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Transcriptional Regulation of Ribosomal Proteins during a Nutritional Upshift in *Saccharomyces cerevisiae*

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The relative rates of synthesis of *Saccharomyces cerevisiae* ribosomal proteins increase coordinately during a nutritional upshift. We constructed a gene fusion which contained 528 base pairs of sequence upstream from and including the TATA box of ribosomal protein gene rp55-1 (S16A-1) fused to a *CYC1-lacZ* fusion. This fusion was integrated in single copy at the rp55-1 locus in the yeast genome. During a nutritional upshift, in which glucose was added to cells growing in an ethanol-based medium, we found that the increase in the relative rate of synthesis of the β-galactosidase protein product followed the same kinetics as the change in relative rates of synthesis of several ribosomal proteins measured in the same experiment. This demonstrates that the nontranscribed sequences upstream from the rp55-1 gene, which are present in the fusion, are sufficient to mediate the change in rates of synthesis characteristic of ribosomal proteins under these conditions. The results also suggest that a change in transcription rates is mainly responsible for the increase in relative rates of synthesis of ribosomal proteins during a nutritional upshift in *S. cerevisiae*.

Ribosome synthesis is coordinated at several levels of gene expression. It is generally accepted that the synthesis of ribosomal components is coupled under all growth conditions so there is normally not a large cellular pool of either free rRNA or ribosomal proteins (r-proteins) (for a review see reference 36). The exact molecular mechanisms responsible for this coordinate regulation are still in question for all organisms studied.

In *Escherichia coli*, Nomura and co-workers (32) suggest that the cellular pool of free ribosomes may ultimately regulate the synthesis of rRNA and indirectly r-proteins. Also in *E. coli*, it has been demonstrated that most r-protein mRNAs are subject to a translational feedback regulation in the presence of excess regulatory r-proteins. These excess regulatory r-proteins bind to a specific site on their own polycistronic message and inhibit translation of all products from that message. This type of autogenous control is facilitated by the organization of many r-protein genes into bacterial polycistronic operons (25). This autogenous translational control plays a major role in growth rate regulation of r-protein synthesis in *E. coli* (22).

Although polycistronic organization is not found in eucaryotes, nor has autogenous regulation been demonstrated, there are several reports that suggest that posttranscriptional regulation mechanisms are utilized to modulate the synthesis of r-proteins in eucaryotes. Evidence for posttranscriptional control of r-protein synthesis has been observed in gene dosage compensation experiments in yeasts (12, 27, 35) and *Xenopus laevis* (5); during growth stimulation of mouse fibroblasts (8); and during *Xenopus* (2) and *Drosophila* (1) development.

The strongest evidence for transcriptional regulation of r-protein genes comes from investigations of heat shock response in *E. coli* and *Saccharomyces cerevisiae*. Zengel and Lindahl (37) have shown that the S10 r-protein operon in *E. coli* is down regulated at the levels of both transcriptional initiation and attenuation after a heat shock. Kim and Warner (16) have measured the rates of transcription of several yeast r-protein genes and found a transient two- to threefold reduction in the transcription rate during the first 30 min of a heat shock response. This is consistent with previous studies which showed a transient coordinate decrease in rates of synthesis of a large number of yeast r-proteins during a similar temperature shift (34).

A coordinate change in the rates of synthesis of r-proteins is also seen during a nutritional upshift. Kief and Warner (15) state that upon the addition of glucose to an ethanol-grown yeast culture, there is a coordinate increase in the relative rates of synthesis of 30 r-proteins they examined. In the first 60 min after glucose addition, the r-proteins reach a synthesis rate that is three to four times faster than the average cellular protein synthesis rate. It is not known at what level of expression this coordinate increase is being mediated.

In this study, we wished to determine whether r-proteins are coordinately regulated at the transcriptional or posttranscriptional level during an ethanol-to-glucose carbon source upshift. More specifically, we wished to determine whether nontranscribed sequences 5′ to a typical r-protein gene are sufficient to cause the increase in rates of synthesis of r-proteins during such a shift. If these sequences alone are sufficient to mediate this change without the presence of any part of the r-protein transcript, it would strongly suggest that changes in rates of transcription are responsible for the coordinate changes in rates of synthesis of r-proteins during this shift.

Our approach was to fuse the entire 5′ nontranscribed region of an r-protein gene to a previously constructed iso-1-cytochrome *c-lacZ* (*CYC1-lacZ*) fusion. The r-protein gene used in this study was rp55-1 (S16A-1), which codes for a small subunit r-protein. The gene for this protein is duplicated (rp55-1, rp55-2) (23), as are many other r-protein genes in yeasts (17). Both genes have been sequenced (23; Pearson and Donovan, unpublished data), and both are known to produce functional proteins (26). We measured the rate of synthesis of the β-galactosidase protein product of this fusion (integrated in the yeast genome in a single copy at the rp55-1 locus) and several r-proteins by the same method, during an ethanol-to-glucose upshift experiment. We found that the relative rates of synthesis of several r-proteins (including rp55) and of the β-galactosidase protein increased

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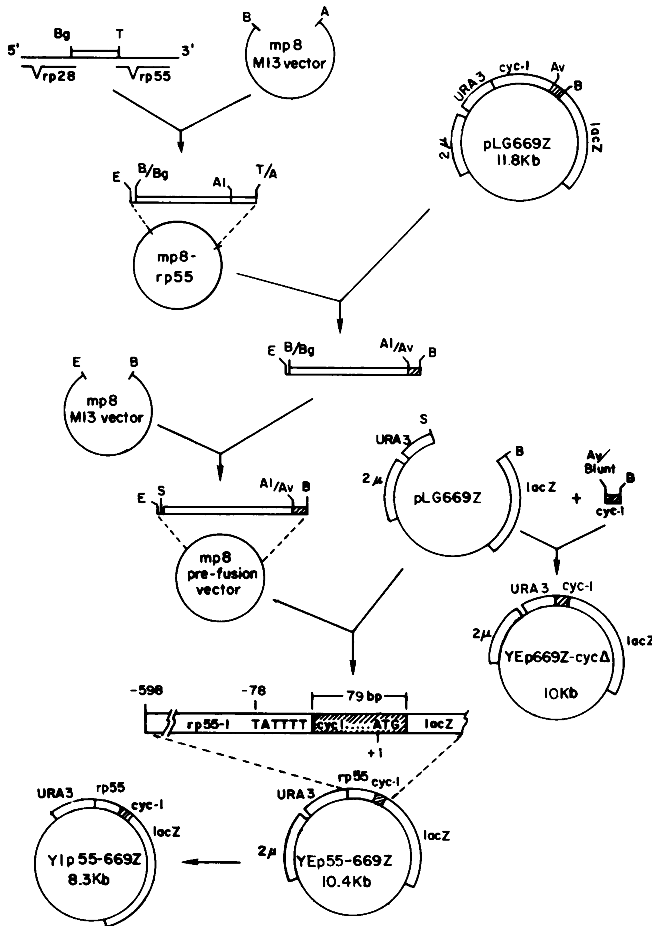

FIG. 1. Construction of YEp55-669Z, YEp669Z-cycΔ, and YIp55-669Z. A complete description of the constructions diagramed in this figure appears in Materials and Methods. A, *Acc*I; Al, *Alu*I; Av, *Ava*II; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; S, *Sma*I; T, *Taq*I. Kb, kilobases.

with the same kinetics during this upshift. This result demonstrates that nontranscribed sequences 5′ to an r-protein gene are sufficient to mediate the observed change in the rates of r-protein synthesis during an ethanol-to-glucose upshift. Therefore, these results strongly suggest that the regulation of yeast r-proteins during the shift occurs mainly at the level of transcription.

MATERIALS AND METHODS

Strains. The following strains were used: *S. cerevisiae* JL6B (*Mat*α *ura3-52 leu2-112 leu2-3 trp1 cry1*) (kindly supplied to us by J. L. Woolford); *E. coli* HB301 (F^- Δ*lacU169*) (used in all *lacZ* constructions and supplied to us by R. Wolf). *E. coli* JM103 was used as the host for M13 constructions (20).

Media. Haploid yeast strain JL6B was grown at 25°C in synthetic medium lacking uracil to maintain replicating plasmids and in synthetic medium lacking methionine for radioactive labeling experiments. Synthetic complete medium is described in reference 29. The EMM medium used contained 6.7 g of yeast nitrogen base per liter, all the nucleotides and amino acids contained in complete medium except methionine, 0.05% yeast extract, and 2% ethanol (vol/vol). To make GMM medium, 2% glucose was added (wt/vol) (15). The glycerol-ethanol (G-E) medium is described in reference 9.

Vectors. pLG669Z is a yeast episomal plasmid derived from YEp24 which contains the 2μm origin of replication, a yeast *URA3* gene, and a *CYC1-lacZ* fusion. It was constructed and characterized by L. Guarente and M. Ptashne (10). M13mp8 was obtained from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.).

Plasmid construction. The construction of YEp55-669Z, YEp669Z-cycΔ, and YIp55-669Z is diagramed in Fig. 1 and described here. The 630-base-pair (bp) *Bgl*II-*Taq*I DNA fragment containing the entire rp28-rp55 copy 1 intergenic region was cloned into the multiple cloning site of M13mp8 at the *Bam*HI and *Acc*I compatible sites. From this construct (mp8-rp55), a 539-bp *Eco*RI-*Alu*I fragment was then isolated and purified by electroelution from a polyacrylamide gel. The *CYC1* transcriptional and translational start sites were isolated on a 79-bp fragment. This fragment contains 71 bp of the native *CYC1* sequence plus 8 bp of *Bam*HI linker present in the original pLG669Z construction. This 79-bp fragment was prepared by blunt ending an *Ava*II-digested pLG669Z plasmid and then digesting with *Bam*HI. The resulting 79-bp fragment was purified by electroelution and ligated to the purified 539-bp *Eco*RI-*Alu*I fragment. The blunt-ended *Ava*II end on the 79-bp fragment should ligate to the *Alu*I end of the 539-bp fragment. The ligation mix was electrophoresed directly on an 8% polyacrylamide gel, and the expected 618-bp ligation product was electroeluted and cloned into the *Eco*RI and *Bam*HI sites of M13mp8. The fusion joint was verified by dideoxy sequencing (28). The fusion joint between rp55 upstream sequences and *CYC1* sequences is 2 bp downstream from the presumptive rp55 TATA-like box. The 610-bp *Sma*I-*Bam*HI fragment in this M13mp8 prefusion vector was isolated. This fragment contains only 4 bp of M13mp8 at the *Sma*I site and fuses the rp55 upstream sequences directly to the *CYC1* sequences. This 610-bp fragment was cloned into pLG669Z at the *Sma*I and *Bam*HI sites. The resulting plasmid thus contains a 2 μm origin of replication and a yeast *URA3* gene, in addition to the rp55-*CYC1*-*lacZ* fusion.

The 2μm sequences were deleted from the plasmid to create YIp55-669Z. The control plasmid, YEp669Z-cycΔ, was constructed by ligating the 79-bp *CYC1* fragment described above directly into pLG669Z cut at *Sma*I and *Bam*HI. Therefore this plasmid contains neither *CYC1* nor rp55 upstream regulatory sequences.

Nucleic acid techniques. DNA was isolated from yeast cells essentially as described by Sherman et al. (29). Yeast RNA was isolated by the method described by Warner and Gorenstein (34). Nick translations and DNA polymerase I (Klenow) fill-in reactions were done by the methods of Maniatis et al. (18). DNA polymerase I (Klenow) was purchased from Bethesda Research Laboratories. The deoxynucleotide triphosphates were from Sigma Chemical Co. (St. Louis, Mo.).

Southern analysis. Electrophoresis of DNA was done on 1% agarose gels in a buffer system of 50 mM Tris acetate (pH 8), 20 mM sodium acetate, and 2 mM EDTA. DNA was transferred to nitrocellulose as described by Southern (31). Hybridization with DNA nick-translated probes and subsequent filter washes were performed as described by Bergman et al. (3).

Northern analysis. Total RNA was electrophoresed on 1.5% agarose gels containing 6% formaldehyde and blotted to nitrocellulose as described by Pearson et al. (27).

In vivo labeling, extraction, and electrophoresis of yeast

r-proteins. The protocol of Kief and Warner (15) was followed for pulse-labeling r-proteins with [^{35}S]methionine during an ethanol-to-glucose upshift. This method is described briefly in the Results section. In the experiment described here, 10 μCi of [^{3}H]methionine per ml was used for the steady-state label in ethanol medium, and 100 μCi of [^{35}S]methionine per ml was used for short pulses. Radioactive methionine was purchased from New England Nuclear Corp. (Boston, Mass.). The separation of r-proteins on two-dimensional gels and the quantitation of radioactivity in each r-protein spot is described by Warner and Gorenstein (34).

S1 nuclease mapping. The method of Berk and Sharp (4) was used to determine the 5′ transcriptional start sites of the fusion gene. YIp55-669Z was linearized with *Bam*HI and end labeled with T4 polynucleotide kinase (Bethesda Research Laboratories). This double-stranded, 5′-end-labeled DNA fragment was hybridized to 750 μg of total yeast RNA in 35 μl of 80% formamide–40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid] (pH 7.2)–400 mM NaCl–1 mM EDTA. The hybridization mix was incubated at 85°C for 20 min and then immediately cooled to 52°C. After 3 h at 52°C, the mix was gradually cooled to 37°C over a 3-h period. S1 nuclease (300 μl; 200 U/ml) in 280 mM NaCl–30 mM sodium acetate–4.5 mM zinc acetate–20 μg of denatured salmon sperm DNA per ml was added directly to the hybridization mix and incubated for 30 min at 37°C. Reaction products were then precipitated in 50% isopropanol and electrophoresed on an 8% polyacryamide-urea sequencing gel. A dideoxy sequencing ladder was used as a sizing standard.

Immunoprecipitation and quantitation of β-galactosidase protein. Antibody to β-galactosidase was purchased from Cooper Biomedical, Inc. (West Chester, Pa.). The immunoprecipitation and resolution of the immunoprecipitated products on 10% acrylamide-sodium dodecyl sulfate gels were performed by a modification of the method of Kessler (14, 24). β-Galactosidase protein was located by staining with Coomassie brilliant blue and excised from the gel, and the radioactivity in the protein was quantitated by the same method used for r-proteins.

β-Galactosidase activity assay. Yeast cells were broken by vortexing with glass beads in Z buffer (0.06 M Na_2HPO_4, 0.04 M NaH_2PO_4, 0.01 M KCl, 0.001 M $MgSO_4$, 0.05 M β-mercaptoethanol, pH 7.0). Samples of the extract were assayed as described by Miller (21). Cell debris was removed from each sample by centrifugation for 10 min at 12,000 rpm in a Sorvall SS34 rotor before optical density determinations. β-Galactosidase activity was expressed as enzyme activity per milligram of total protein in the sample. Protein determinations were done by the method of Bradford (6).

Yeast transformation. Yeast transformation was performed by the LiCl method (13). Transformants were selected on synthetic medium lacking uracil.

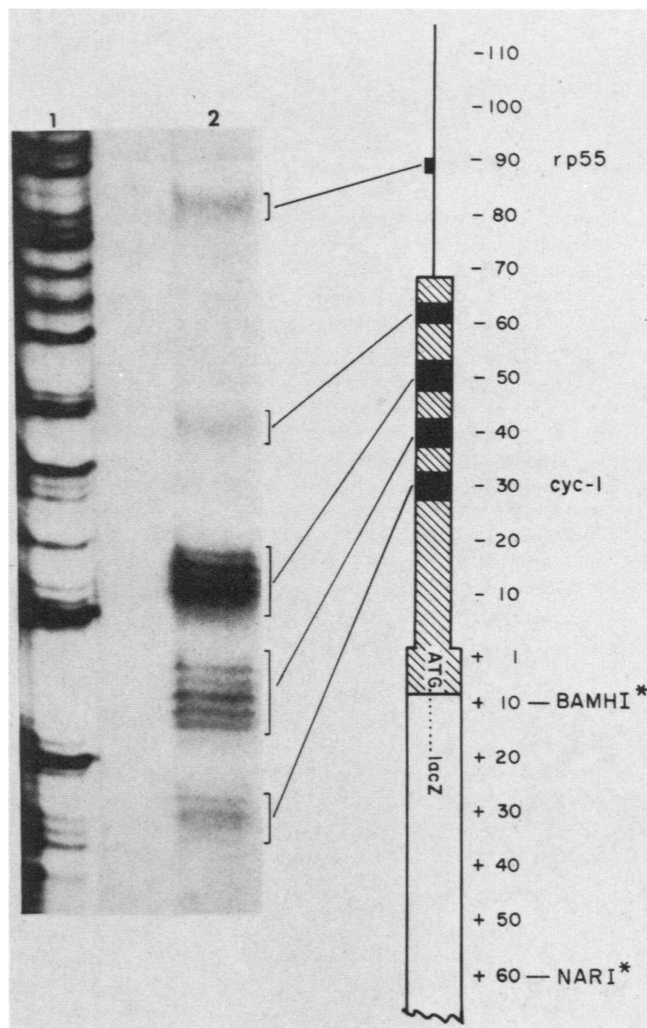

FIG. 2. Determination of transcriptional start sites by S1 mapping. S1 nuclease mapping was performed as described in Materials and Methods. Lane 1, Dideoxy chain termination sequencing ladder size markers; lane 2, DNA fragments protected from S1 nuclease digestion with 750 μg of total RNA from strain JL6B(YIp55-669Z). DNA was end labeled at the *Bam*HI site as indicated. ▨, *CYC1* sequences; ——, rp55 sequences. The exact sizes of the protected fragments around −90 and −63 were not determined. The other fragments start at positions −30 to −33, −37 to −42, and −45 to −49. Identical results were obtained when RNA was isolated from the same strain harboring the replicating plasmid [JL6B(YEp55-669Z)]. No protected fragments were observed when total RNA from the host JL6B was used. Finally, identical start sites were detected when DNA was end labeled at the *Nar*I site shown in the diagram.

RESULTS

Construction of the YEp55-*lacZ* and YIp55-*lacZ* fusion plasmids. The constructions of the gene fusions used in this report are diagramed in Fig. 1 and described in the Materials and Methods section. These fusions are derivatives of pLG669Z constructed by Guarente and Ptashne (10). The final construct contains only rp55 5′ nontranscribed sequences (528 bp) and no *CYC1* transcriptional regulatory sequences (9). The only *CYC1* sequences that remain in the fusion are 71 bp 5′ to and including the ATG translational start codon. These 71 bp provide the necessary yeast transcriptional and translation start sites and presumably a yeast ribosome-binding site. They do not include any of the sequences shown to regulate the *CYC1* gene (9).

The fusion joint is 2 bp downstream from the rp55 TATA-like box. Closely linked upstream from rp55 in the genome is another rp gene, rp28-1 (23). Our fusion contains the last seven amino acid codons of rp28-1 and its translational and transcriptional stop sites. This should prevent readthrough transcription occurring from far upstream sites on the plasmid. As shown in Fig. 1, we constructed both an autonomously replicating fusion plasmid (YEp55-669Z) and an

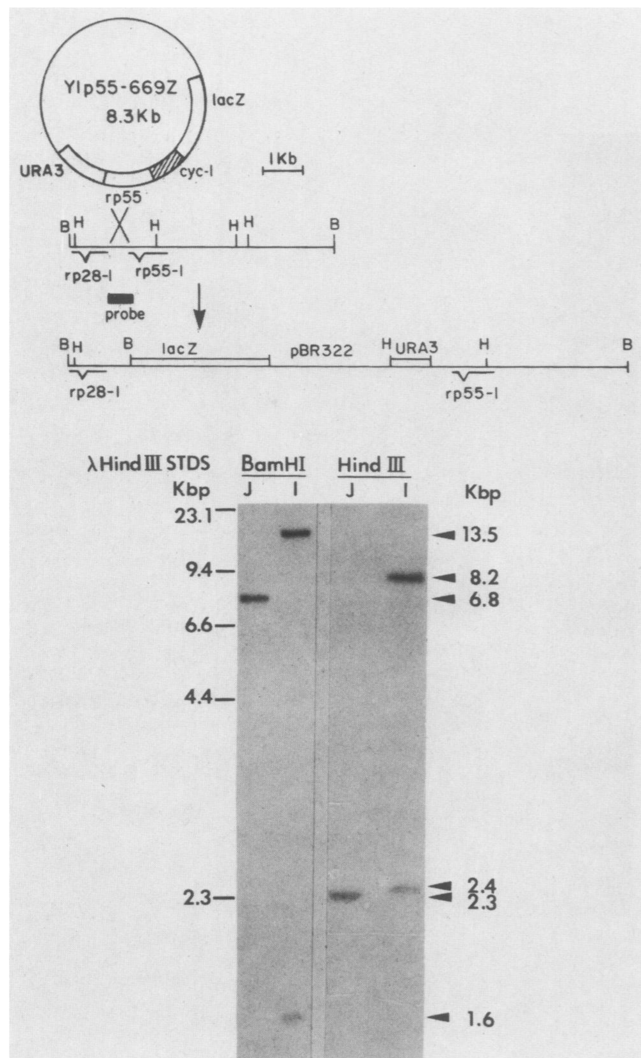

FIG. 3. Southern analysis of YIp55-669Z integrated at the rp55-1 locus in strain JL6B. A schematic of the integration event is shown in panel A. The nick-translated probe used to probe southern blots in this experiment was a 630-bp *Bgl*II-*Taq*I fragment (cloned into mp8) from the intergenic region between rp28-1 and rp55-1. Yeast DNA from strains JL6B and JL6B(YIp55-669Z) was cut with *Bam*HI and *Hin*dIII (B). Integration of a single copy of the fusion plasmid at the rp55-1 locus in JL6B produces unique 13.5- and 1.6-kilobase (kb) *Bam*HI fragments hybridizable to the probe and unique 8.2- and 2.4-kilobase *Hin*dIII fragments. JL6B without the integration produces a 6.8-kilobase *Bam*HI fragment and a 2.3-kilobase *Hin*dIII fragment. J, JL6B host strain; I, host strain with plasmid integrated. The genomic representations are drawn to scale, the circular plasmid is not. STDS, Standards; B, *Bam*HI; H, *Hin*dIII.

integrating fusion plasmid without the 2μm origin of replication (YIp55-669Z). In addition, we constructed a plasmid which lacks both the rp55 upstream region and the *CYC1* upstream region (YEp669Z-cycΔ).

S1 nuclease mapping of the rp55-669Z transcriptional start sites. To confirm that there are no r-protein sequences present in the transcripts produced by the fusion, we determined the transcriptional start sites by the S1 nuclease mapping procedure of Berk and Sharp (4). All of the major start sites were within the 71 bp of *CYC1* sequences (Fig. 2). The same pattern of site usage was observed with both YIp55-669Z and YEp55-669Z (data not shown). There was a weakly expressed transcript that appeared to have its origin in the rp55 sequences (Fig. 2). We estimate that this start site may contribute maximally 5 to 10% of the total message from the fusion. The fact that greater than 90% of the transcripts produced from this fusion start in the *CYC1* region indicate that no rp55 sequences are present in the vast majority of the fusion mRNAs. Therefore, if β-galactosidase expression is regulated like rp55, then this regulation must be mediated by 5′ nontranscribed sequences from rp55 present in the fusion.

Expression of rp55-669Z fusion during steady-state growth in ethanol- and glucose-based media. Yeast strain JL6B was transformed with plasmids YEp55-669Z, pLG669Z, YIp55-669Z, and YEp669Z-cycΔ in separate experiments, and transformants were selected as described in Materials and Methods. It was demonstrated by Southern analysis that YIp55-669Z was integrated at the rp55-1 locus in single copy in the exact location of the native rp55 gene downstream from rp28 (Fig. 3). This integrant is very stable. After over 50 generations of nonselective growth, no loss of plasmid marker was detected in >300 colonies screened. YEp55-669Z and pLG669Z were shown to be replicating plasmids by Southern analysis (data not shown) and by the fact that plasmids were rapidly lost when cells were removed from selective media.

The level of β-galactosidase activity per milligram of protein was measured from cells harboring these fusions which were growing logarithmically in EMM medium, G-E medium, or glucose-based medium (described in Materials and Methods). These results are summarized in Table 1.

For cells harboring the parent plasmid, pLG669Z, which contains the full *CYC1* upstream regulatory region, the level of β-galactosidase activity was significantly increased in glycerol-ethanol-based medium compared with that in glucose-based medium. This control is consistent with previous studies. The transcription of *CYC1* is high in ethanol medium and glucose repressed (10). But the opposite was true of the cells harboring YEp55-669Z. In this case there was a threefold increase in β-galactosidase activity in glucose cultures as compared with that of cells grown in ethanol-based medium. Thus, simply by changing the 5′ nontranscribed regions one reverses the relative steady-state levels of β-galactosidase activity in logarithmically growing cells in these two media. This clearly demonstrates that the rp55 5′ nontranscribed sequences play a role in regulation of this r-protein gene under these conditions. The increased levels of expression and the magnitude of the increase are also consistent with the increase in relative rates of synthesis of the r-proteins during this shift (15). This experiment also

TABLE 1. β-Galactosidase activity levels in different growth media

Strain	Growth conditions	β-Galactosidase levels[a]
JL6B(pLG669Z) replicating	Glycerol-ethanol medium	3,140
	2% glucose	1,225
JL6B(YEp55-669Z) replicating	2% ethanol	78
	2% glucose	255
JL6B(YIp55-669Z) integrated	2% ethanol	51
	2% glucose	107

[a] Measured as β-galactosidase activity per milligram of protein.

provides functional evidence that sequences necessary to promote and regulate expression of rp55 are in the intergenic region between rp28 and rp55 genes as has been suggested by the fact that this region contains many of the short conserved sequences found 5′ to other r-protein genes (17, 33). Furthermore, yeast cells containing the plasmid construct which is deleted for the 528-bp rp55 5′ region (YEp669Z-cycΔ) showed no detectable β-galactosidase activity, demonstrating that there is no significant readthrough transcription from 5′-adjacent regions on the plasmid resulting in expression of the fusion product. Finally, the fact that the fusion gene containing the rp55 upstream sequences is expressed and regulated on a replicating plasmid like a r-protein gene indicates that these regulatory sequences need not be in the genome adjacent to the rp28 gene to promote expression and proper regulation.

It is possible that the differences in absolute values for the β-galactosidase activity in the cells with the two different replicating plasmids are due to differences in plasmid copy number. Since we cannot control copy number in these experiments and do not know what effects shifting from ethanol to glucose medium might have on copy number and segregation of plasmids during mitotic divisions, we decided to measure β-galactosidase levels from the integrated form of this plasmid (Fig. 3). In this case, a twofold increase in β-galactosidase activity per milligram of protein was seen in

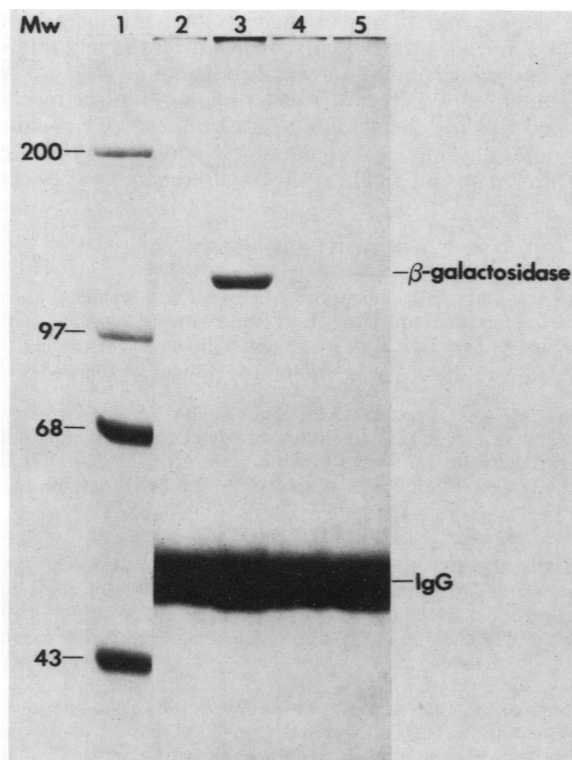

FIG. 4. Sizing of the immunoprecipitated β-galactosidase protein on an 8% polyacrylamide-sodium dodecyl sulfate gel. Immunoprecipitation with β-galactosidase antibody from yeast cell extracts was carried out as described in Materials and Methods. The precipitated products were then run on an 8% polyacrylamide gel and stained with Coomassie brilliant blue (30). Lanes: 1, molecular weight (Mw) markers (×10³); 2, yeast strain JL6B; 3, JL6B(pLG669Z); 4, JL6B(YEp55-669Z); 5, JL6B(YIp55-669Z). The amount of protein loaded in each lane is not identical. IgG, Immunoglobulin G.

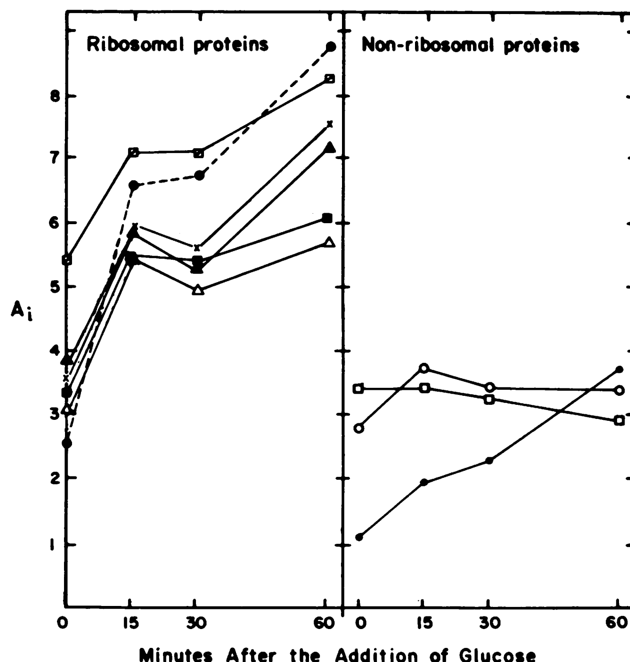

FIG. 5. Relative rates of synthesis (A_i) of r-proteins and β-galactosidase during an ethanol-to-glucose upshift for JL6B(YIp55-669Z). A_i values were determined for several r-proteins and β-galactosidase protein at 0, 15, 30, and 60 min during the upshift as described in the Results. Five r-proteins and β-galactosidase are graphically displayed. Symbols: □, rp55; ×, rp53; ▲, rp52; △, rp39; ■, rp38; ●, β-galactosidase. The non-r-proteins are unidentified but appear as recognizable spots on the two-dimensional gel used to resolve r-proteins. These results are the average of two independent experiments. Differences in values were <20%. A total of eight r-proteins were measured. All showed similar kinetics.

the glucose-grown cells as compared with that of the ethanol-grown cells (Table 1). The magnitude of this increase is slightly less than that seen in experiments with replicating plasmids, but is actually more consistent with the results described in the next sections.

Expression of the integrated rp55-669Z fusion during the first hour of an ethanol-to-glucose shift. The experiments described above demonstrate that the level of β-galactosidase activity in cells harboring the rp55-669Z fusion is two- to threefold greater in steady-state growth in glucose as compared with that in ethanol. However, we were more interested in knowing how this r-protein gene was regulated during the first hour of the shift when the relative rates of synthesis of r-proteins are rapidly changing. Before the shift, the generation time of the cells growing in ethanol was 6 to 8 h. Immediately after the addition of glucose, the cells appeared to stop growing for a period of 1 to 1.5 h and then resumed logarithmic growth at a new generation time of 3 to 4 h. Interestingly, it was during this period of arrested growth that the relative rates of synthesis of r-proteins accelerated. A similar phenomenon was observed by Kief and Warner (15). Based on microscopic observation, the cells do not appear to pause at a specific stage of the cell cycle.

To directly and accurately compare the relative rates of synthesis of β-galactosidase protein to rates of synthesis of r-proteins, as has been previously measured during the shift, it was necessary to devise a method to measure the rate of synthesis of β-galactosidase protein rather than just its

accumulation. This was facilitated by immunoprepitation of β-galacactosidase with a commercially available polyclonal antibody, followed by resolution of the protein on a one-dimensional sodium dodecyl sulfate-polyacrylamide gel (Fig. 4).

To measure the relative rates of synthesis of β-galactosidase and several r-proteins in an upshift experiment, we followed the protocol of Kief and Warner (15). Briefly, strain JL6B with the integrated YIp55-669Z was grown in EMM medium for several generations and then labeled with [^{3}H]methionine in the early log phase for approximately two generations. While the culture was still in the log phase, glucose was added to give a final concentration of 2%. At time points immediately before and after the addition of glucose, 5-min pulses with [^{35}S]methionine were done. Cells were harvested, and a portion of the cell pellet was used for immunoprecipitation of β-galactosidase. From another portion, proteins were acid extracted and applied to a two-dimensional gel system used to resolve r-proteins (34). In each case unlabeled cold protein was added to locate the position of proteins of interest by staining with Coomassie brilliant blue. The β-galactosidase protein band and the r-protein spots on the gels were punched out and processed as described in the Materials and Methods, and the ^{35}S and ^{3}H counts were determined in a scintillation counter. The ^{35}S-to-^{3}H ratio for each protein spot was calculated for each time point. This was divided by the same ratio for total protein at that time point. This gives a relative rate of synthesis of an individual protein relative to the average rate of total protein synthesis at that time point. This number is designated A_i (34).

The results from this experiment are shown in Fig. 5. Over a period of 1 h we saw approximately a twofold increase in the A_i values for both β-galactosidase and r-proteins. This change was slightly less than that seen by Kief and Warner (15). This may be due to strain differences. But this twofold difference is consistent with our steady-state measurements of β-galactosidase activity described above. Most importantly, it can readily be seen that the change in rate of synthesis of the β-galactosidase protein during an ethanol-to-glucose upshift follows the same kinetics as the coordinate change in the rates of synthesis of the r-proteins measured, including rp55. We also measured the change in rates of synthesis of several non-r-proteins which appeared on the two-dimensional gel. Although these showed a variety of changes, they did not appear to be coordinated with r-proteins.

This result strongly suggests that the nontranscribed sequences 5′ to rp55 are necessary and sufficient to mediate the acceleration in the rates of synthesis of r-proteins seen immediately after the addition of glucose in this carbon source upshift.

DISCUSSION

The coordinate regulation of ribosome synthesis is complex. In yeasts, there is evidence to suggest that r-proteins may be regulated on several different levels. Some examples are given in the Introduction. Our aim in this report is to determine at what level of synthesis r-proteins are regulated under a specific physiological condition, namely, a nutritional upshift from growth in an ethanol-based medium to growth in a glucose-based medium. Our experiments show that a change in transcription rates accounts for the observed change in rates of r-proteins during this upshift. In addition, we showed that untranscribed sequences 5′ to the gene mediate this response in the r-protein gene we studied.

It is interesting to note that our fusion utilizes similar but not identical transcriptional start sites as those used by the native *CYC1* gene (7, 19). This is consistent with the hypothesis that other factors in addition to consensus sequences determine the position of start sites of *CYC1* such as the position of the functional TATA elements (11).

Our experiments do not prove that all r-proteins are regulated in a similar manner during this upshift. However, we think that this is likely to be true for the following reasons. The change in relative rates of synthesis of all the r-proteins (and β-galactosidase) that we measured during the shift followed very similar kinetics, suggesting some common underlying mechanism. In addition, the accumulation of mRNAs of a few r-proteins we measured by Northern analysis during the shift also appeared qualitatively similar (data not shown). rp55 is a very typical r-protein gene. It has an intron near the 5′ end of the protein-coding sequence, it is duplicated, and it has all the conserved consensus sequences which appear upstream to approximately 80% of the r-protein genes examined (17, 33). Therefore, we have no reason to believe it would be exceptional and not be representative of other r-protein genes in general.

A significant observation during this nutritional upshift experiment is that the growth of cells is arrested immediately upon the addition of glucose. They resume growth at a faster rate about 1 to 1.5 h later. The rapid acceleration of r-protein synthesis also occurs during this arrested growth period, possibly because an increase in the number of ribosomes per cell is required before the new, faster growth rate is established. This correlation also suggests that the increase in rates of synthesis of r-proteins signalled by the addition of glucose is connected in some way to a higher level of growth rate regulation. Whether a transcriptional control mechanism mediates the change in rates of synthesis of r-proteins under other physiological conditions in addition to this type of nutritional upshift and heat shock (16) remains to be seen.

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