Altered response to growth rate changes in *Kluyveromyces lactis* versus *Saccharomyces cerevisiae* as demonstrated by heterologous expression of ribosomal protein 59 (CRY1)

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ABSTRACT

We report the cloning, characterization and preliminary analysis of the regulation of the gene coding for ribosomal protein 59 (RP59) from the budding yeast *Kluyveromyces lactis*. The RP59 gene is present as a single copy, contains an intron within the amino terminal coding portion of the gene, and harbors conserved *S.cerevisiae* splicing signals. Sequence elements upstream of the transcriptional start site are homologous to UAS_{RPG}, known to regulate the transcription of numerous genes in *S.cerevisiae* via their interaction with the *trans*-activating factor RAP1. These elements are necessary for transcription of RP59 in both *K.lactis* and *S.cerevisiae* hosts. UAS_{RPG} in *S.cerevisiae* rp genes also modulate the transcription of rp RNA synthesis in response to a growth rate upshift. In *K.lactis*, the RP59 gene does not respond to growth rate upshift. Reciprocal expression of RP59 and CRY1 in heterologous hosts demonstrates that glucose upshift occurs in *S.cerevisiae* but not *K.lactis*. These results demonstrate that a factor or factors required for growth upshift are lacking in *K.lactis*, and provide further evidence that the UAS_{RPG} are sufficient signals for modulating this response.

INTRODUCTION

Ribosomes are composed of 4 different rRNAs and approximately 75 different ribosomal proteins (rp) in *S.cerevisiae* (1–3). The ribosomal proteins, which can account for greater than 15% of the total cellular protein, are produced stoichiometrically along with the various rRNAs. Ribosomal proteins are highly conserved among prokaryotes and between both higher and lower eukaryotes, suggesting equivalent functional roles (4,5). Recently, a mouse rp has been shown to be interchangeable with its yeast homolog, albeit with reduced functionality (6). We therefore reasoned that phylogenetic comparisons between ribosomal encoding sequences from closely related organisms would serve

to identify functionally conserved regulatory regions and provide insight into the mechanism of ribosomal protein regulation.

The production of equimolar amounts of mRNAs for the majority of ribosomal proteins is the primary method by which *S.cerevisiae* regulates rp production. UAS_{RPG}, (consensus, 5'ACACCCATACATTT³) upstream of most rp genes are responsible for modulating this effect (7–9). A *trans*-activating factor RAP1 (TUF, GRF1) is believed to specifically bind UAS_{RPG} sequences coordinately stimulating the transcription of genes bearing this site (10–13). UAS_{RPG} are also necessary and sufficient for mediating an increase in the transcriptional rate of rp genes in response to growth rate changes; typically, such as when cells are shifted from a nonfermentable (ethanol or pyruvate) to a fermentable carbon source (glucose)(14). The increased transcription rate in response to growth rate upshift is thought to reflect enhanced binding of RAP1 to UAS_{RPG}.

K.lactis genes have been shown to functionally complement several *S.cerevisiae* mutants (15,16). Additionally, the presence of an UAS upstream of *K.lactis* LAC4 (UAS_{LAC}) and its homology to UAS_{GAL} led to the discovery that *K.lactis* LAC9 can *trans*-activate the galactose regulon in a *S.cerevisiae* gal4 mutant (17). We have previously shown that ACT1 precursor mRNA can be reciprocally spliced in *K.lactis* and *S.cerevisiae* (18). Thus major similarities exist in the regulatory mechanisms between *K.lactis* and *S.cerevisiae*. To examine the regulation of rp synthesis in *K.lactis* we utilized the *S.cerevisiae* rp59 gene (CRY1) to isolate the ribosomal protein 59 gene (RP59) from *K.lactis* and have analyzed the regulation of this gene in *K.lactis*.

Our results demonstrate the RP59 gene is present as a single copy gene and has greater than 95% homology with the coding regions of the *S.cerevisiae* CRY1 gene. A single intron interrupts the fourth codon of the gene, and retains the conserved *cis*-acting splicing signals common to *S.cerevisiae* introns (19). Two copies of a sequence homologous to the *S.cerevisiae* consensus UAS_{RPG} are located in the 5' regulatory region. We demonstrate that at least one copy of this element is required for transcription of RP59 in both *K.lactis* and *S.cerevisiae* hosts. Transcription

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of this gene appears to be unregulated in *K.lactis*, as evidenced by a constitutively high level of transcription under all growth conditions. In contrast, the *RP59* gene is down regulated when transformed into a *S.cerevisiae* host, and transcription can be increased 3–5 fold by the growth rate shift accompanying a carbon source change from ethanol to glucose. Our data demonstrate that a factor, or factors required for transcriptional regulation of ribosomal protein genes is absent in *K.lactis* and suggest a novel form of regulation for the ribosomal components in *K.lactis*.

MATERIALS AND METHODS

Yeast, bacterial strains and yeast transformation

Plasmids were propagated in *E.coli* MV1190 (Bio-Rad) or MC1061 (20) grown in LB medium supplemented with ampicillin (30mg/l). The yeast strains *K.lactis* 5D298 (*lac4-8, ura3-1, ade3-1*) (furnished by M. Riley and R. Dickson, Univ. Kentucky) and *S.cerevisiae* JM43 (*his3-11,15, ura3, leu2-3, trp1*) were used (21). *S.cerevisiae* and *K.lactis* were grown in yeast complete medium (YPD) unless otherwise noted (22). *S.cerevisiae* and *K.lactis* were transformed using lithium acetate and selected as URA⁺ (23).

Plasmids

A *K.lactis* genomic library was constructed by partially digesting *K.lactis* strain Y1140 with *Sau3A* and 10–20kb fragments were cloned into the *Bam*HI site of p1B3 (kindly supplied by M. Riley and R. Dickson) (17). All subcloning of fragments was done into pTZ18U (Bio-Rad) using standard techniques (24,25). A *S.cerevisiae* plasmid harboring the complete *K.lactis* *RP59* gene was constructed by cloning the 1.95kb *HinP I-Bgl II* fragment using polylinker flanking *Bam HI-Sal I* sites into *Bam HI-Sal I* digested pYEp24 (26). The *S.cerevisiae* *CRY1* gene (27) was cloned into the *K.lactis* vector pKEp24 (this laboratory) by first replacing the 2 micron *S.cerevisiae* replication origin of pYEp24 with a 1.1kb *KARS1B* fragment derived from p1B3 to render it capable of replication in *K.lactis*. Next, a 2.2kb *Hind III* fragment from pJT22 (kindly supplied by J. Woolford) bearing the intact *S.cerevisiae* *CRY1* gene was rendered flush ended, *Bam HI* linkers added, and inserted into the *Bam HI* site of pKEp24.

DNA sequence analysis

DNA sequence analyses were carried out on denatured plasmid subclones using the dideoxy chain termination method (Sequenase, US Biochemicals) (28,29).

β -Galactosidase fusions

In-frame transcriptional/translational β -Galactosidase fusion plasmids were constructed by first creating a *Sal I* site at codons 5/6 in exon 2 of *RP59* by site directed mutagenesis (Muta-gene, Bio-Rad) (30). Subsequently a 1.2kb *Bam HI-Sal I* fragment containing the *K.lactis* *RP59* promoter, exon 1, intron and the 5' end of exon 2 was cloned into the β -galactosidase expression vectors p2UB (*S.cerevisiae*) or pKUB (*K.lactis*) (this laboratory). p(-UAS) plasmids were created by deleting both endogenous UAS elements by cleavage with *Bst BI* (pos. –222), rendering the site flush-ended, cleaving with *Sal I*, and subsequently cloning into p2UB and pKUB. p2UB(UAS_{SYN}) and pKUB(UAS_{SYN}) were created by cloning into the *Bam HI* site of each UAS deletion mutant an 18bp synthetic oligonucleotide duplex containing the UAS sequence located at –265 flanked by *Sau 3A* sites (5'GA-

TCCACCCAGACATTGT3'). β -galactosidase fusions activities were determined from glucose grown log phase cells as described (25).

Nucleic acid preparation and blot analyses

Plasmid DNA was purified and manipulated using standard techniques (24). Yeast RNA was prepared from mid log phase cells ($A_{600}=2.0$) grown in YPD by the hot phenol extraction method (31). Poly A⁺ RNA was fractionated on oligo dT cellulose as described (24). RNA was fractionated on 1.5% agarose gels containing MOPS/formaldehyde and transferred to a nylon membrane (Zeta-Probe, Bio Rad)(25). Blots were hybridized to a 0.43kb random-prime ³²P labeled exon 2 specific *Hae III-Hinc II* fragment in the presence of 6×SSPE, 1% SDS, and 0.5% non-fat dry milk at 65°C (Amersham)(32). Filters were subsequently washed in 0.1×SSC at 25°C and autoradiographed.

RNA sequence analysis and transcript mapping

The splice junction was sequenced and transcriptional start sites identified by sequencing Poly A⁺ RNA. An 82bp, 5' end labeled *Dde I-Hpa II* fragment labeled at the *Hpa II* site was used to prime AMV reverse transcriptase (Life Sciences) mediated cDNA synthesis as described (33). The products were separated on a 6% polyacrylamide, 7M urea sequencing gel. Analysis of the 3' end of the *RP59* transcript was accomplished with S1 nuclease analysis as described (34). Briefly, total RNA was annealed to a 253bp, 3' end labeled *Hpa II-Hinc II* restriction fragment labeled at the *Hpa II* site. After annealing, S1 nuclease was added and the reaction incubated at 37°C for 30 min. The reactions were phenol extracted, ethanol precipitated, and the products separated on a 6% polyacrylamide 7M urea sequencing gel. The 3' labeled fragment was subjected in parallel to chemical sequencing (35).

Glucose upshift

Cells for glucose-upshift were grown in 0.67% yeast nitrogen base, 0.04% glucose, 2% ethanol supplemented with 20 mg/l uracil and 20 mg/l adenine (*K.lactis*); 20 mg/ml adenine (*K.lactis* transformants); 20 mg/l histidine, 20 mg/l leucine, 20 mg/l tryptophan, and 20 mg/ml uracil (*S.cerevisiae*); or without uracil (*S.cerevisiae* transformants). Cells were grown to app. $A_{600}=0.8$, then 0.1 volume of 20% glucose was added and the cells rapidly mixed. Samples were taken immediately prior to (0'), 15, 30, and 60 min. after glucose addition. Cell pellets were immediately frozen in dry ice prior to RNA extraction as described above.

Quantitative primer extension analysis

Total RNAs were annealed to oligonucleotide primers complementary to the unique 3' untranslated region of either *S.cerevisiae* *CRY1* (SC3'UT, 5'AATACGGCAATACAAGTACAATACATGCATAAC3') or *K.lactis* *RP59* (KL3'UT, 5'GTGATGAAATACCGAGAAAATACTAATGGCAAA3') in the presence of a 23mer (ACT, 5'ACGTAAAGTCAAGATACCTCT3') complementary to a conserved region of exon 2 in both *K.lactis* and *S.cerevisiae* *ACT1* mRNAs (18). Briefly, 5μg total RNA was annealed to either 0.5pmol ³²P kinase labeled SC3'UT or KL3'UT primer in the presence of 0.5pmol kinased *ACT1* primer in 10μl. The mixture was held at 80°C for three minutes and cooled to 37°C over a period of 60 min. Reverse transcription was carried out at 45°C for 30 min. as described above. The products were analyzed on 6% polyacrylamide, 7M

urea sequencing gels, autoradiographed, and later quantitated on a radioanalytic imaging system analyzer (AMBIS Systems, San Diego, CA). *K.lactis* *RP59* and *S.cerevisiae* *CRY1* transcripts were normalized to *ACT1* mRNA levels at each time point.

RESULTS

Characterization of the *K.lactis* *RP59* gene

A *K.lactis* genomic DNA bank was prepared and colonies screened with a *S.cerevisiae* *CRY1* exon 2 specific restriction fragment (36). A 1.95kb *Hin* *PI-Bgl* *II* fragment containing the *RP59* gene was identified and subsequently sequenced (data not shown, EMBL accession number X59860). Initial sequence analyses revealed a potential intron with a branch point sequence (TACTAAC) identical to that found in *S.cerevisiae* introns. The presence of an intron in *RP59* was confirmed by sequencing poly A⁺ RNA with an exon 2 specific primer (Fig. 1). The intron interrupts the fourth codon and is 756 nucleotides in length. By contrast, the *S.cerevisiae* *CRY1* intron interrupts codon 3 and is only 307bp (27). The conserved *S.cerevisiae* intron splicing signals are all present: 5' splice site (GTACGT) (pos. +36), branch point sequence (TACTAAC) (pos. +747), and 3' splice site (CAG) (pos. +790). The 5' splice site varies by a single nucleotide from the yeast preferred sequence GTATGT (19). The coding region of *RP59* is over 95% homologous with *CRY1*, and encodes a predicted protein of 137 amino acids.

To determine the copy number of the *RP59* gene, genomic DNA was digested with a variety of restriction enzymes. When

an exon 2 specific fragment was hybridized to DNA blots from digests with a panel of restriction enzymes, a single band was seen in each case indicating a single copy gene for *RP59* (data not shown).

Examination of sequences upstream of the transcriptional start sites reveals two sequences highly homologous to the *S.cerevisiae* UAS_{RPG}. These sequences are located at pos. -264 (ACACCC-AgACATT) and pos. -310 (ACAaCCGTGgAgT) (Fig. 1, lower case denotes variations from the *S.cerevisiae* consensus UAS_{RPG}). Such motifs have been shown to play a role in the transcriptional regulation of ribosomal protein genes during steady state growth and are required for enhanced transcription following carbon source upshift (14,7). Besides these motifs and multiple TATA boxes located at app. -50, no other sequence similarities to *S.cerevisiae* rp genes were observed in the flanking regions.

Analysis of the *RP59* transcription unit

RNA blot hybridization analysis of total and Poly A⁺ RNA, probed with an exon 2 specific fragment identified one transcript of approximately 560 nucleotides (Fig. 2). A faint band of about 1300 nucleotides believed to represent unspliced precursor RNA was also detected. When a duplicate blot was hybridized with an intron specific probe, only the 1300 nucleotide transcript hybridized indicating that this fragment corresponded to precursor RNA (data not shown).

The 5' and 3' termini of the *RP59* transcript were identified using primer extension and S1 nuclease analyses respectively. Primer extension analysis yielded two products of 127 and 119 nucleotides (Fig. 1), suggesting the transcriptional start sites are at the two cytidines indicated in Figure 1, 25 and 18 nucleotides respectively, upstream of the ATG translational initiation codon. The 3' terminus of the *RP59* transcription unit was identified

Figure 1. Mapping the splice junction and 5' ends of the *RP59* transcript. An exon 2 5' end-labeled 82nt *Dde* *I-Hpa* *II* fragment was hybridized to Poly A⁺ RNA from *K.lactis*, and cDNA was synthesized from the resulting hybrids in the presence of reverse transcriptase, dNTPs, and ddNTPs. The reaction mixes were denatured and subjected to electrophoresis on 6% polyacrylamide, 7M urea sequencing gels. Above each lane are the indicated ddNTPs. The DNA sequence shown is complementary to the mRNA coding strand. The arrow indicates the location of the intron. The primer used is shown below. Hatched box, intron; solid, exon. MW, pBR322 digested with *Hpa* *II* was used as a molecular weight standard.

Figure 2. Determination of *RP59* transcript size. RNA was isolated from mid-log phase cultures grown in YPD, subjected to electrophoresis in formaldehyde/agarose gels, and blotted to a nylon membrane. Lane 1, 1μg total RNA; lane 2, 10μg Poly A⁺ RNA; lane 3, 1μg Poly A⁺ RNA. Hybridization was carried out with a random-prime labeled exon 2 specific probe shown below. Lambda DNA molecular weight markers in kbp are shown.

using S1 nuclease analysis (Fig. 3). A 3' end labeled restriction fragment was annealed with *K. lactis* total RNA and the hybrids digested with S1 nuclease. A protected fragment of 209 nucleotides was produced, indicating that the *RP59* transcript is polyadenylated at the thymidine residue at pos. +1316, 119 nucleotides downstream from the termination codon. The spliced transcript is thus 555 nucleotides in length.

UAS_{RPG} sequences are required for efficient expression of *RP59*

Since *RP59* retained sequences homologous to *S. cerevisiae* UAS_{RPG} known to control the transcription of numerous genes in *S. cerevisiae*, we examined the transcriptional activating potential of these motifs in both *K. lactis* and *S. cerevisiae*. *E. coli*-*lac Z* fusions containing the *RP59* 5' flanking region, exon 1, intron, and 5' end of exon 2 were constructed. β -galactosidase activities of mutants harboring specific deletions in the 5' flanking region of *RP59* were subsequently measured in glucose grown *K. lactis* and *S. cerevisiae* transformants (Table I). The deletion of the two endogenous UAS reduced β -galactosidase activity 15 fold and 6 fold in *K. lactis*, and *S. cerevisiae* respectively. A single synthetic UAS element (UAS_{SYN}), identical to that located at pos. -264 (5'GATCCACCCAGACATTGT^{3'}), restored transcription to near wild type levels in both organisms. These results demonstrate that UAS homologs present in the 5' flanking region of *RP59* are functionally similar to the *S. cerevisiae* UAS_{RPG} homologs.

Figure 3. S1 nuclease mapping the 3' ends of the *RP59* mRNA. A 3' end-labeled 253bp *Hpa II-Hinc II* fragment was annealed to total RNA from *K. lactis*. S1 nuclease analysis was performed and the products separated on a DNA sequencing gel. Lane 1, fragment without S1; lane 2, fragment with S1 (100U/ml); lane 3, 1µg total RNA plus S1 (100U/ml); lane 4, 10µg total RNA plus S1 (100U/ml). G, G+A, T+C, and C represent Maxam-Gilbert sequencing reactions of the 3' labeled fragment run in parallel. The asterisk indicates the major protected fragment. MW, pTZ18U digested with *HpaII* was used as a molecular weight standard. The 3' end-labeled restriction fragment is depicted below.

K. lactis RP59 does not respond to glucose upshift

UAS_{RPG} present upstream of *rp* genes in *S. cerevisiae* are known to be necessary for mediating a 3–5 fold increase in the transcription rate of these genes when cells are growth rate upshifted from ethanol (six hour generation time) to glucose (two hour generation time)(14). Since UAS_{RPG} are necessary for the efficient transcription of *RP59* in both *K. lactis* and *S. cerevisiae*, we decided to further test if *RP59* transcription was growth rate dependent. Growth rate experiments demonstrated that *K. lactis* cells responded like *S. cerevisiae* cells in that they showed comparable growth rate kinetics when shifted from ethanol to

Table I. *RP59* contains UAS_{RPG} which function in *K. lactis* and *S. cerevisiae*.

Construct	Relative β -Galactosidase Activity	
	<i>K. lactis</i>	<i>S. cerevisiae</i>
pKUB/p2UB	0.02	0.01
pKUB(<i>RP59</i>)/p2UB(<i>RP59</i>)	1.00	1.00
pKUB(-UAS)/p2UB(-UAS)	0.06	0.17
pKUB(UAS _{SYN})/p2UB(UAS _{SYN})	0.61	0.83

Extracts were prepared from *K. lactis* or *S. cerevisiae* transformants grown in glucose minimal media containing adenine (*K. lactis*), or containing histidine, leucine, and tryptophan (*S. cerevisiae*). β -galactosidase activities listed are relative to those obtained from the parental plasmids pKUB(*RP59*) and p2UB(*RP59*) in their respective host. β -Galactosidase activities represent levels obtained from duplicate samples and varied by no more than 20% between duplicate samples. Absolute β -galactosidase activities were, in general, 2–3 times higher in *K. lactis* than *S. cerevisiae*.

Figure 4. *K. lactis RP59* does not respond to glucose upshift. RNAs from glucose upshifted *S. cerevisiae* and *K. lactis* cells immediately prior to (0'), 15, 30, and 60' after glucose upshift were analyzed by quantitative primer extension analysis as described in Materials and Methods. *S. cerevisiae* RNAs prepared from ethanol to glucose upshifted cells (left) were reverse transcribed in the presence of SC3'UT and ACT oligonucleotide primers. *K. lactis* RNAs (right) were reverse transcribed in the presence of KL3'UT and ACT primers. SC3'UT and KL3'UT primers yield extension products of nearly identical size in the gel system used. ACT (sc), actin from *S. cerevisiae*; ACT(kl), actin from *K. lactis*. MW, marker pGEM-3Z (Promega) digested with *Hpa II*.

glucose as carbon sources. This shift is accompanied by an increase in ribosome biosynthesis as determined from measurements of the steady state level of 17S rRNA (data not shown).

Ethanol grown cultures of *S.cerevisiae* and *K.lactis* were glucose upshifted and total RNAs isolated. Quantitative primer extension analyses using oligonucleotide primers unique to the 3' untranslated region of *CRY1* (SC3'UT), and *RP59* (KL3'UT) were performed on total RNAs isolated from these cultures (Fig. 4). An exon 2 specific primer (ACT) complementary to both *ACT1* mRNAs was used to normalize *RP59* and *CRY1* RNA levels at each time point. A characteristic 3–5 fold increase in steady state *CRY1* mRNA levels was observed within 15' for *S.cerevisiae* cells upshifted over a 60 minute time period, while steady state *RP59* mRNA levels remained constant in upshifted *K.lactis* cells. Apparent increases in *RP59* transcript levels in *K.lactis* cells at 30' and 60' after upshift, when normalized to *ACT1* transcript levels, indicated no net increase in *RP59* transcript levels. The basal level of *RP59* transcription in ethanol grown *K.lactis* cells remains as high as in glucose grown cells.

rp mRNA synthesis in response to growth rate upshift is dependent upon host background

Since *RP59* mRNA levels did not show a glucose upshift in *K.lactis*, we next asked if UAS_{RPG} elements were capable of effecting an upshift in *RP59* mRNA levels in a *S.cerevisiae* host subjected to the same growth rate upshift. We inserted the entire *RP59* transcription unit into pYEp24 and transformed a *S.cerevisiae* host. Transformants were glucose upshifted and their RNAs isolated as described above. Since the oligonucleotide primers were specific for the 3' untranslated region of either *RP59* or *CRY1*, this allowed the unique identification of either transcript. Primer extension analyses demonstrated that *K.lactis* *RP59* responded to glucose upshift in the heterologous host in

a pattern similar to the endogenous *S.cerevisiae* *CRY1* gene (Fig. 5). *RP59* primer extension products generated in *S.cerevisiae* differed somewhat in size from those of *K.lactis*. These products probably arise as a result of the use of alternative start points in *S.cerevisiae*. We have noted this when other *K.lactis* genes are expressed in *S.cerevisiae* (18).

To confirm that the upshift of rp mRNA levels was host specific, the normally glucose responsive *S.cerevisiae* *CRY1* was placed in the *K.lactis* plasmid pKEp24 and transformed into *K.lactis*. Transformants were growth rate upshifted, total RNAs isolated, and primer extensions analyses carried out. *CRY1* transcription was shown to be unresponsive to a glucose upshift in a *K.lactis* host (Fig. 6, right). These results demonstrate that although UAS_{RPG} in *RP59* are essential transcriptional elements, their ability to respond to a carbon source induced growth rate upshift is dependent on the host background.

DISCUSSION

Utilizing a *CRY1* gene probe from *S.cerevisiae*, we have isolated the single copy gene coding for ribosomal protein 59 (rp59) from the budding yeast *K.lactis*. This rp is 95% homologous at the predicted amino acid level to *S.cerevisiae* rp59 encoded by *CRY1* (24). Five of the seven amino acid substitutions represent highly conserved changes. This is not unexpected since rp59 is one of the most highly conserved rp (4). Aside from coding sequence information, all other sequences in this gene were highly divergent from *CRY1* with the exception of splice site signals, TATA elements, and UAS_{RPG} sites.

The *K.lactis* *RP59* intron is accurately spliced when expressed in *S.cerevisiae*. This is based on two lines of evidence. First, β -galactosidase activity in the gene fusion system employed to measure the transcriptional activating potential of UAS elements,

Figure 5. *K.lactis* *RP59* responds to glucose upshift in a *S.cerevisiae* host. RNAs from *S.cerevisiae* cells transformed with *K.lactis* *RP59* were glucose upshifted and analyzed as in Fig. 4. *S.cerevisiae* *CRY1*, primer extension in the presence of SC3'UT and ACT primers (left), *K.lactis* *RP59*, primer extension in the presence of KL3'UT and ACT primers (right). ACT, *S.cerevisiae* actin. MW, marker pGEM-3Z digested with *Hpa* II.

Figure 6. *S.cerevisiae* *CRY1* is unresponsive to glucose upshift in *K.lactis*. RNAs from *K.lactis* cells transformed with *S.cerevisiae* *CRY1* were glucose upshifted and analyzed as in Figs. 4 and 5. *K.lactis* *RP59*, primer extension in the presence of KL3'UT & ACT primers (left), *S.cerevisiae* *CRY1*, primer extension in the presence of SC3'UT & ACT primers (right). ACT, *K.lactis* actin. MW, marker pTZ18U digested with *Hpa* II.

relies on the precise excision of the intron to generate an in-frame fusion transcript and subsequent enzymatic activity. Second, primer extension products in *S.cerevisiae* transformants harboring *RP59* correspond to the spliced message (Fig. 5). This supports previous data demonstrating that *K.lactis* transcripts can be accurately spliced in *S.cerevisiae* (15,18).

In the present study, two potential UAS_{RPG} elements were identified 264 and 310 nucleotides upstream of the transcriptional start point in *RP59*. UAS_{RPG} in many *S.cerevisiae* genes have been shown to be necessary for the transcription of *rp* and other genes regulated by RAP1 (12). It was of interest to see if these sequences served similar functional roles. A single copy of the UAS at pos. -264 was able to restore transcriptional activity in either *K.lactis* or *S.cerevisiae* transformants harboring a *RP59-lacZ* mutant lacking the two endogenous UAS indicating, that the UAS homologs are required for efficient transcription in both organisms (Table I).

Since UAS_{RPG} elements are required for transcription of *RP59*, we asked whether these sequences were responsive to growth rate upshift. Adding glucose to ethanol grown *S.cerevisiae* cells results in a 3–5 fold increase in *rp* transcription within 15 minutes (37). The increased mRNA synthesis is thought to reflect the increased need for ribosomal proteins, and hence ribosomes, at an increased growth rate. It is believed that RAP1 binding to UAS_{RPG} is affected by the growth rate, perhaps via modification of RAP1. Growth rate experiments indicated that *K.lactis* cells had a three-fold faster growth rate in glucose versus ethanol, and the steady state ribosome content of cells growing in glucose is twice that of ethanol grown cells (as determined from 17S rRNA levels, data not shown). Thus it is conceivable that *RP59* transcription in *K.lactis* would show a similar growth rate upshift. Surprisingly, despite these growth rate changes, *K.lactis* showed no change in *RP59* transcript levels (Fig. 4, right).

The ability of a synthetic UAS_{RPG} to stimulate the transcription of the *RP59-lacZ* fusion in a *S.cerevisiae* host suggests that *RP59* transcription could be growth rate responsive in *S.cerevisiae*. Primer extension analyses on RNAs prepared from glucose upshifted *S.cerevisiae* transformants harboring the complete *RP59* transcription unit demonstrate that *RP59* transcription (Fig. 5, left) is now regulated in a pattern identical to the endogenous *S.cerevisiae CRY1* gene (Fig. 5, right). *RP59* and *CRY1* mRNA levels increase app. 4 fold within 15' of upshift. It appeared that the ability of UAS_{RPG} to respond to growth rate changes may be species specific. Therefore, we asked if *S.cerevisiae CRY1* transcription was glucose inducible in *K.lactis*. Primer extension analyses on RNAs prepared from glucose upshifted transformants harboring *S.cerevisiae CRY1* were carried out (Fig. 6). *S.cerevisiae CRY1* (Fig. 6, right) was not responsive to glucose upshift when in a *K.lactis* background. From these experiments we conclude that the sequence information necessary to effect an increase in the transcription of *RP59* following glucose upshift is present in the upstream region of *RP59*, but accessory factors that mediate the transcription increase may be absent or nonfunctional in *K.lactis*. A second possibility is that *K.lactis* might lack a factor necessary for down regulating transcription under conditions of a poor carbon source. This would be reflected in the high basal levels of *RP59* transcription even under poor carbon source growth (ethanol).

The steady state level of *RP59* mRNA in *K.lactis* remains constant upon glucose upshift. One possible explanation for this phenomenon could be that a factor which interacts at UAS_{RPG} is absent or nonresponsive to growth rate changes. In *S.cerevisiae*

RAP1 has been postulated as the factor which mediates the transcriptional increase in *rp* mRNA synthesis through its interaction with UAS_{RPG} in response to a growth rate increase. Preliminary data indicate that *K.lactis* does not contain a *RAP1* homolog. Low stringency Southern blot hybridizations using the *S.cerevisiae RAP1* gene versus various restriction digests of *K.lactis* total DNA failed to reveal homologous cross-hybridizing fragments (Larson and Rossi, data not presented).

We conclude *RP59* transcription in *K.lactis* is insensitive to growth rate changes. If an increase of *rp59* synthesis occurs in response to a growth rate change, it does not seem to take place at the level of translational initiation. *K.lactis* transformants harboring *RP59-lacZ* fusions grown under ethanol or glucose conditions have no apparent differences in β -galactosidase activities (data not presented). Since it is highly unlikely that *rp* synthesis is not regulated in *K.lactis*, regulation may occur posttranslationally as has been demonstrated to occur in *S.cerevisiae* transformants overexpressing *rp*'s (38).

We assume a functional homolog to RAP1 exists in *K.lactis* since the UAS_{RPG} are conserved and required for transcription of *RP59*. The expression of *S.cerevisiae RAP1* in *K.lactis* is insufficient by itself to bring *rp* gene transcription under growth rate regulation (Larson and Rossi, unpublished). Since no growth rate regulation at the level of transcriptional initiation is observed in *K.lactis*, it is not possible to determine whether or not RAP1 itself is responsible for mediating this. Assuming a RAP1 functional homolog exists in *K.lactis*, it should be possible to identify a gene encoding this by complementation of a *S.cerevisiae rap* mutant. We are currently pursuing this strategy with the eventual goal of comparing the two proteins. If, in fact, growth rate upshift is mediated by a modification of RAP1 or a particular functional domain of RAP1, we should be able to identify it using this approach.

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