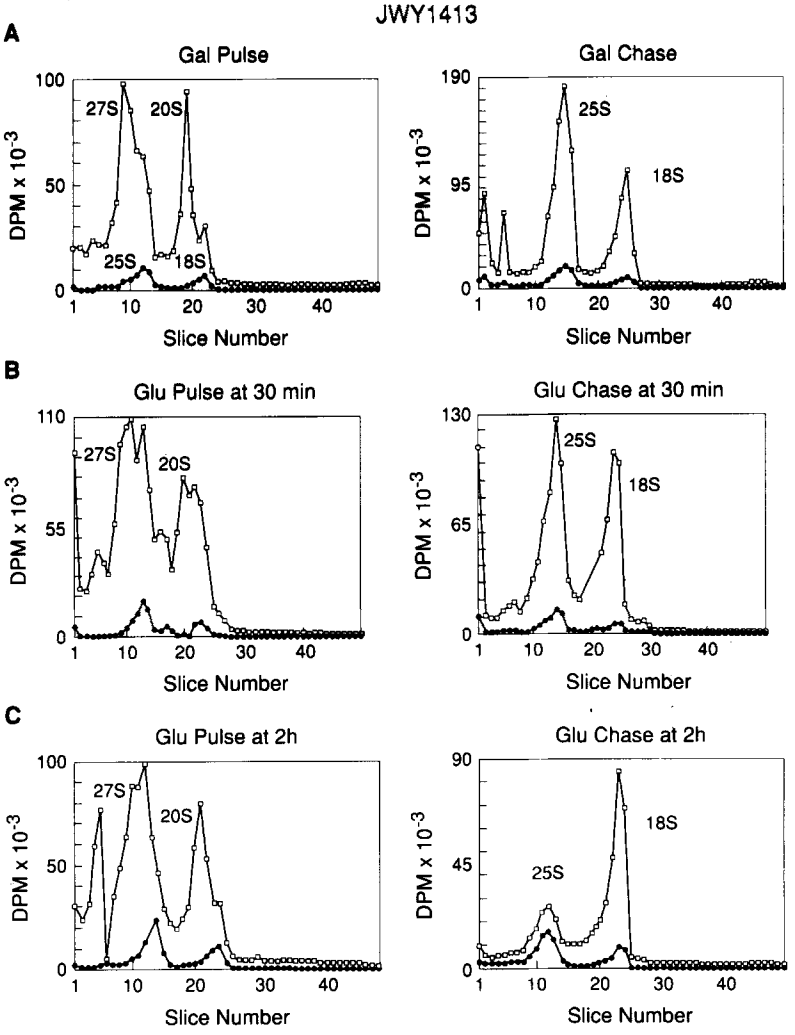

Figure 4. Accumulation of 25 and 18S rRNAs in JWY1413 grown in galactose or shifted to glucose for 30 min (*b*) and 2 h (*c*). RNA was pulse-labeled (*left*, *a–c*), the label was chased (*right*, *a–c*), and rRNA species were separated by gel electrophoresis. Peaks corresponding to 27 and 20S rRNA precursors (*left*, *a–c*) and mature 25 and 18S rRNAs are labeled (*left*, *a*; *right*, *a–c*). (□) ^{3}H disintegrations per minute (short-term label); (●) ^{14}C disintegrations per minute (long-term label).

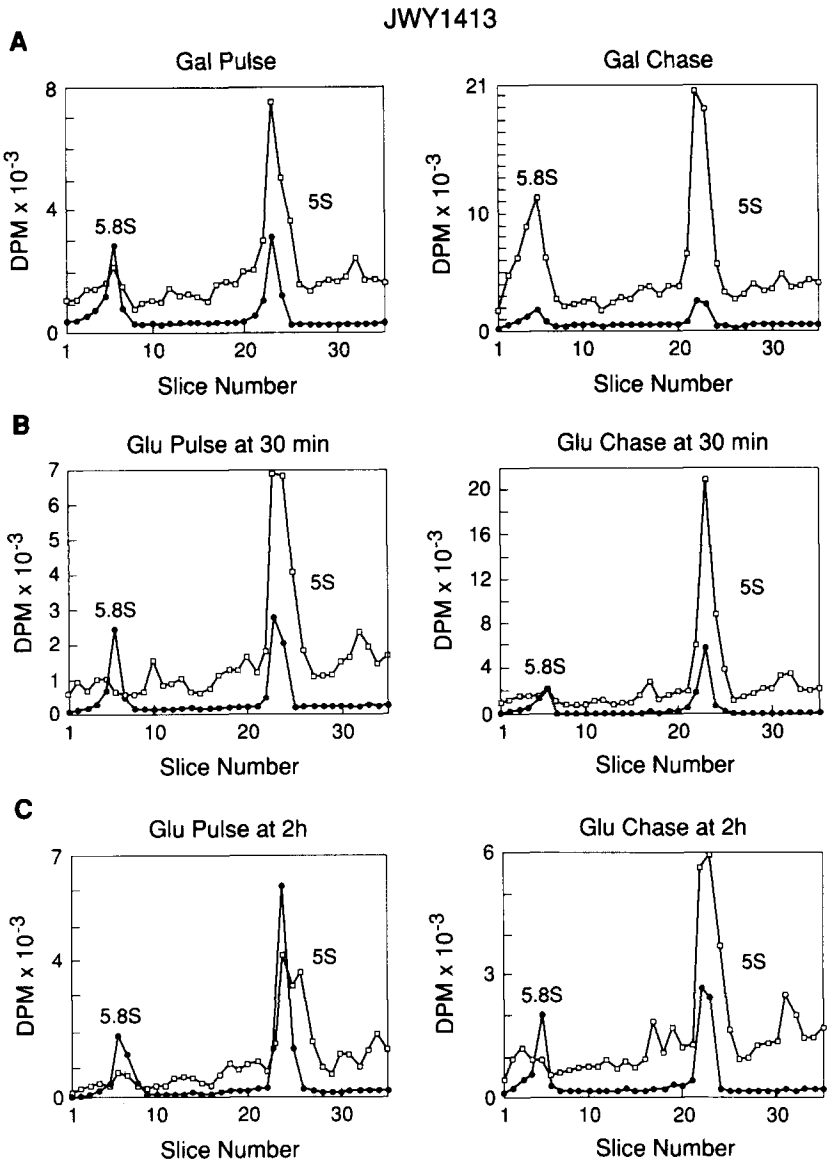

Figure 5. Accumulation of 5.8 and 5S rRNAs in JWY1413 grown in galactose (*a*) or shifted to glucose for 30 min (*b*) and 2 h (*c*). RNA was pulse-labeled (*left*, *a–c*), the label was chased (*right*, *a–c*), and RNA species were separated by gel electrophoresis. Peaks representing 5.8 and 5S rRNAs are labeled. (□) ^{3}H disintegrations per minute (short-term label); (●) ^{14}C disintegrations per minute (long-term label).

Termination of Synthesis of RP59 Results in Degradation of 40S Ribosomal Subunit Proteins and RNAs, But Has No Effect on 60S Subunit Constituents

We also assayed the effect of terminating synthesis of the 40S subunit r-protein, rp59, on the synthesis and stability of other r-proteins and rRNAs. Pulse-chase experiments to follow synthesis and degradation of r-proteins and rRNAs were performed on the *GAL-CRY1* stain, JWY1884, essentially as described above for the *GAL-RPL16A* strain. In this case, we found that components of the 40S subunit were synthesized at normal rates relative to control cells, but many were rapidly degraded upon depletion of rp59, whereas 60*S* subunit constituents were largely unaffected.

As was the case for the *GAL-RPL16A* strain, synthesis rates of most r-proteins were not affected when JWY1884 was shifted to glucose for 30 min or 2 h (Table III). Two exceptions appear to be S3 and S4. Again, there are a few other proteins (e.g., S11, S13) whose rates of synthesis appear to decrease, but are not considered to be biologically significant since the chase A_is in these cases indicate that significant amounts of these proteins accumulated.

Although, for the most part, synthesis of r-proteins did not appear to be affected by depletion of rp59, the stability indices (Table III, Fig. 6) show that 7 out of 17 40S subunit proteins tested were more rapidly degraded (stability index >50) after 30 min in glucose compared to galactose-grown controls. After 2 h, 10 out of 17 40*S* subunit proteins assayed were more rapidly degraded than in the controls. Neither synthesis nor degradation of 18 out of 21 60S subunit proteins tested were changed significantly by depletion of rp59 (Table III, Fig. 6).

The 40S subunit proteins that were affected by depletion of rp59 varied in stability, as was observed for 60S subunit proteins upon depletion of L16. There are also 40S subunit proteins that remained stable even after JWY1884 had been in glucose for 2 h (e.g., S12, S13, Table III, Fig. 6).

Synthesis and stability of rRNAs were also assayed in the

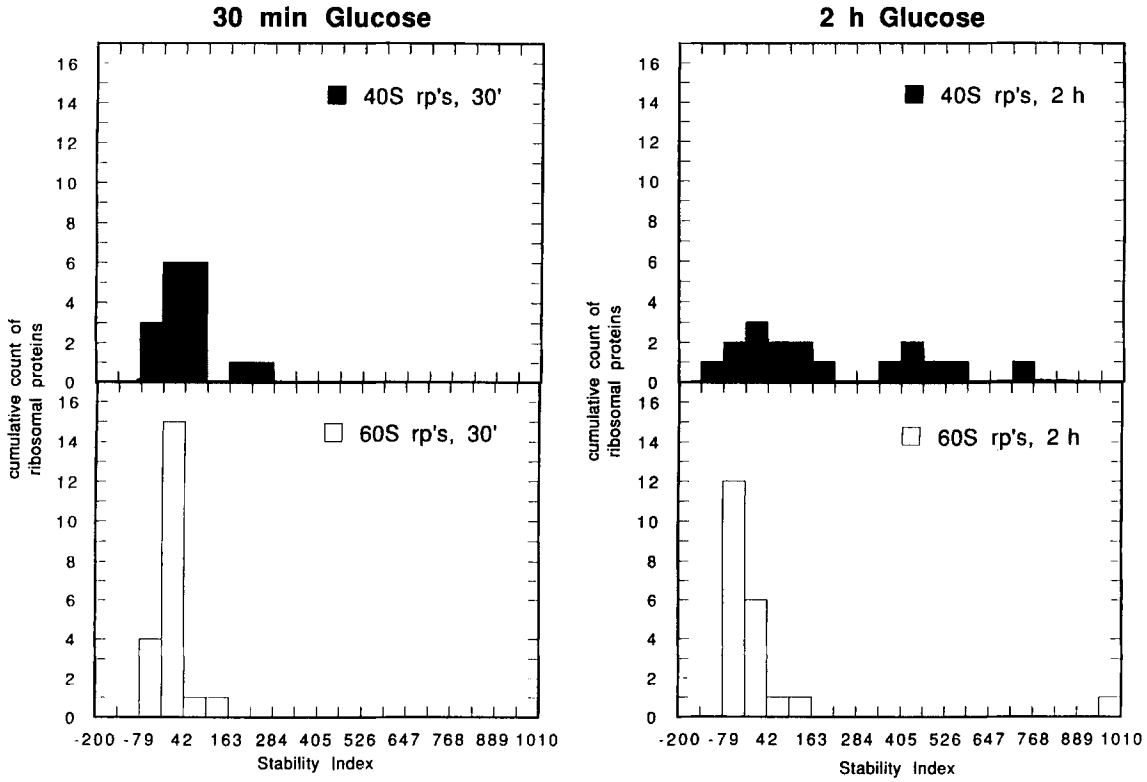

Figure 6. Most 40S subunit r-proteins become increasingly unstable with time (30 min and 2 h) after termination of rp59 synthesis. Most 60S subunit r-proteins remain stable. The number of r-proteins (*rp's*) falling within each range of stability indices is plotted versus that range of indices. The stability index of each protein (defined in the text) is shown in Table II.

GAL-CRY1 strain. 25 and 18S rRNA species were synthesized and accumulated normally after 30 min in glucose (Fig. 7). However, after 2 h less 18S rRNA accumulated than in the galactose-grown control or in the cells shifted to glucose for 30 min. 25S rRNA accumulated to normal levels after 2 h in glucose.

The 5.8 and 5S rRNAs were analyzed in a separate pulse-chase experiment in the same manner as described for the *GAL-RPL16A* strain. Depletion of rp59 had no effect on the levels of these small rRNAs even after the cells had been shifted to glucose medium for 2 h (Fig. 8).

Thus, when rp59 was no longer synthesized most other components of the small subunit were synthesized but rapidly degraded. Depletion of rp59 did not affect synthesis or stability of most 60S subunit proteins or rRNAs. Therefore, the experiments with both the *GAL-RPL16A* and the *GAL-CRY1* strains show that the accumulation of the two ribosomal subunits is uncoupled at the posttranscriptional level.

Discussion

Depletion of Ribosomal Proteins L16 or RP59 Does Not Affect Synthesis of Most Other Ribosomal Constituents

We tested how yeast cells respond to termination of synthesis of the 60S subunit protein, L16, or the 40S subunit protein, rp59, using strains that are conditional for synthesis of these r-proteins. Pulse-chase experiments were performed to determine rates of synthesis and degradation of r-proteins and rRNAs 30 min and 2 h after terminating synthesis of L16 or rp59. Depletion of either L16 or rp59 did not affect synthesis of most r-proteins (except L3, L29, S3, and S4). Ribosomal RNA synthesis was not affected by depletion of L16. Since 5.8S rRNA, which is derived from the 35S rRNA precursor, accumulated to normal levels in JWY1884 (Fig. 8), it does not seem likely that rRNA synthesis was affected by the depletion of rp59 either.

Our conclusions differ in some ways from those of Nam and Fried (1986) who assayed the effect of terminating synthesis of two 60S subunit proteins, L3 and L29, on the synthesis of other r-proteins and 25S and 18S rRNAs. They concluded that depletion of L3 and L29 resulted in a decrease in apparent synthesis rates for most 60S r-proteins. However, they used relatively long pulse-labeling periods (3 min vs. our 45 s). We suggest that this is the reason for our differing conclusions. A_i values from a 3-min pulse probably reflect both synthesis and degradation for many proteins. Experiments subsequent to those of Nam and Fried showed that r-proteins that could not assemble into ribosomes because they were produced in excess of other ribosome constituents, were degraded extremely rapidly (El-Baradi et al., 1986; Maicas et al., 1988; Tsay et al., 1988).

Our conclusion that most r-proteins are synthesized and then rapidly degraded in the absence of their ability to assemble into ribosomes agrees with the work of Wittekind et al. (1990). In this study, cells were depleted of the large subunit of RNA polymerase I, which results in termination

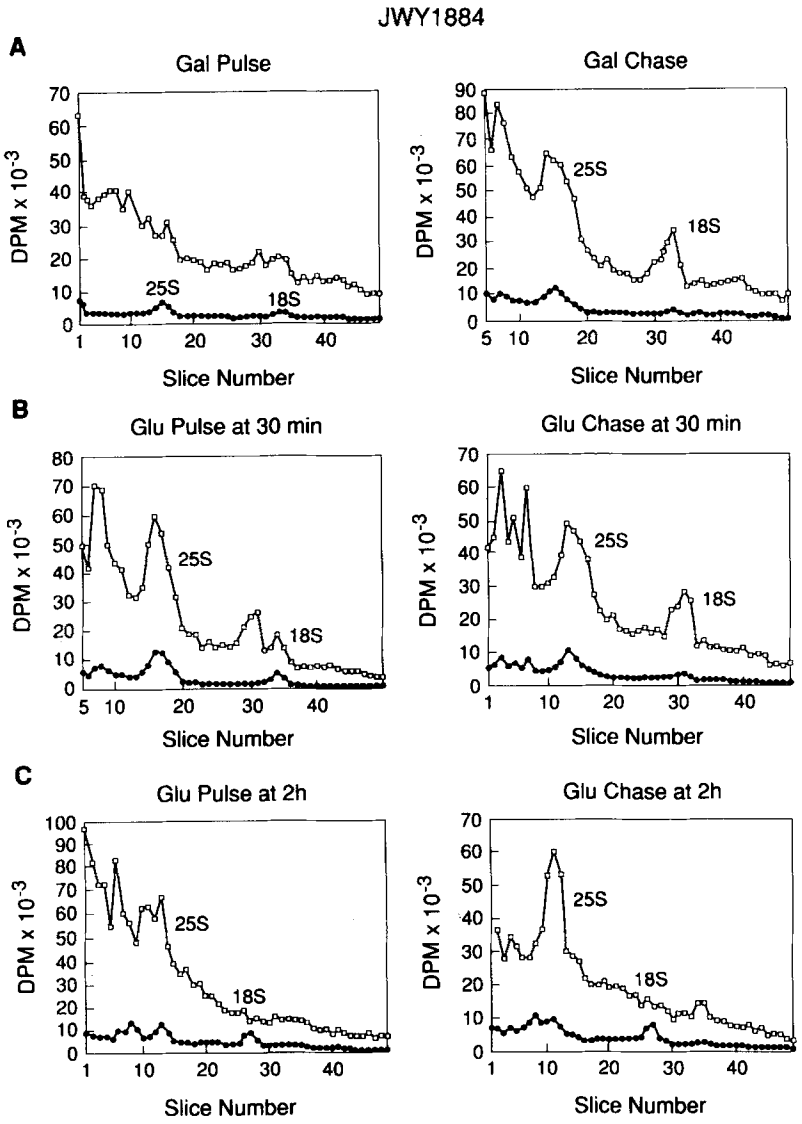

Figure 7. Accumulation of 25 and 18S rRNAs in JWY1884 grown in galactose (*a*) or shifted to glucose for 30 min (*b*) and 2 h (*c*). RNA was labeled and analyzed as described in Materials and Methods. Peaks representing 25 and 18S rRNAs are labeled. (□) ^{3}H disintegrations per minute (short-term label); (●) ^{14}C disintegrations per minute (long-term label).

of rRNA synthesis. Wittekind et al. (1990) showed that most r-proteins were made and rapidly degraded in the absence of rRNA synthesis. Termination of 35S rRNA synthesis affected the stability of r-proteins from both the 40 and 60S subunits.

Our results (Tables II and III) suggest either that the synthesis of r-proteins L3, L29, S3, and S4 was diminished upon depletion of L16 (for L3 and L29) or rp59 (for S3 and S4), or that these proteins were turned over within the 45 s required for the pulse-labeling. Nam and Fried (1986) found that the level of L3 mRNA dropped by 50% upon depletion of L29, and vice versa. Wittekind et al. (1990) also observed that pulse A_i values were low for L3 and L29, but found that L3 and L29 mRNA levels did not decrease and the mRNAs did not shift to smaller polyribosomes in the absence of rRNA. Wittekind et al. (1990) concluded that these proteins were degraded extremely rapidly, within the 30-s period of pulse-labeling. The reason for this discrepancy between the results of Nam and Fried (1986) and Wittekind et al. (1990) is unclear.

Failure of Subunits to Assemble and Turnover of Ribosome Constituents

Although we found no evidence for alterations of synthesis rates of most r-proteins or rRNAs upon depletion of L16 or rp59, most proteins and rRNAs in the same subunit as the depleted protein were degraded. There are a few exceptions to the observation that proteins of the opposite subunit were stable. In the L16-depletion experiment, one 40S subunit r-protein became unstable by the 2-h time point (S27, Table II). Upon depletion of rp59 there were three 60S subunit proteins that became unstable (rpl0, rp27, and L29, Table III; note that rp27 and S27 are different proteins). Perhaps rpl0 and S27 have been misassigned to the 60S and 40S subunits, respectively, since rpl0 was stable when L16 was depleted and S27 was stable upon depletion of rp59. The apparent degradation of rp27 in the rp59-depletion experiment may be artifactual because its pulse A_i in galactose was only 0.33 at the 2-h time point (Table III), whereas its pulse A_i was about one in all other experiments (Tables II and III). It is

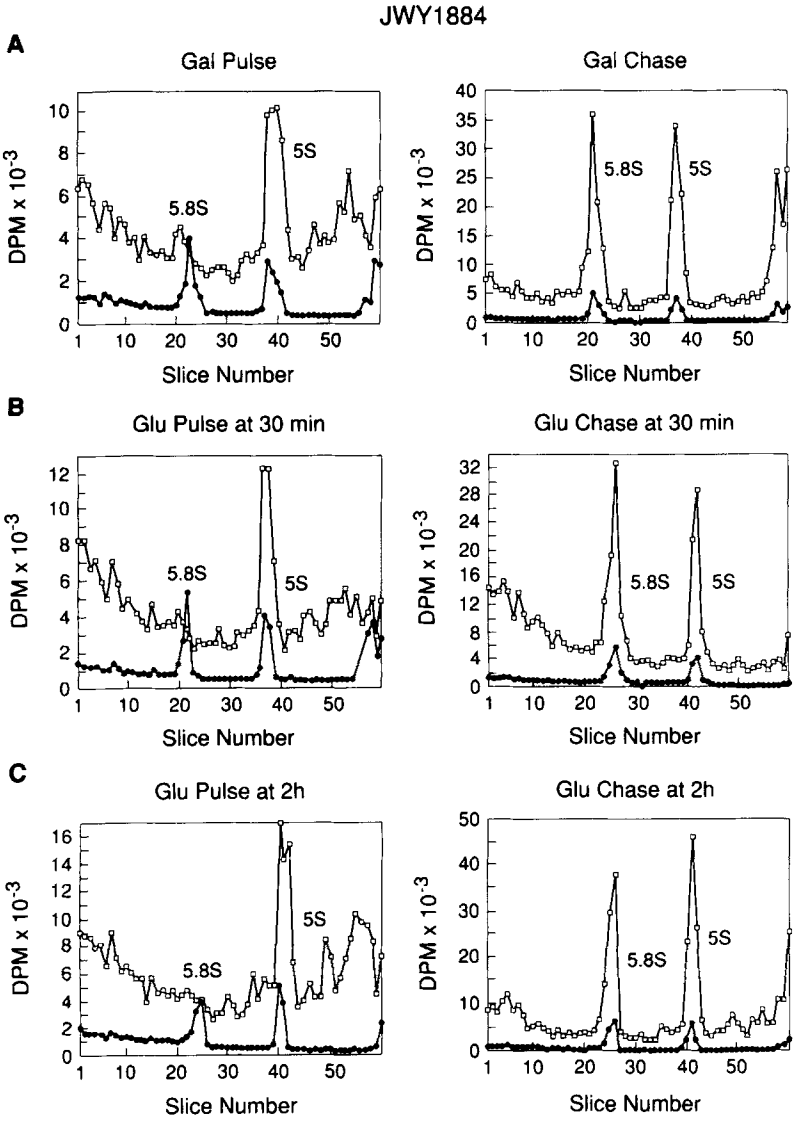

Figure 8. Accumulation of 5.8 and 5S rRNAs in JWY1884 grown in galactose medium (*a*) or shifted to glucose medium for 30 min (*b*) and 2 h (*c*). RNA was labeled and analyzed as described in Materials and Methods. Peaks representing 5.8 and 5S rRNAs are labeled. (□) ^{3}H disintegrations per minute (short-term label); (●) ^{14}C disintegrations per minute (long-term label).

difficult to conclude whether or not L29 was affected upon depletion of rp59. It has a high stability index, but an inspection of its A_i values leaves some doubt as to its instability (Table III).

Presumably, the degradation of proteins and RNAs from the same subunit as L16 or rp59 was a result of the inability of these proteins and rRNAs to assemble into subunits in the absence of L16 or rp59. It is noteworthy that under these conditions, the stability of r-proteins varied: turnover of some proteins was evident by 30 min after termination of L16 or rp59 synthesis in glucose, degradation of some proteins was not apparent until the 2-h time point, and other proteins were not affected at all. A similar wide range of turnover rates for r-proteins was observed upon termination of rRNA synthesis or processing in *Schizosaccharomyces pombe* or *Saccharomyces cerevisiae* (Yamagishi and Nomura, 1988; Wittekind et al., 1990; Gorenstein and Warner, 1977). When r-proteins were overproduced, their half-lives varied from 30 s to 24 min (El-Baradi et al., 1986; Maicas et al., 1988; Tsay et al., 1988). A trivial explanation for this variation in stability is that some proteins are inherently unstable when not assembled into ribosomes and turn over at different rates as a function of their secondary or tertiary structure.

Do Ribosomal Protein Stabilities Imply an Assembly Pathway?

A more interesting possibility is that variation in r-protein stability results from the position of each protein in the ribosome assembly pathway, or from the existence of preassembly complexes of r-proteins. Proteins most unstable upon depletion of L16 or rp59 may be those that normally assemble into ribosomes after L16 or rp59. In the absence of L16 or rp59, binding sites for later assembling proteins may never be formed and these unprotected proteins may be rendered targets for proteases. r-proteins that normally assemble before L16 or rp59 might be more stable if they continue to bind to nascent rRNA or to preribosomal particles regardless of the absence of L16 or rp59. Stable proteins might also include those that assemble after L16 or rp59, but whose binding site is not determined by the assembly of L16 or rp59 into the ribosome. In addition, some proteins may be pro-

tected from degradation if they are members of subassembly particles that contain a small number of r-proteins.

Studies of assembly of *E. coli* 30S subunits in vitro (Traub and Nomura, 1968; Mizushima and Nomura, 1970; Held et al., 1973) indicated a strict order of assembly of r-proteins into the subunit and that complicated interdependencies exist between r-proteins as they assemble. Nearly every r-protein in the 30S subunit changes the conformation of 16S rRNA upon assembly and there is cooperativity in the assembly of the r-proteins (Stern et al., 1989 and references therein). Thus, binding sites for later assembling proteins are likely to be created by the assembly of earlier r-proteins.

Since not all proteins were rendered unstable in our experiments and only one subunit was affected in each of our strains it follows from our model that L16 and rp59 assemble at a middle step in the pathway. This step is probably after processing of the 35S rRNA precursor, which separates what will ultimately be the 40 and 60S subunits. Since the 27S rRNA precursor was processed to yield at least some mature 25S rRNA upon depletion of L16, this processing step may not be dependent on assembly of L16.

Components of the 5S RNA-L1 Protein Complex Are Stable in the Absence of 60S Ribosomal Subunit Assembly

The 25 and 5.8S rRNAs (60S subunit components) are unstable in the absence of L16, but 5S rRNA is more stable (Figs. 4 and 5). In *Xenopus*, mouse, and HeLa cells (Picard and Wegnez, 1979; Rinke and Steitz, 1982; Steitz et al., 1988), 5S rRNA is associated with TFIIIA, La protein, or r-protein L5, in stable RNP particles before assembly of 5S rRNA and L5 into ribosomes. Yeast TFIIIA (Brow and Geiduschek, 1987; Wang and Weil, 1989) and r-protein L1 (also known as YL3), are yeast 5S rRNA binding proteins. L1 is the yeast homologue of the *Xenopus* and mammalian L5 proteins (Nazar et al., 1979). Upon depletion of L16, L1 is stable (Tables II and III). These results suggest that L1 and 5S RNA may form an RNP particle prior to assembly into the 60S subunit that remains stable in the absence of 60S subunit assembly.

Implications for the Regulation of Ribosome Assembly

We demonstrated that although the two ribosomal subunits normally accumulate in equal amounts, yeast cannot maintain this situation when one ribosomal component is missing. There are many examples of this uncoupling of subunit accumulation in yeast and in bacteria (e.g., Nam and Fried, 1986; Musters et al., 1989; Andrew et al., 1976; Gorenstein and Warner, 1977; Carter and Cannon, 1980; Dabbs, 1986; Marvaldi et al., 1979; Nashimoto and Nomura, 1970). Upon termination of synthesis of rRNAs (Gorenstein and Warner, 1977; Yamagishi and Nomura, 1988; Dennis, 1974) or r-proteins (Shulman and Warner, 1978; Nam and Fried, 1986) other ribosomal components are degraded. By performing very short pulses to label r-proteins, we have shown that there is no regulation of synthesis of most r-proteins or rRNAs upon depletion of one component from either subunit. Thus, our results provide further evidence that yeast cells, unlike bacteria, do not use posttranscriptional feedback regulation of r-protein synthesis as a major means of controlling ribosome biosynthesis.

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