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The Accumulation of Three Yeast Ribosomal Proteins under Conditions of Excess mRNA Is Determined Primarily by Fast Protein Decay

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The suggestion that compensation for overabundant mRNA of the genes for *Saccharomyces cerevisiae* ribosomal protein (r-protein) L3, L29, or rp59 occurs by translation repression has been reinvestigated. First, analysis of the distribution of these three mRNAs in polysome profiles revealed no differences between normal and mRNA-overproducing strains, indicating that initiation of r-protein translation is not repressed under conditions of mRNA overaccumulation. Second, experiments involving radioactive pulse-labeling of proteins were done by using a modified method of data collection and analysis that allows quantitation and correction for fast decay during the pulse. These measurements revealed that the synthesis rate of the three r-proteins is increased when their mRNA levels are elevated and that their decay rate is also high, with half-lives ranging from a fraction of a minute to more than 10 min. We conclude that accumulation of excess r-protein mRNA has no effect on translation rate; rapid decay of protein during the course of the labeling period can account for the apparent discrepancy between mRNA levels and protein synthesis rates. Yeast r-proteins, when produced in excess, are among the most rapidly degraded proteins so far described.

Saccharomyces cerevisiae ribosomes contain approximately 75 different proteins, in nearly equimolar amounts (35). Several studies have shown that the synthesis of these ribosomal proteins (r-proteins) responds to various physiological changes in a highly coordinated fashion (7, 12, 19, 20, 26). It has been suggested that this coordinated control occurs at the levels of transcript synthesis (7, 20).

The synthesis of the 52 r-proteins of the procaryote *Escherichia coli* is also regulated in a coordinated fashion. In this case regulation occurs, at least in part, through a translational feedback mechanism on the basis of the ability of some r-proteins to repress the translation of their own polycistronic messengers when they accumulate in excess relative to rRNA (6, 9, 22). One consequence of this feedback repression mechanism is that an artificial increase of the copy number of a r-protein operon (when carried on a multiple-copy plasmid, for example) leads to a higher level of the corresponding mRNA but not to a proportionately higher rate of synthesis of the proteins coded by it (6, 9, 24).

When cloned *S. cerevisiae* r-protein genes became available, similar experiments were performed. As we observed with *E. coli*, an artificial increase in the copy number of some of these genes led to a higher level of mRNA, but the synthesis rate of the corresponding protein did not appear to increase to the same extent (14, 25, 36). This phenomenon was interpreted as a reflection of a translational control mechanism used to control the synthesis of r-proteins under natural conditions.

In the experiments presented here, we set out to determine whether the accumulation of three r-proteins, L3, L29, and rp59 (the products of the genes *tcm1*, *cyh2*, and *cry1*, respectively) is indeed regulated at the level of translation, or whether the apparently low rates of synthesis might instead be due to fast decay of excess proteins synthesized during the pulse period, as proposed for rp51 and L25 by Abovich et al. (1) and El Baradi et al. (8). First, it is shown that the rate of translation of overproduced mRNA for L3, L29, or rp59 is not repressed, as deduced from their distribution in a polysome profile. Second, by using a modification of the pulse-labeling protocol and data analysis used by previous workers to measure r-protein rates of synthesis, it is shown that the synthesis rates of L3, L29, and rp59 are elevated approximately in proportion to the artificial increase in their mRNA level. Finally, by using a mathematical treatment of the experimental data, quantitative estimates are made of the decay rate of these proteins when they are overproduced.

MATERIALS AND METHODS

Strains, media, and plasmids. *S. cerevisiae* SC252 (*ho MATα ade1 leu2-3,2-112 ura3-52*) was obtained from James Hopper. It is sensitive to 2 μg of trichodermin per ml (a gift from Leo Pharmaceuticals, Ballerup, Denmark) and to 1 μM cryptopleurine (Chemasea, Sydney, Australia). Strain J409 was obtained from Jonathan Warner. It is sensitive to 1 μg of cycloheximide per ml (Sigma Chemical Co., St. Louis, Mo.). Growth media were prepared as described by Sherman et al. (29).

Plasmid pEM39 was constructed by inserting the *Bam*HI-*Ava*I segment (3.2 kilobases) from the *tcm1* (trichodermin resistance) gene (27) into the multiple-copy plasmid vector YEp13 (5). This plasmid accumulates in strain SC252 to four to five copies per cell and renders it resistant to 10 μg of trichodermin per ml.

Plasmid pGAL-TCM1 consists of a fusion of the *tcm1* coding region (including the initiation codon and the entire 5′ leader sequence) to the promoter and transcription start site of the *GAL10* gene. Transcription of this hybrid message is completely dependent on the *GAL10* promoter, and hence

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this plasmid renders strain SC252 resistant to 10 μg of trichodermin per ml on galactose medium, but not on glycerol-lactate or glucose medium. This *GAL10-tcm1* fusion is carried on a 2μm *LEU2*-based vector called pGAL.

YEpCYH1 has been described by Warner et al. (36). It contains the cycloheximide resistance allele of the *cyh2* gene in the multiple-copy plasmid YEp24 (5). It renders strain J409 resistant to 1 μg of cycloheximide per ml.

pAV12 has been described by Himmelfarb et al. (14). It contains the cryptopleurine resistance allele of the *cry1* gene in the multiple-copy plasmid pYF91 (31). SC252 transformants are resistant to 1 μM cryptopleurine.

Quantitation of mRNA levels. Total RNA was extracted from cultures containing 2×10^7 cells per ml. Specific transcripts were detected by RNA blot hybridization (23) by using radiolabeled DNA probes (10); the autoradiograms were quantitated by using a Bio-Rad 620 densitometer (Bio-Rad Laboratories, Richmond, Calif.). The level of each overproduced mRNA was normalized to one of the other r-protein mRNAs used in this study or to the actin mRNA to correct for variations in the RNA yield; this ratio was then normalized to the corresponding normalized mRNA level in the strain transformed with vector alone (referred to as the normal level in the text). It was found that overproducing the L29 mRNA resulted in an accumulation of unspliced precursors, as reported by Fried et al. (11). Since these transcripts do not code for a normal protein, the level of the mature form only was quantitated. Overproduction of the rp59 mRNA did not lead to a similar accumulation of the unspliced form.

Separation and analysis of polysomes. The overproducer strains and the control strains (transformed with plasmid vector alone) were grown in synthetic selective medium to a concentration of 2×10^7 cells per ml. Polysomes were prepared and analyzed as described by Baim et al. (3). Ribosome runoff during sample preparation was inhibited with 33 μg of cycloheximide per ml for all strains except J409(YEpCYH1) (cycloheximide resistance allele) and its control J409(YEp24), for which 165 μg of cycloheximide per ml was used.

Protein labeling and separation. Ten milliliters of culture at a density of 2×10^7 cells per ml in complete synthetic medium without methionine was pulse-labeled for various lengths of time with L-[^{35}S]methionine (New England Nuclear Corp., Boston, Mass.; greater than 1,000 Ci/mmol). A total of 900 μCi was used for a 30-s pulse, 400 μCi was used for a 2-min pulse, and 200 μCi was used for a 5-min or 15-min pulse. The labeling was stopped by pouring the cultures onto 10 g of crushed ice and swirling to mix. Under these circumstances, the culture cools from 30 to 0°C in less than 5 s. The liquid was then pipetted out of the ice, filtered on a nitrocellulose membrane (2.5-cm diameter, 0.45-μm pores) under suction, and washed with 5 ml of ice-cold TM buffer (50 mM Tris [pH 7.5], 5 mM $MgCl_2$), and the moist filters were frozen quickly on a glass surface kept on dry ice. All these steps were done in a cold room, as quickly as possible. Less than 2 min elapsed between the cooling of the culture and the freezing of the filters.

The frozen filters were then broken in pieces (without thawing them) and the fragments were placed in 1.5-ml microcentrifuge tubes. A 600-μl quantity of TM buffer, containing 5×10^8 cells transformed with vector alone and labeled for two generations with 80 μCi of L-[*methyl*-^{3}H]methionine (Amersham Corp., Arlington Heights, Ill.), was added to the frozen filters, and the tubes were vortexed for 30 s. The liquid was transferred to a microcentrifuge tube containing 660 mg of glass beads (450 to 500 μm), and the cells were broken in a cell homogenizer (B. Braun Instruments, Burlingame, Calif.) for 80 s with CO_2 cooling. The liquid and an extra 600 μl of TM buffer used to rinse the glass beads were transferred to a second tube containing 120 μl of 1 M $MgCl_2$ and centrifuged in an Eppendorf model 5414 centrifuge for 10 s. The supernatant was then mixed with 2.4 ml of glacial acetic acid and precipitated for at least 30 min on ice. The protein extraction protocol was done in a cold room, as fast as possible. Approximately 6 min elapsed between the suspension of the frozen ^{35}S-labeled cells and the addition of acetic acid.

Acid-soluble proteins were then dialyzed against 1% acetic acid, lyophilized, and separated by two-dimensional gel electrophoresis as described by Gorenstein and Warner (12). Radioactivity in various protein spots was quantitated by using a Packard 4000 scintillation counter, correcting for counting efficiency and quenching. The counts were processed as shown by the example in Table 1, footnote *b*.

RESULTS

Distribution of overproduced mRNAs in polysome profiles. A reduction in the rate of translation initiation can be detected by a shift in the distribution of the mRNA toward smaller polysomes. This technique was used to determine whether translation initiation of the L3, L29, or rp59 mRNA is repressed when these messages are overaccumulated.

Figure 1A shows two polysome profiles from strain SC252 transformed with plasmid vector YEp13 or with plasmid pEM39 (L3 mRNA 2.4-fold higher than with vector alone). Figure 1B shows profiles from strain SC252(pGAL) or SC252(pGAL-TCM1) (L3 mRNA fourfold higher). In both cases, L3 mRNA overproduction resulted in a general reduction in polysome size (see profile of optical density at 260 nm in Fig. 1A and B), probably a reflection of the slower growth rate of the overproducer cells [320 min doubling time for SC25(pEM39) versus 210 min for SC25(YEp13) in synthetic selective glucose medium]. Nevertheless, the distribution of L3 mRNA and actin mRNA (two transcripts of similar size) in the polysomal fractions is very similar between control and overproducer strains. Moreover, excess L3 mRNA does not accumulate in small complexes, but rather most of it is associated with polysomes and thus is actively translated. Therefore, these results do not support the proposition that L3 translation can be regulated at the level of initiation under conditions of mRNA overaccumulation. Some L3 and actin mRNA can be detected in the region of the sucrose gradient where particles smaller than 60S would migrate. Most likely, these transcripts do not come from untranslated complexes, but rather from membrane-bound polysomes which, because of the lighter density of lipids in the complex, are arrested at the zone in the gradient corresponding to their buoyant density. This interpretation is supported by the detection of rRNA in these fractions upon ethidium bromide staining of the agarose gel (not shown) and by the similar ratio of the various mRNAs in these and in polysomal fractions.

Figures 1C and D show the results of a similar analysis performed on strains accumulating approximately a four- to eightfold excess of L29 and rp59 mRNAs. Here, too, no shift in mRNA distribution among polysomes from the control and overproducing strains can be detected. In the case of L29, there is a striking accumulation of precursor mRNA in the region of the gradient that corresponds to large struc-

TABLE 1. Calculation of ARROS values for 12 protein spots by using 30-s or 5-min pulses of L-[^{35}S]methionine[a]

Spot	Pulse length					
	30 s			5 min		
	NLR[b]		ARROS[c]	NLR[b]		ARROS[c]
	SC252(YEp13)	SC252(pEM39)		SC252(YEp13)	SC252(pEM39)	
nrpA	0.60	0.53	0.88	1.40	1.40	1.00
nrpB	0.51	0.45	0.88	1.37	1.33	0.97
rp1 (L3)	0.67	1.49	2.22	0.69	1.30	1.88
rp2	0.10	0.13	1.30	0.75	0.76	1.01
rp6,9	0.50	0.50	1.00	0.94	0.92	0.98
rp33,38	1.04	0.87	0.84	0.94	0.93	0.99
rp39	1.08	0.98	0.91	0.91	0.89	0.98
rp45	1.49	1.49	1.00	1.16	1.02	0.88
rp52	1.37	1.45	1.06	0.81	0.87	1.07
rp59	0.99	1.05	1.06	0.90	0.94	1.04
rp61	1.79	1.69	0.94	0.94	1.02	1.08
rp64	1.51	1.85	1.22	0.85	0.93	1.09

[a] The overproducer strain is SC252(pEM39) (accumulating 3.1 times more L3 mRNA than normal), and the control strain is SC252(YEp13). The numbers shown in the field correspond to two of the four pulses presented graphically in Fig. 1.

[b] The disintegrations per minute values for ^{3}H and ^{35}S in each spot, already corrected for counting efficiency and quenching, were additionally corrected for nonspecific background by subtracting from them the counts obtained with a blank spot. The ratio of ^{35}S to ^{3}H disintegrations per minute was then determined for each spot, and the average was calculated for all of these ratios, except the one corresponding to the overproduced protein (in this case, L3). Each individual ratio was then normalized to this average to obtain a series of NLR values.

[c] The ARROS value was calculated by dividing each NLR value from the overproducer strain, SC252(pEM39), by the corresponding value from the control strain, SC252(YEp13). The average and the standard deviation (σ_{n-1}) were calculated for the ARROS values for all spots, excepting the one corresponding to the overproduced protein (in this case, L3). For the 30-s pulse spots, the average ARROS value was 1.00 and the σ_{n-1} was 0.14; for the 5-min pulse spots, the average ARROS value was 1.00 and the σ_{n-1} was 0.06. These two series of ARROS values fit well the theoretical ARROS value obtained by using equation 3 from the appendix when K_s = 2.25 and the half-life = 4.5 min.

tures. Accumulation of precursor mRNA for this transcript has been observed previously (11, 36). Its presence in the gradient might indicate either (i) association of unprocessed (i.e., intron-containing) transcript with ribosomes or (ii) unprocessed transcripts in association with aggregates of spliceosomes (28). At present we do not know which of these is correct.

r-protein synthesis rates. The yeast r-protein pulse-labeling protocol and data analysis of Warner et al. (36) were modified to minimize the possible effects of protein decay, both during and after the pulses (see Materials and Methods; Table 1).

Figure 2 shows, in graphic form, the apparent relative rate of synthesis (ARROS) values (see Table 1, footnote *c*, for a sample calculation) obtained from a series of pulse-labeling experiments, by using a strain that accumulates 3.1-fold more L3 mRNA than normal [SC252(pEM39)] and a control strain transformed with the plasmid vector alone [SC252(YEp13)]. The ARROS values for all of the 11 proteins used as internal controls fluctuate around a value of 1.0. In contrast, the values for L3 are always significantly higher than 1, falling during the course of the experiment from 2.22 to 1.59. These results suggest that when the level of L3 mRNA is increased by more than threefold, L3 synthesis increases significantly, but synthesis of several other proteins, including at least nine r-proteins, is not affected. It appears likely that the decrease in the L3 ARROS values with longer pulses can be explained by the decay of the protein during the pulse.

The Appendix contains a mathematical model that can be used to extrapolate the experimental ARROS values to a hypothetical pulse length of zero, equivalent to the true relative rate of synthesis (K_s). By using this approach, it was found that synthesis of L3 in SC252(pEM39) was very close to 2.25 times higher than the normal rate. The same mathematical approach also allowed an estimation of the half-life of excess L3 as approximately 6 min.

These measurements were repeated by using the same pair of transformed strains [SC252(pEM39) compared with SC252(YEp13)] and with two pulse lengths, 30 s and 5 min. In this case, the rate of synthesis of L3 was close to 2.7-fold higher than normal, for an mRNA level 3.5-fold higher than normal, and the half-life of surplus L3 was estimated at 15 min (data not shown). The rate of synthesis of L3 in strain NP6B(YEpTCM5) compared with NP68(YEp24) (25) was also measured by using pulse lengths of 30 s and 5 min. In this case, L3 synthesis was close to 2.7-fold higher than normal, with 4.0-fold more mRNA available, and the excess protein decayed with a half-life close to 10 min (data not shown).

Synthesis of L3 was also measured in strain SC252 transformed with plasmid pGAL-TCM1 (L3 mRNA 10-fold higher than normal) and in a control strain transformed with vector alone [SC252(pGAL)], both grown for one generation on galactose medium. The ARROS values, shown in graphic form in Figure 3A, are consistent with a rate of synthesis 9.6-fold higher than normal and a half-life of approximately 2 min for the excess protein.

Pulse-labeling experiments were performed with r-proteins L29 and rp59, whose synthesis was previously reported to be controlled at the level of translation (14, 36). Figure 3B shows the ARROS values for L29 and 11 other proteins when the level of mature L29 mRNA is 4.9-fold higher than normal. The data are compatible with a rate of synthesis 3.3-fold higher than normal and a half-life for the surplus L29 protein close to 13 s. Figure 3C shows the ARROS values for rp59 and 11 other proteins when the rp59 mRNA is 4.3-fold more abundant than normal. These values are compatible with a rate of synthesis 3.5-fold normal and a half-life close to 1.4 min.

The small but systematic discrepancy between calculated synthesis rates and mRNA levels for all three proteins (see Fig. 2 and 3 and above) could be the result of protein decay during extraction, which cannot be corrected for.

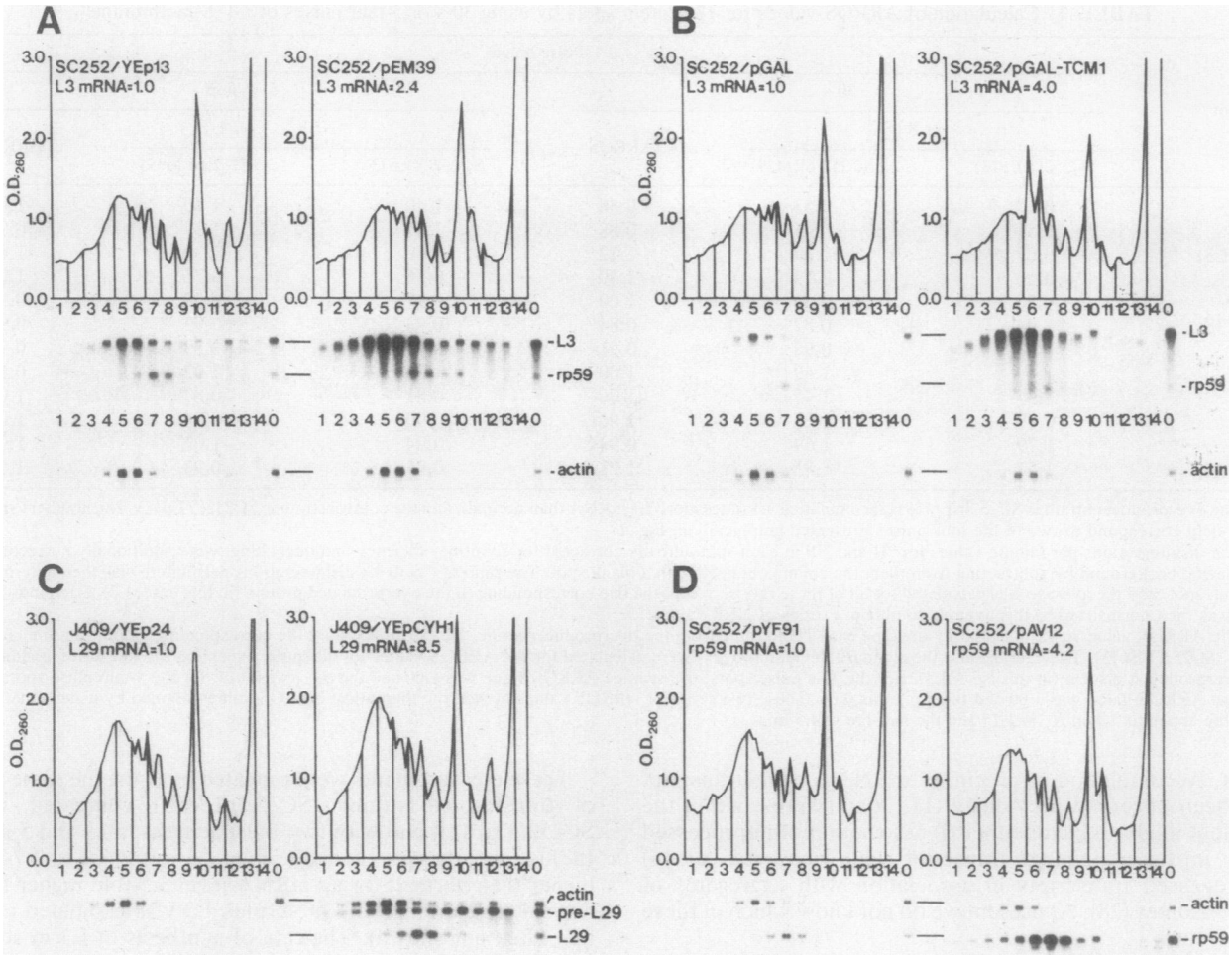

FIG. 1. Analysis of mRNA distribution in polysome profiles. The sucrose gradients were separated in 70 fractions of approximately 0.5 ml, and the optical density at 260 nm of each was recorded and plotted. The fractions were then pooled in groups of five, and RNA was extracted from the pools (numbered 1 to 14). Of the RNA recovered from each pool, 1/20 was analyzed by RNA-blot hybridization. Lanes 0 correspond to 6 μg of RNA extracted from total cell extracts. To compare the distribution of the L3 and actin mRNAs, which have a similar size, the same blots were probed sequentially. mRNA levels were normalized to the actin mRNA level. (A) Analysis of strains SC252 transformed with vector YEp13 or plasmid pEM39; (B) analysis of strain SC252 transformed with vector pGAL or plasmid pGAL-TCM1, both grown for one generation on galactose medium; (C) analysis of strains J409 transformed with vector YEp24 or plasmid YEpCYH1; (D) analysis of strain SC252 transformed with vector pYF91 or plasmid pAV12.

DISCUSSION

The aim of this series of experiments was to determine whether, in *S. cerevisiae*, the accumulation of high amounts of mRNA coding for the r-proteins L3, L29, and rp59 is compensated by translational repression (14, 25, 36) or whether these proteins are in fact produced in excess and then rapidly degraded, as has been proposed for r-proteins rp51 and L25 (1, 8).

A reduction in the translation initiation rate of an mRNA can be detected by a shift in the distribution of this message in a polysome profile. Results obtained from such an analysis are not affected by fast decay of the protein product. Figure 1 shows polysome profile analyses of strains overaccumulating L3, L29, or rp59 mRNA. None displays the shift toward smaller polysomes which is to be expected of a mechanism maintaining normal r-protein synthesis rates through repression of translation initiation. These results are in agreement with those obtained for r-protein L25 (8). It has been reported that a threefold overaccumulation of L3 mRNA results in a partial shift in the distribution of this message toward smaller polysomes (36). A possible explanation for the discrepancy between these and our results might lie in the greater resolution with which we analyzed our polysome profiles.

Analysis of polysome profiles is not in itself sufficient to rule out all forms of translational control. To confirm the conclusion derived from this analysis, we wanted to demonstrate the enhanced synthesis rate of the corresponding r-proteins. To that end, we used a modified version of the pulse-labeling protocol and data processing used by previous workers (36) to limit to a minimum the effect of fast protein decay on estimates of synthesis rates. The first of these modifications is the use of very short pulses, as short as 30 s. We found that normalized labeling rate (NLR) values obtained with such short pulses were far from the average (1.0) for some proteins (see Table 1). (The NLR value [defined in Table 1, footnote *b*] is not completely identical to the original Ai value as defined by Gorenstein and Warner [12], since it is normalized to a r-protein average, not to a total protein average, but is comparable.) This discrepancy could be the

consequence of labeling for a period shorter than the time required for complete synthesis of some proteins (synthesis of a 43-kilodalton protein like L3 takes approximately 60 s; calculated from Boelhke and Friesen [4]). Whatever their cause, these differences in NLR values are reproducible, not random, and can be corrected. We thus introduced a second modification, this one in the analysis of data: normalization of the NLR value for a given protein from the overproducer strain to the corresponding value from the control strain. We call this last number the ARROS (see Table 1, footnote *c*, for a calculation example). Therefore, the ARROS values compare the rate of synthesis of a protein with its normal rate, determined experimentally, without any assumption required about this normal rate. This property is particularly critical in the case of L3, as described below.

The third modification was contained in steps aimed at minimizing protein decay during extraction (see Materials and Methods). Special precautions should be taken to limit this decay, since it cannot be corrected for.

Finally, we attempted to describe mathematically the synthesis and decay of r-proteins in *S. cerevisiae* (see Appendix). An equation was obtained that can be used to calculate the most probable synthesis and decay rate from two or more experimental ARROS values. To estimate the accuracy of this model, we obtained four different ARROS values from an L3 overproducer strain (Fig. 2) and determined by trial and error the K_s and K_d values most compatible with these ARROS values (K_d = ln2/half-life). The fact that a theoretical curve can be fitted closely to four experimental values suggests that the kinetics of decay of surplus L3, and probably of other r-proteins, is adequately described by the model.

On the basis of data collected and analyzed according to this modified protocol, we conclude that, for L3, L29 and rp59, an increase in mRNA level is not compensated signif-

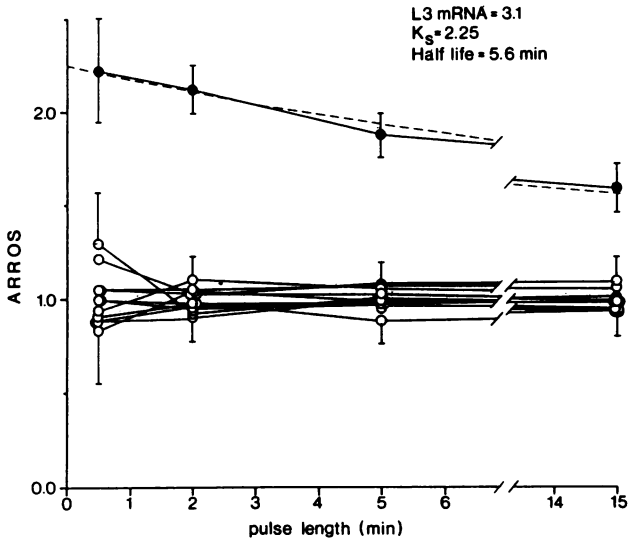

FIG. 2. Graphic representation of ARROS values obtained with strain SC252(pEM39) (L3 mRNA level 3.1-fold higher than normal) compared with SC252(YEp13), by using four different pulse lengths (30 s and 2, 5, and 15 min). Symbols: ●, ARROS for rp1 (L3); ○, ARROS for 11 other protein spots; – – –, theoretical ARROS values calculated from equation 3 (see Appendix) and the K_s and half-life values given at the top of the figure, which were found to yield a theoretical curve fitting closely to the experimental ARROS values. The error bars represent two standard deviations calculated as described in Table 1; footnote *c*.

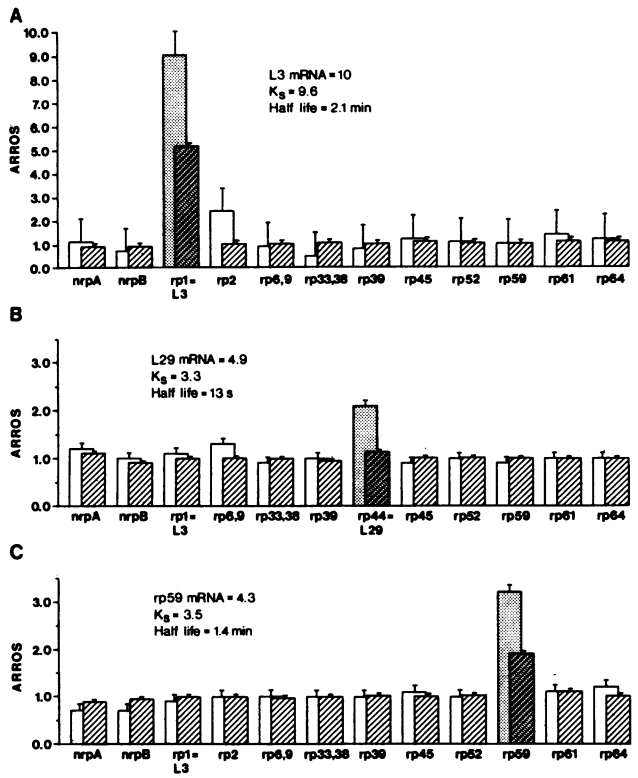

FIG. 3. Graphic representation of ARROS values. (A) Strain SC252(pGAL-TCM) (10 times more L3 mRNA than normal) compared with strain SC252(pGAL), both grown one generation on galactose medium; (B) J409(YEpCYH1) (4.9 times more mature L29 mRNA than normal) versus J409(YEp24); (C) SC252(pAV12) (4.3 times more rp59 mRNA than normal) compared with strain SC252(pYF91). Symbols: ▭, 30-s pulses; ▨, 5-min pulses; ▩ and ▩, ARROS values for the over-produced protein. The error bars extend plus or minus two standard deviations calculated as described in Table 1, footnote *c*. The K_s and half-life values given at the top of each diagram yield theoretical ARROS values very similar to those obtained experimentally for the overproduced protein, as calculated from equation 3 (see Appendix).

icantly by translation repression. Instead, excess quantities of the corresponding proteins are synthesized and then degraded at a rate which can be surprisingly fast (Fig. 3B and C). This can explain why pulse-labeling results obtained with a single 3- or 5-min pulse led to the conclusion that in yeast cells high levels of L29 and rp59 mRNA can be compensated by translational repression (14, 36). In the case of L3, the main difference between our results and those of Pearson et al. (25) and Warner et al. (36) does not reside in the correction for fast protein decay, but in the use of the ARROS value instead of the Ai value as an indication of the synthesis rate. We and others have reproducibly obtained normal synthesis rates for L3 that are lower than average (8, 25, 36; Table 1). Ai values are not corrected for such natural fluctuations in the synthesis rates, but ARROS values are. This seemingly trivial difference is critical for the L3 data, since it reveals a significant difference between two Ai values or NLR values that were previously considered equivalent (25, 36). The experiment was performed four times (Results; Fig. 2 and 3A). In all these experiments, the ARROS values for L3 were significantly higher than normal, suggesting that a normal rate of L3 synthesis cannot be maintained when excess mRNA accumulates.

Mathematical treatment of pulse-labeling data allows one to estimate that excess L3 decays with a half-life ranging between 2 and 15 min and that excess L29 and rp59 decay with a half-life of a few seconds and less than 2 min, respectively. A pulse-and-chase approach was used by Warner et al. (36) to estimate that excess r-protein S10 decays with a half-life of 5 to 10 min. Abovich et al. (1) and El Baradi et al. (8) showed that surplus rp51 or L25 is eliminated within a few minutes. From those and the present results, it appears that free yeast r-proteins are among the most rapidly degraded proteins so far described. Relatively fast decay of unassembled r-proteins has also been observed in mammalian cells (17, 33, 34). It is important to note that we and others have scored as decay any event that prevents the protein from migrating at its normal position in a two-dimensional gel electrophoretic separation. Therefore, the rate we have measured is in fact the rate of the first reaction in the decay process, which could be, for example, a single proteolytic cleavage or chemical modification. The complete breakdown of the protein to small peptides or amino acids will most likely occur more slowly. Little is known about the molecular mechanism responsible for this fast decay. Since r-proteins accumulate in the nucleus, where ribosome assembly takes place, their fate is probably determined there. Therefore, it is likely that their degradation, or at least marking for degradation, occurs in the nucleus. We cannot say whether fast degradation of r-proteins is a specific regulatory mechanism or merely a facet of the general scavenging of proteins that fail to form part of a larger structure.

r-protein synthesis was shown to be controlled at the level of translation in a wide variety of organisms ranging from bacteria to mammals (2, 18, 22, 30). Our results suggest that a similar mechanism does not exist in *S. cerevisiae* to regulate the synthesis of ribosomes. Instead, transcription emerges as the major control point for ribosome biosynthesis in yeast cells (7, 20). The identification of sequence elements common to most r-protein gene promoters (21, 32) and of a protein that binds to them (15, 16) provides a likely mechanism for the coordinated transcriptional regulation of these genes.

APPENDIX

A mathematical model describing the synthesis and decay of excess r-protein. To correct ARROS values to obtain a true relative rate of synthesis independent of degradation and also an estimate of the rate of decay, we attempted to describe mathematically the degradation mechanism responsible for decay of surplus r-proteins. The model is based on the following assumptions: (i) proteins are stable once assembled into ribosomes, (ii) the unassembled proteins decay rapidly, (iii) r-proteins are normally produced in quantities very close to the amount needed for ribosome assembly, and (iv) synthesis is zero order and decay is first order. To facilitate the mathematical treatment, the protein population was divided into two independent subpopulations; the first is stable and has a normal rate of synthesis, and the second is in excess and decays with a short half-life.

A mathematical analysis inspired by the approach-to-steady-state treatment (13) was used. Briefly, the rate of labeling of the stable subpopulation is described by the equation

$$dP_s = K_{s1}dt \quad (1)$$

where P_s (in arbitrary units, P) is proportional to the amount of stable labeled molecules at time t (in min), and K_{s1} (in P/min) is the rate of labeling of the stable subpopulation. The total amount of labeled protein that has accumulated from zero to t min is given by integration of equation 1.

The rate of labeling of the unstable (excess) subpopulation is described by the equation

$$dP_u = K_{s2}dt - K_dP_udt \quad (2)$$

where P_u (in P units) is proportional to the amount of unstable labeled molecules at times t (in min), K_{s2} (in P/min) is the rate of labeling of the unstable population, and K_d (in min^{-1}) is the decay rate of the excess labeled protein. The total amount of unstable labeled protein that has accumulated after a period of t min is given by integration of equation 2 from zero to t.

The amount of total labeled protein, P, that has accumulated after t min is the sum of P_s and P_u, that is, of the integrals of equations 1 and 2 from zero to t.

If K_{s1}, which is the normal rate of synthesis, is arbitrarily set at 1.0 P/min (corresponding to an ARROS of 1.0), and if the value P is expressed as ARROS $\times$ t (the ARROS value is in fact a rate of synthesis, in P/min), then the relationship between the true relative rate of synthesis, K_s, which is defined as $K_{s1} + K_{s2}$, or $1.0 + K_{s2}$, and ARROS, K_d, and t, is given by the following expression:

$$\text{ARROS} = 1 + (K_s - 1)[(1 - e^{-K_dt})/K_dt] \quad (3)$$

Only two of the four variables of equation 3 are determined experimentally (ARROS and t). Therefore, one needs at least two values of ARROS obtained with two different pulse lengths to solve the equation for the variables K_s and K_d. Since no solution of equation 3 for K_d is known, we were obliged to estimate the best values of K_s and K_d for a given set of ARROS values by trial and error. In Fig. 2 and 3 and in Table 1, we propose a set of values for K_s and the half-life (K_d = ln2/half-life) compatible with the experimental ARROS values.

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