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The ribosomal protein L2 in *S.cerevisiae* controls the level of accumulation of its own mRNA

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The expression of the yeast L2 r-protein gene is controlled at the level of mRNA accumulation. The product of the gene appears to participate in this regulation by an autogenous feedback mechanism. This control does not operate at the level of transcription but instead affects L2 mRNA accumulation. This autogenous regulation of mRNA accumulation provides an interesting analogy to the autogenous translational regulation of r-proteins in *Escherichia coli*.

Key words: autogenous regulation/ribosomal protein/RNA stability/yeast

Introduction

In the yeast *Saccharomyces cerevisiae*, the expression of the genes coding for ribosomal proteins is controlled at the transcriptional and post-transcriptional levels (Mager, 1988; Planta and Raué, 1988; Woolford *et al.*, 1988). Transcription regulates the r-protein genes as a group and cannot vary the activity of individual r-protein genes. Common regulatory elements have been found in the promoters of this set of genes (Teem *et al.*, 1984; Huet *et al.*, 1985; Leer *et al.*, 1985; Rotenberg and Woolford, 1986) suggesting that the co-ordinated transcriptional activation is achieved through the action of two or perhaps a few common transcriptional factors (Dorsman *et al.*, 1988, 1989; Capieaux *et al.*, 1989; Della Seta *et al.*, 1990).

In contrast, post-transcriptional controls seem to provide the mechanisms for fine tuning the expression of individual r-proteins. For the majority of these proteins turnover seems to be the only level of control utilized. In one case, autogenous inhibition of splicing has been shown to control the accumulation of the L32 r-protein mRNA (Dabeva *et al.*, 1986).

In higher eukaryotes, in addition to splicing control (Bozzoni *et al.*, 1984; Caffarelli *et al.*, 1987) and protein turnover (Kay and Jacobs-Lorena, 1985; Pierandrei-Amaldi *et al.*, 1985), translational regulation has been shown, likewise, to be involved in the regulation of expression of r-protein genes (Al-Atra *et al.*, 1985; Pierandrei-Amaldi *et al.*, 1985; Bowman, 1987; Meyuhas *et al.*, 1987; Mariottini and Amaldi, 1990).

In previous studies we have analyzed the regulation of expression of the L1 r-protein gene in *Xenopus laevis* which undergoes a specific post-transcriptional control operating at the level of splicing. Other evidence suggested that this gene, in analogy with the *Escherichia coli* system, is under an autogenous feedback control in which the L1 r-protein controls its synthesis by regulating the efficiency of splicing of its own pre-mRNA (Bozzoni *et al.*, 1984; Caffarelli *et al.*, 1987; Pierandrei-Amaldi *et al.*, 1987).

The study of the regulation of expression of the genes coding for the same protein in two distantly related species and the comparison of the processes involved in their control are of great importance in understanding the evolution of regulatory processes with respect to the physiology and growth conditions of the cell. For this reason we isolated and analyzed the structure of the gene which in *S.cerevisiae* encodes the L2 r-protein which is homologous to the *X.laevis* L1 protein (Lucioli *et al.*, 1988; Presutti *et al.*, 1988). In the present study we have analyzed the regulation of expression of this gene and found that, as in *X.laevis*, it is also controlled at the post-transcriptional level, by regulating the accumulation of mature mRNA. Evidence is presented that this regulation is mediated by the product of the gene itself, the r-protein L2, by a mechanism of autogenous feedback control.

Results

The expression of the L2 r-protein gene is regulated at the level of mRNA accumulation

The classical way of studying the regulation of expression of a specific r-protein gene is to increase its copy number and then analyze the regulatory response that the cell utilizes to ensure the balanced presence of all the ribosomal components. The gene coding for the r-protein L2, which is the *S.cerevisiae* counterpart of the *X.laevis* r-protein L1, has previously been isolated and shown to be present in two copies (A and B) per haploid genome (Presutti *et al.*, 1988).

The A copy, which is more active transcriptionally (Lucioli *et al.*, 1988; Della Seta *et al.*, 1990), was cloned in the pEMBLYe30 multicopy plasmid and in the insertional vector pEMBLYi27 (Baldari and Cesareni, 1985). Strain e30-L2A, which has 10-15 episomal copies of the L2A gene, and strain i27-L2A, which contains one extra copy of L2A together with vector sequences integrated in the L2A locus, were obtained. These two genotypes were confirmed by Southern blot analysis (not shown). These strains were analyzed together with control S150-2B cells transformed with the pEMBLYe30 vector alone (strain e30) for the accumulation of L2 transcripts. The Northern blot in Figure 1a shows that the e30-L2A strain (lane 3) accumulates only twice as much L2 mRNA as strain e30 (lane 1). The same filter was hybridized with a LEU2 probe in order to normalize for the gene copy number (Figure 1b). Comparison of LEU2 hybridization signals on RNA from strain i27-L2A (lane 2), which has only one active LEU2 gene (the one carried by the integrated vector), and from strains e30 (lane 1) and e30-L2A (lane 3) shows that the latter contain 10-15 transcriptionally active copies of the corresponding plasmids. The mere 2-fold accumulation of

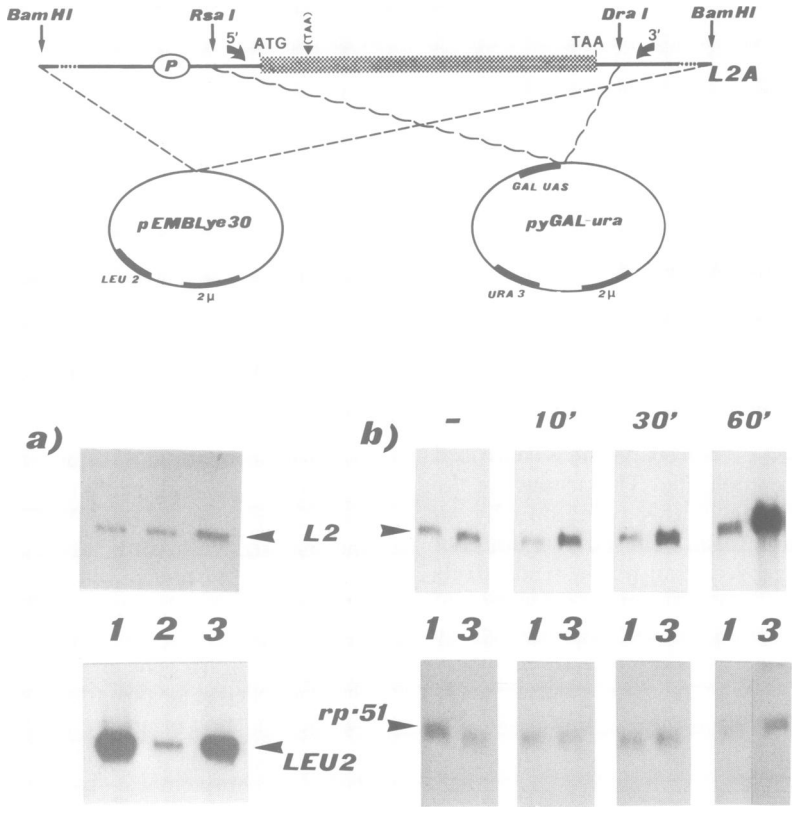

Fig. 1. Panel a: Northern blot analysis of RNA extracted from strains e30 (lane 1), i27-L2A (lane 2) and e30-L2A (lane 3) grown in SC-Leu$^-$ medium to an OD_{600} of 0.6. The filter was hybridized with L2A (upper panel) and *LEU2* (lower panel) probes. **Panel b:** strains e30 (lanes 1) and e30-L2A (lanes 3) were grown in SC-Leu$^-$ medium to an OD_{600} of 0.6 and cycloheximide was added to a final concentration of 200 μg/ml. RNA was extracted before and after CH addition at the indicated times. The filter was hybridized with L2 (upper panel) and rp-51 (lower panel) probes. A diagrammatic representation of the different plasmid constructs used in the paper is shown above. The insertional vector pEMBLyi27 is a pEMBLye30 plasmid lacking the 2μ sequence.

L2 mRNA in strain e30-L2A is then diagnostic of the occurrence of some specific regulation.

The extrachromosomal L2A sequences cloned in the pEMBLYe30 vector are functional and active copies of the L2 gene as previously shown by their ability to restore normal growth conditions in strains in which the endogenous L2A gene copy was inactivated by gene disruption (Lucioli *et al.*, 1988).

The results obtained with the Northern analysis were confirmed on the same RNA preparations by S1 mapping of the 3′ portions of L2 mRNAs (Figure 2). The probe, which is derived from the L2A gene, gives three bands of protection when annealed to L2 mRNA from wild type cells: the a′ and a″ bands reveal L2A transcripts whereas band b identifies those derived from the L2B gene. This is due to sequence divergency in the 3′ untranslated regions between the A and B copies. Figure 2a shows, in agreement with the Northern analysis, that the level of L2 mRNA accumulation in the e30-L2A strain (lane 2) does not correlate with the gene copy number, but that the overall amount of L2 mRNA is only twice that of the control strain (lane 1). The S1 analysis gives additional information, i.e. that the overloading of L2A sequences affects the accumulation of L2B transcripts, as indicated by the diminution of the b signal in the e30-L2A RNA (band b of lane 2). These results indicate that the observed levels of L2 mRNA accumulation are not due to the intrinsic stability of the transcripts but instead are controlled by a specific *trans*-acting mechanism. In order to check whether the L2A endogenous transcripts are also affected by the presence of extra copies of the L2 gene and have a strong transcriptional activation independent of the plasmid copy number, we constructed a plasmid in which the L2A coding sequences are cloned under the control of the CYC promoter and GAL-UAS regulatory sequences of plasmid pyGAL-URA (see Materials and methods). The corresponding strain was derived (gal-L2A) and, after galactose induction, the RNA was extracted and analyzed by S1 mapping. As shown in Figure 2b, in addition to bands a and b, a protected band is obtained (band g′) which corresponds to the size expected for transcripts originating from the gal-L2A plasmid (see schematic representation in Figure 1 and Materials and methods). The g″ band is probably an alternative site of polyadenylation and is found only when RNA derived from gal-L2A strains is analyzed. This experiment, which allows the distinguishing of endogenous transcripts from those originating from the episomal plasmids, shows that in the presence of extra copies of the L2 gene, the accumulation of both the L2A and L2B endogenous transcripts is affected; in fact, no a or b signals can be visualized (Figure 2b, lane 2). Furthermore, in consideration of the fact that gal induction produces a very high activation of transcription (~100-fold), it is very likely also that the L2 transcripts derived from the gal controlled promoter undergo regulation. In fact, the accumulation of L2 mRNA in these strains is ~10- to 20-fold greater than that in the control (compare

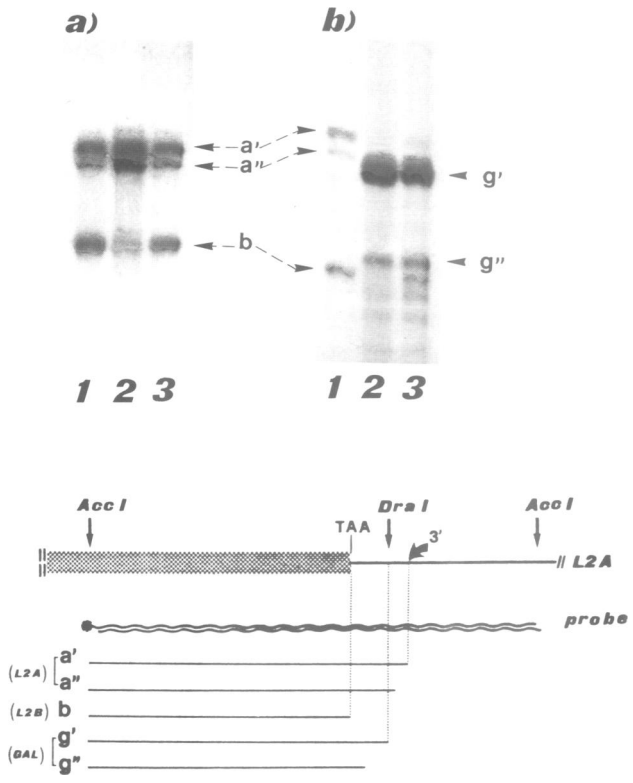

Fig. 2. S1 analysis of the 3′ portions of L2 transcripts. The probe was annealed (**panel a**) to RNA extracted from strains e30 (lane 1), e30-L2A (lane 2), e30-L2Ataa (lane 3); or (**panel b**) to RNA extracted from strains gal-ura (lane 1), gal-L2A (lane 2) and gal-L2Ataa (lane 3) after 6 h of galactose induction. Bands a and b identify the L2A and L2B transcripts respectively, whereas bands g correspond to L2 RNAs transcribed from the gal plasmids. The diagram below shows a schematic representation of the probe and of the S1 resistant fragments.

the intensity of the S1 protected bands in lanes 1 and 2).

The down-regulation of the endogenous L2A and L2B transcripts observed in the gal-L2A strain, where massive L2 transcription is directed from a heterologous *cyc-gal* promoter, demonstrates that the disappearance of the L2B RNA in the e30-L2A strain is not due to competition of the promoters of the extra copies of L2 for specific transcriptional factors.

The r-protein L2 is involved in the regulation of accumulation of its own mRNA

In order to test the hypothesis that the L2 protein could regulate the accumulation of its own mRNA, we repeated the experiments described above utilizing as episomal L2 sequence a specific mutant (L2Ataa) in which a termination codon was created in the proximal region of the L2A gene. Transcripts derived from this mutated gene encode only the first 25 amino acids of the L2 protein (see Materials and methods). This mutant gene was cloned both in pEMBLyE30 (e30-L2Ataa) and in pyGAL-ura (gal-L2Ataa) vectors and the corresponding transformed strains were obtained. S1 mapping on RNA from strain e30-L2Ataa shows that the accumulation of the L2B endogenous transcripts is restored (see reappearance of band b in Figure 2a, lane 3). This strain has a plasmid copy number similar to that of the e30-L2A strain (~ 10 copies per cell, not shown), yet no increase in the accumulation of L2Ataa transcripts proportional to the

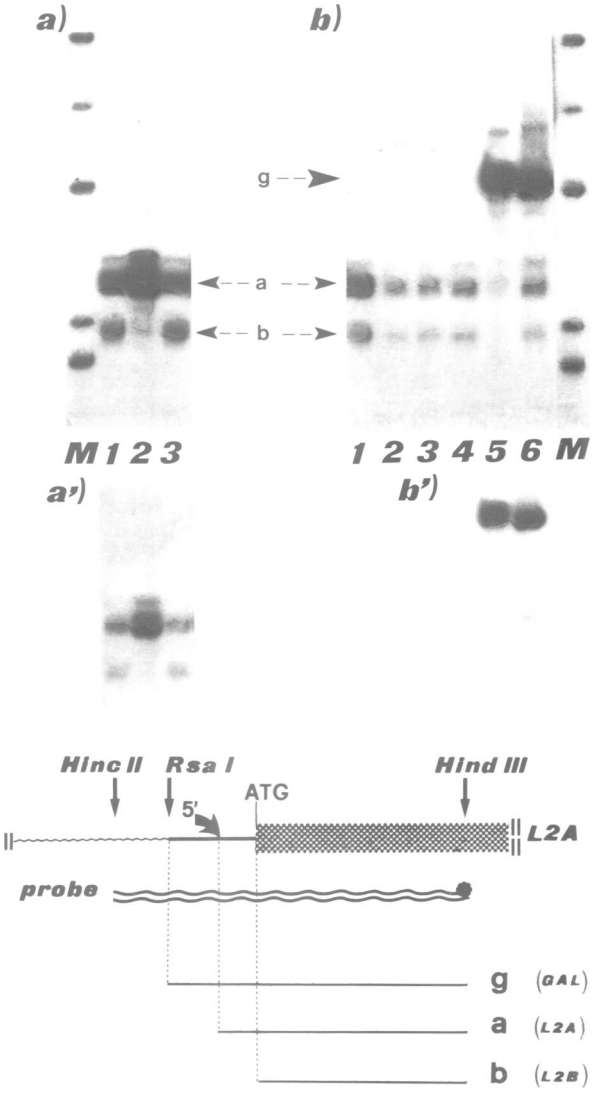

Fig. 3. S1 analysis of the 5′ portions of L2 transcripts. The probe was annealed with RNA from strains e30 (lane 1), e30-L2A (lane 2), e30-L2Ataa (lane 3) (**panel a**), and from strains gal-ura (lanes 1 and 4), gal-L2A (lanes 2 and 5) and gal-L2Ataa (lanes 3 and 6) (**panel b**). In lanes 1, 2 and 3 of panel b the RNA was extracted from uninduced strains; in lanes 4, 5 and 6 the cells were induced with galactose for 6 h. Bands a and b correspond to L2A and L2B transcripts respectively, whereas bands g indicate L2 RNA transcribed from the gal plasmids. Lanes M indicate the mol. wt markers. A schematic representation of the probe and of the S1 resistant fragments is shown below.

gene copy number is observed. We suggest that this reflects the intrinsic low stability of this RNA due to its poor translatability as already shown in similar cases (Losson and Lacroute, 1979. The use of the gal-L2Ataa strain once again allows the problem of plasmid copy number variation to be overcome. S1 mapping on gal-L2Ataa RNA shows, in agreement with the previous experiment, that the accumulation of both the L2A and L2B endogenous transcripts is restored (see reappearance of bands a and b in Figure 2b, lane 3).

S1 analysis of the 5′ regions of the L2 RNAs was performed on the same RNA preparations used for the previous experiments (Figure 3). The probe used allows the L2A (band A) to be distinguished from the L2B (band B)

transcripts which differ also in the 5′ untranslated region (see diagram in Figure 3). The results show once again that there is a correlation between the coding capacity of the genes present in extra copies and the accumulation of the L2 transcripts. In the presence of extra copies of wild type L2A genes, the accumulation of L2B RNA is reduced (panel a, lane 2) while it is unaffected when the extra copies code for a non-functional L2 protein (panel a, lane 3).

The same results are obtained when the corresponding gal constructs are used. In the absence of the inducer, all the different strains show only the endogenous L2 transcripts (Figure 3b, lanes 1−3); after gal induction, strain gal-L2A shows a drastic decrease in the accumulation of the L2A and L2B transcripts (lane 5) which is instead restored in strain gal-L2Ataa (lane 6).

A time course of galactose induction was performed in order to follow the timing of disappearance of the endogenous L2 transcripts in strain gal-L2A. Northern blot analysis was performed on RNA extracted at 10, 30 and 60 min after galactose induction. Figure 4 shows that after 10 min the transcripts deriving from the *cyc-gal* promoter can already be visualized and that the endogenous L2 transcripts are present in both gal-L2A (lane 2) and gal-L2Ataa (lane 3) strains. After 30 min of induction, the accumulation of gal-derived transcripts is still increasing; at the same time the endogenous L2 mRNAs are almost undetectable in strain gal-L2A while they are still present in strain gal-L2Ataa. After 1 h of galactose induction the accumulation of gal-derived transcripts reaches a maximum; no L2A and L2B mRNAs are detected in gal-L2A strain while they are still present in gal-L2Ataa (these results have also been confirmed by S1 mapping, not shown).

In order to check that there were no secondary effects on RNA accumulation due to ribosome availability in the e30-L2A and e30-L2Ataa strains, we checked the mRNA accumulation of different but related transcripts, namely the ones for the r-protein rp-51 (Teem and Rosbash, 1983). Figure 5 shows that no difference in rp-51 mRNA levels is observed in these two strains.

The regulated accumulation of L2 mRNA depends on ongoing protein synthesis

In order to test whether ongoing protein synthesis is necessary for the regulated accumulation of L2 mRNA, we analyzed the effects of cycloheximide (CH) treatment on the L2 mRNA levels in strains e30 and e30-L2A. Exponentially growing cells were treated with 200 μg/ml of CH for 10, 30 or 60 min. RNA was extracted and equal amounts were analyzed on Northern blots using L2 and rp-51 probes. The upper panel of Figure 1b shows that, in strain e30-L2A, the accumulation of L2 mRNA has already increased after 10 min of CH treatment and after 1 h reaches levels proportional to the gene dosage, while the accumulation of L2 mRNA in the e30 control strain increases only slightly. Hybridization of the same filter with the rp-51 probe (lower panel) shows that the CH treatment does not produce any detectable stabilization of a different r-protein mRNA. These results show that high levels of accumulation of L2 mRNA in strain e30-L2A depend on the inhibition of protein synthesis and are not simply due to the mRNA stabilization effect of polysome freezing determined by CH treatment (Pachter *et al.*, 1987). It can be concluded that the regulated accumulation of L2 mRNA is determined by the translation of a specific protein factor. In line with these results, the CH treatment of gal-L2A strain produces levels of L2 mRNA proportional to the transcriptional activation described for the *gal-cyc* promoter (not shown).

The feedback regulation of the L2 r-protein does not act at the transcriptional level

The data presented show that the L2 r-protein feedback regulates its own synthesis by controlling the amount of mRNA accumulated in the cell but do not allow us to distinguish whether the protein affects a transcriptional or a post-transcriptional process. The experiment of increasing the L2 gene copy number in the cell has already suggested the possibility that the control was not transcriptional. In fact, if the mRNA accumulated in wild type cells is the result of a regulatory loop determined by the ratio between the r-protein L2 and its own promoter, in strains with extra copies of the L2 gene in which this ratio is the same as in control strains, one should have found a proportional increase in the accumulation of L2 transcripts.

In order to analyze this aspect in greater detail, we made a construct (L2-lacZ) in which the L2 promoter (from position −1420 to +70, see Materials and methods) is fused in frame to *lacZ* sequences of plasmid Yep355 (Myers *et al.*, 1986). Strains e30, e30-L2A and e30-L2Ataa were transformed with this plasmid, giving e30-lacZ, L2A-lacZ and

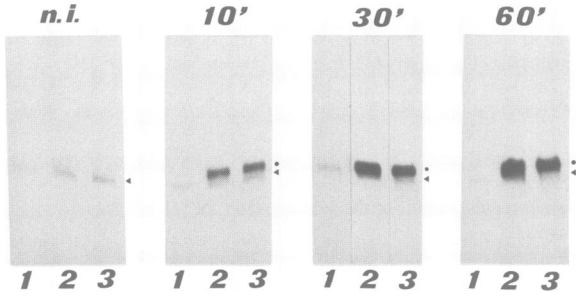

Fig. 4. Time course of galactose induction of strains gal-URA (lanes 1), gal-L2A (lanes 2) and gal-L2Ataa (lanes 3). RNA was extracted before and after galactose addition at the indicated times and analyzed on Northern blots with an L2A probe. Transcripts deriving from the *gal-cyc* promoter (indicated by the dot) have a larger size than the endogenous L2 mRNA (indicated by the arrowhead).

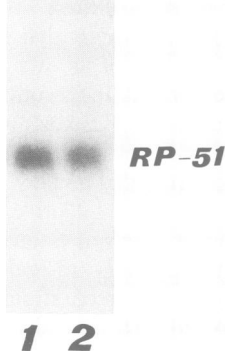

Fig. 5. Northern blot analysis of RNA extracted from strains e30-L2A (lane 1) and e30-L2Ataa (lane 2) grown in SC-Leu$^-$ at an OD_{600} of 0.6 and hybridized with the rp-51 probe.

L2Ataa-lacZ strains. Many independent transformants were analyzed for the gene copy number of the two plasmids. Figure 6a shows the Southern analysis performed with URA3 and LEU2 probes (the marker genes from the Yep355 and pEMBL vectors) on single representatives of each strain. It appears that the different strains contain a similar number of the URA and LEU plasmids. On the same strains, Northern analysis with lacZ and L2 probes was performed. Figure 6b shows that no strong differences in the level of accumulation of lacZ mRNA are observed in strains containing extra copies of the wild type L2 gene (lane 2) or of its mutated copy (lane 3).

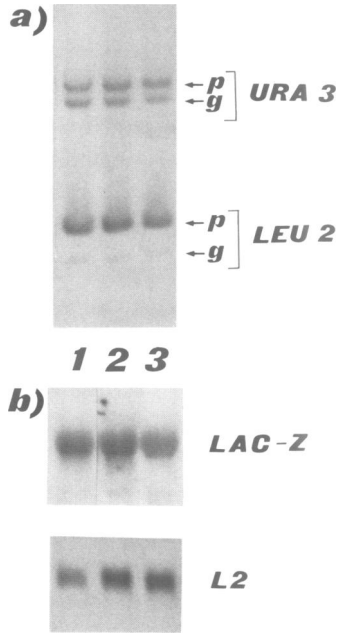

Fig. 6. Panel a: Southern blot analysis of 15 μg of DNA from strains e30-lacZ (lane 1), L2A-lacZ (lane 2) and L2Ataa-lacZ (lane 3) hybridized with *URA3* and *LEU2* probes. The hybridizations to the plasmid and chromosome copies of the *URA3* and *LEU2* genes are indicated by p and g respectively. **Panel b:** Northern blot analysis of 15 μg of RNA from strains e30-lacZ (lane 1), L2A-lacZ (lane 2) and L2Ataa-lacZ (lane 3) hybridized with *lacZ* (upper panel) and L2 (lower panel) probes.

Destabilization of L2 transcripts produces truncated molecules

Northern blot analysis and S1 mapping of the 3′ regions showed that in strains containing extra copies of the L2 gene, accumulation of full length mRNAs is not proportional to the gene copy number. On the other hand, S1 mapping of the 5′ portions of L2 transcripts performed on the same RNA preparations utilized for the previous experiments, reveals an interesting difference (Figure 3). The intensity of band a in the e30-L2A RNA is closer to the gene copy number (compare the intensity of band a in Figure 3a', lanes 1 and 2). The differential accumulation of 5′ and 3′ ends of the wild type L2 transcripts was also observed in gal-L2A strains where the intensity of band g compared with bands a and b in the control strain (compare Figure 3b', lanes 4 and 5) is higher than in Figure 2b (lanes 2 and 3). The differential abundance of the 5′ and 3′ portions of the L2 transcripts would suggest the presence of truncated molecules and offer an experimental approach to the understanding of the molecular mechanism at the base of this regulation. Whether this is due to an attenuation process during transcription or to endonucleolytic cleavage of terminated transcripts has still to be demonstrated. In contrast with the observation made on e30-L2A RNA, the abundance of 5′ and 3′ portions is the same in the case of the L2Ataa transcripts (Figures 2a and 3a) and is never proportional to the gene copy number. We suggest that this reflects the intrinsic instability of these transcripts due to their poor translatability. In comparison, the differential abundance of 5′ and 3′ portions of the wild type L2 RNA is indicative of a different pathway of RNA degradation.

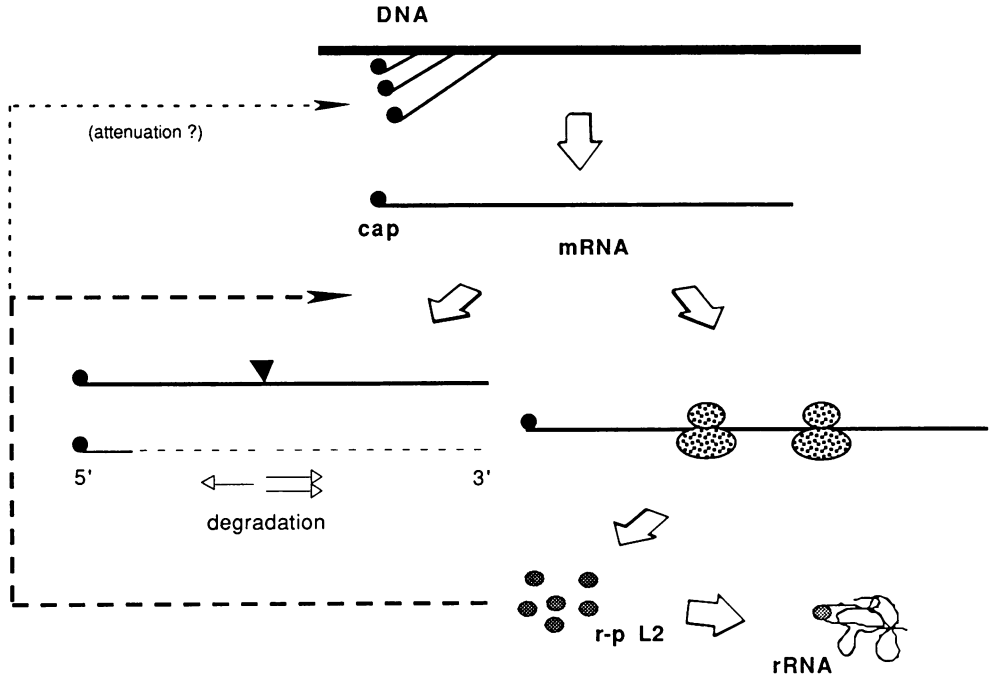

Fig. 7. Model of the autogenous regulation of the L2 r-protein gene.

Discussion

The best known example of ribosomal protein gene regulation is given by the autogenous translational control of r-proteins in *E.coli* (Nomura *et al.*, 1984). When synthesized in excess, specific regulatory r-proteins function as translational feedback repressors which control the synthesis of several r-proteins within their own polycistronic regulatory units.

In eukaryotes, there are at least two cases in which there is evidence for a feedback regulation of r-protein synthesis. In *X.laevis* and in *S.cerevisiae*, the r-proteins L1 and L32 respectively seem to be involved in the splicing control of their own pre-mRNA (Bozzoni *et al.*, 1984; Dabeva *et al.*, 1986; Pierandrei-Amaldi *et al.*, 1987). It is interesting to note that in both cases the target of the control is nuclear. This is explained by the fact that the nucleus is the cellular compartment where ribosomes are assembled and where regulation mediated by free unassembled r-proteins can occur.

In the present study we have analyzed the regulation of expression of the yeast gene coding for the r-protein L2, which is homologous to the *X.laevis* r-protein L1 (Presutti *et al.*, 1988). We have shown that the increase of the L2 gene dosage does not lead to a proportional increase of L2 mRNA accumulation. Similar experiments performed with the majority of other r-protein genes in yeast have shown that gene overdosage is normally accompanied by the accumulation of excess mRNA and proteins which are subsequently rapidly degraded (Abovich *et al.*, 1985; El-Baradi *et al.*, 1986; Maicas *et al.*, 1988; Tsay *et al.*, 1988).

The increase in the L2A gene copy number affects not only the accumulation of L2A transcripts but also that of L2B mRNA. This is indicative of a specific regulation occurring *in trans*. If the L2 extra copies are cloned under the *gal-cyc* promoter, a decrease in the accumulation of both the L2A and L2B endogenous transcripts is observed. The use as episomal sequences of the L2Ataa gene in which a termination codon was created such that only the first 25 amino acids can be translated allowed us to demonstrate that the L2 protein itself is involved in the control of the accumulation of its own mRNA. In fact, in the presence of extra copies of the L2Ataa gene, under its original promoter or under the gal UAS, the accumulation of the endogenous L2 transcripts is restored. The use of protein synthesis inhibitors confirmed that translation must occur in order to obtain the regulated accumulation of L2 mRNA.

We have also shown that this feedback control does not operate at the transcriptional level, in fact the level of transcription of the *lacZ* reporter gene fused to the L2 promoter is the same in strains containing extra copies of the wild type L2A or of the L2Ataa genes. If the L2 protein was inhibiting transcription from its own promoter, one should have observed lower levels of *lacZ* RNA in strains containing extra copies of the wild type L2 gene.

The comparison of the S1 analysis performed on the 5′ and 3′ portions of the L2 mRNAs can provide some clues on the possible mechanism of down-regulation of these transcripts. While the number of 3′ ends corresponds to the level of accumulation of the mature RNA, the 5′ ends are more abundant. This is diagnostic of the presence of truncated molecules. Truncated molecules were previously described as the initial products of the L1 pre-mRNA turnover in *X.laevis* where endonucleolytic cleavage inside the third intron of the gene represents the first step towards RNA degradation (Caffarelli *et al.*, 1987). The cleaved molecules undergo subsequent degradation which is faster for the 3′ halves because they lack the cap structure at the 5′ end. This explains the unbalanced presence of 5′ and 3′ half molecules. A similar example has been reported also in *E.coli* where the ribosomal protein S8 triggers nucleolytic cleavage of the *spc* operon mRNA followed by exonucleolytic mRNA degradation (Mattheakis *et al.*, 1989). In both cases, mRNA processing and degradation are important for tighter regulation. Endonucleolytic cleavage seems to provide an efficient method for taking RNA molecules towards a degradation pathway and has been proposed in other cases where RNA stability plays a crucial role in the regulation of gene expression (Cleveland, 1988; Mattheakis *et al.*, 1989).

Cleaved molecules can be easily detected in the *X.laevis* oocyte where RNA turnover proceeds quite slowly. In a rapidly growing cell, like yeast, the half life of these molecules is much shorter. If trimmed truncated molecules were the products of this turnover process, one could explain why they can be visualized by S1 analysis (our probe allows the visualization of molecules containing 150 nucleotides from the 5′ end) and not by Northern analysis. The similarity with the *X.laevis* system would also favor the idea that in yeast the differential abundance of the 5′ and 3′ portions of the L2 mRNA could be due to specific endonucleolytic cleavage and differential stability of the resulting molecules. Our data at the moment do not exclude the possibility that the same effect on mRNA accumulation and the same molecules could originate by a process of transcriptional attenuation. Nuclear run-on experiments were unsuccessfully tried in our laboratory; other approaches are currently being tested in order to discriminate between the two possibilities.

The results presented allow us to conclude that the gene encoding the ribosomal protein L2 in yeast is under the autogenous feedback control operating at the post-transcriptional level (Figure 6). It is interesting to note the evolutionary conservation of this regulatory process and its adaptation to the different structure and physiology of the cells. In *E.coli*, where there is no cellular compartmentalization, the regulatory target is on the mRNA and translational repression occurs. In consideration of the fact that the L2 gene does not contain introns, it will be interesting to find out whether in this case the target of the regulation is confined to the nucleus, where ribosomes assemble, and which nuclear process is affected. In addition, it will be interesting to find out whether, in analogy with the *E.coli* system, the r-protein L2 controls the accumulation of its own mRNA by direct binding to a region showing homology to its binding site on the 26S rRNA. The encouraging evidence is that the L2 r-protein is among the rRNA binding proteins (El-Baradi *et al.*, 1984, 1985), a feature which has been shown in *E.coli* to be a prerequisite for a ribosomal protein to be regulatory.

Materials and methods

Plasmids and strains

Plasmid e30-L2A was obtained by cloning into the *Bam*HI site of pEMBLye30 vector (Cesareni *et al.*, 1985), a 3.6 kb *Eco*RI genomic fragment containing the whole L2A coding region plus 1 kb of upstream

sequences (Presutti *et al.*, 1988), via Klenow repair and linker ligation. The same fragment was also cloned in the *Bam*HI site of the integrative plasmid pEMBLyi27 (Baldari and Cesareni, 1985) to give clone i27-L2A. Clone gal-L2A was obtained by inserting into plasmid pyGAL-ura, which is the pEMBLyex4 vector (Cesareni and Murray, 1987) with the *LEU* gene deleted, a 1208 bp fragment extending from the *Rsa*I site, 54 bases upstream of the ATG codon, to the *Dra*I site located 70 nucleotides downstream of the TAA codon of the L2A gene. This fragment was initially cloned into the *Sma*I−*Hin*cII sites of the Bluescript vector (BS-L2). The *Bam*HI−*Xho*I insert was recovered from the BS-L2 plasmid and cloned into the corresponding sites of pyGAL-ura. This construct has lost the polyadenylation signal of the L2A gene and utilizes the one carried by the vector. The mutants containing the termination codon (e30-L2Ataa and gal-L2Ataa) were obtained by inserting an *Xho*I linker into the *Pvu*II site (at position +70 from the ATG) of the L2A gene. This insertion produces a change in the reading frame at position 75 and termination codon at position 124 from the ATG. The mutants were sequenced according to Maxam and Gilbert (1980). Plasmid L2A-lacZ was obtained by inserting, in the *Eco*RI−*Sma*I sites of plasmid YEp355 (Myers *et al.*, 1986), the 1490 bp *Eco*RI−*Pvu*II fragment extending from position −1420 to +70 with respect to the ATG of the L2A gene. This construct maintains the L2 and *lacZ* sequences in the same reading frame. The recipient for all these constructs was the haploid *S.cerevisiae* strain S150-2B (a *leu2-3 leu2-112 ura3-52 trp1-289 his3-* Δ1) which was transformed according to Sherman *et al.* (1982). The transformed strains were grown at 30°C in SC medium lacking leucine or uracil (Sherman *et al.*, 1982). Integrative transformants (i27-L2) were controlled by plating on non-selective medium and then replica-plating on Leu$^-$ medium. Galactose induction was performed according to Sherman *et al.* (1982).

Inhibition of protein synthesis

Strains e30 and e30-L2A were grown in SC Leu$^-$ medium to a density of 0.6 at OD_{600} then cycloheximide was added to a final concentration of 200 μg/ml. Total RNA was extracted before and after 10, 30 or 60 min of cycloheximide treatment.

Probes

The *Bam*HI−*Xho*I insert of plasmid BS-L2, which contains the complete transcribed portion of the L2 gene, was used as a probe for Northern blot analysis. For 3′ S1 mapping, the 804 nucleotide *Acc*I fragment, from 464 nucleotides upstream of the termination codon to 340 nucleotides downstream of the termination codon of the L2A gene, was used (see diagram in Figure 2). This fragment, 3′ end labeled with Klenow polymerase, protects 564 and 544 bases on L2A transcripts, 464 bases on L2B transcripts and 530 bases on transcripts derived from the gal constructs (see panel d of Figure 2). For S1 mapping of the 5′ regions, the 237 nucleotide fragment extending from the *Hin*dIII site (142 bases downstream of the ATG) to the *Rsa*I site (55 bases upstream of the ATG) of the L2A gene plus 40 nucleotides of the BS polylinker region (see diagram in Figure 3) was utilized. This fragment, 5′ end labeled with T4 polynucleotide kinase, protects 142 bases on L2B transcripts, 170 bases on L2A transcripts and 194 bases on gal driven transcripts. The *LEU* probe is a 3 kb long fragment containing the entire *LEU2* gene, whereas the *URA3* probe is a 1100 bp *Nco*I−*Xho*I fragment. The *lacZ* probe is a 2557 bp *Pvu*II fragment internal to the coding portion of the *lacZ* gene. The rp-51 probe was obtained from *Sal*I−*Bam*HI digestion of plasmid pHZ18 kindly provided by Professor M.Rosbash.

Blot analysis and S1 mapping

Total DNA or RNA was extracted from cultures grown to an OD_{600} of 0.6 and 10 μg were analyzed by Southern or Northern blotting (Bozzoni *et al.*, 1984) or by S1 mapping (Caffarelli *et al.*, 1987). In the case of gal constructs, total RNA was extracted from control cultures grown in glycerol−ethanol after 8 h of galactose induction.

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