Curr Genet (1986) 10:733–739

Current Genetics

The cellular level of yeast ribosomal protein L25 is controlled principally by rapid degradation of excess protein

Tarek T. A. L. ElBaradi, Carine A. F. M. van der Sande, Willem H. Mager, Hendrik A. Raué, and Rudi J. Planta

Biochemisch Laboratorium, Vrije Universiteit, de Boelelaan 1083, NL-1081 HV Amsterdam, The Netherlands

Summary. When the gene dosage for the primary rRNA-binding ribosomal protein L25 in yeast cells was raised about 50-fold, the level of mature L25 transcripts was found to increase almost proportionally. The plasmid-derived L25 transcripts were structurally indistinguishable from their genomic counterparts, freely entered polysomes in vivo and were fully translatable in a heterologous in vitro system. Nevertheless, pulse-labelling for periods varying from 3–20 min did not reveal a significant elevation of the intracellular level of L25 protein. When pulse-times were decreased to 10–45 s, however, we did detect a substantial overproduction of L25. We conclude that, despite the strong RNA-binding capacity of the protein, accumulation of L25 is not controlled by an autogenous (pre-)mRNA-targeted mechanism similar to that operating in bacteria, but rather by extremely rapid degradation of excess protein produced.

Key words: Yeast – Ribosome synthesis – Regulation – Ribosomal protein turnover

Introduction

The biosynthesis of ribosomes is a prime example of a process requiring the coordinate expression of a set of functionally related genes. In both pro- and eukaryotic cells the numerous components making up the ribosome are produced in balanced amounts under virtually all physiological conditions (reviewed by Warner 1982; Planta and Mager 1982; Nomura et al. 1984). In bacteria this balance is maintained principally by controlling the rates of synthesis of the various ribosomal constituents. In eukaryotes in addition, modulation of turnover appears to be employed to achieve the same goal. For instance, subjecting a yeast culture to a temperature shift causes a transient drop in de novo r-protein synthesis while transcription of rRNA genes continues. The newly-formed precursor rRNA, however, fails to be processed and is rapidly degraded (Udem and Warner 1972; Shulman and Warner 1978). Conversely, most unassembled r-proteins are rapidly degraded when accumulation of mature rRNA is impeded, e.g. in a yeast mutant defective in rRNA processing (Gorenstein and Warner 1977). A similar instability of "excess" r-protein has been observed in anucleolate *Xenopus* embryos, which lack rRNA genes (Pierandrei-Amaldi et al. 1985); upon the decrease in rRNA synthesis in terminally differentiating rat myoblasts (Jacobs et al. 1985) and upon inhibition of rRNA synthesis in rat liver cells by low doses of actinomycin D (Stoyanova and Hadjiolov 1979). These observations suggest that in eukaryotes degradation of excess of ribosomal constituents plays an important role in maintaining balanced cellular levels of the various components.

The results of gene dosage experiments investigating the control of individual r-proteins in yeast are indeed consistent with this idea at least as far as some r-proteins are concerned (Warner et al. 1985; Abovich et al. 1985). At the same time, however, these experiments showed other r-proteins to be controlled mainly at the level of processing of their pre-mRNAs or possibly initiation of translation. In these cases the control mechanism could well operate on the same principle employed by bacteria, namely autogenous regulation based upon a direct competition between (pre-)rRNA and (pre-)mRNA for the newly-formed r-protein (Nomura et al. 1984). The most likely candidates for such a competition-based regulation are the primary rRNA-binding r-proteins, the only well-studied examples of which in yeast are L1a (YL3),

Offprint requests to: R. J. Planta

Abbreviations: rRNA, ribosomal RNA; r-protein, ribosomal protein; pre-mRNA, precursor mRNA

binding to 5S rRNA (Nazar 1979; Nazar and Wildeman 1983) and L25 whose binding site on 26S rRNA we have recently elucidated (ElBaradi et al. 1985). L25 also is one of the first yeast r-proteins to be assembled (Kruiswijk et al. 1979) and may well play a crucial role in the assembly process as a whole (ElBaradi et al. 1984, 1985). It was, therefore, of interest to determine whether yeast cells exploit the RNA-binding properties of L25 for direct (pre-)mRNA targeted control of its synthesis or use the more indirect (assembly-mediated) modulation of turnover to keep accumulation of L25 within bounds. In this paper we describe gene dosage experiments addressing this question.

Materials and methods

Yeast strain and vectors. Plasmid pBMCY138 consists of a HindIII-generated yeast DNA fragment carrying the L25 ribosomal protein gene cloned in the vector pBR322 (Bollen et al. 1982). A BglII-generated fragment containing most of the insert was cloned into the unique BamHI-site of the vector pJDB207 (Beggs 1978). The yeast strain AH22 was used as a host for transformation (Beggs 1978).

Isolation and analysis of nucleic acids. Yeast DNA was isolated from 25 ml cultures essentially as described by Davis et al. (1980) Restriction enzyme digestions were performed as recommended by the supplier (Boehringer Mannheim). Yeast RNA was isolated and purified as described previously (Bollen et al. 1980). Southern and Northern hybridization were performed according to standard procedures (Maniatis et al. 1982). A TaqI-HpaI fragment isolated from pBMCY138 and cloned in M13 mp9 (Messing 1982) was labelled using the sequencing primer (Schaap et al. 1984) and employed as an L25 gene-specific probe. Primer extension analysis and S1 mapping were carried out as described previously (Leer et al. 1984a).

In vivo labelling of proteins. 10 ml yeast cultures, grown in minimal medium containing 6.8 g/l yeast nitrogen base (Difco), 20 g/l glucose, 2 ml/l sodium lactate and 20 mg/l histidine were pulse-labelled using 25 μCi/ml [^{35}S]-methionine (Amersham, 800 Ci/mmol). Labelling was stopped by mixing the culture with an equal volume of ice-cold medium containing 100 μg/ml of unlabelled methionine. Cells were harvested by centrifugation and combined with cells uniformly labelled with 1 μCi/ml [^{3}H]-methionine (Amersham, 6.9 Ci/mmol) containing only the vector without the insert. Further details of the labelling procedures are described in Figure and Table legends. The cells were broken by shaking with glass beads in Eppendorf tubes (Bromley et al. 1982) and proteins were extracted with acetic acid (Hardy et al. 1969). Analysis of total protein was carried out on two-dimensional gels according to Mets and Bogorad (1974) or Kaltschmidt and Wittmann (1970). The gels were dried and [^{35}S]labelled protein were visualized by fluorography using Amplify (Amersham).

Results

The yeast genome contains only a single copy of the gene for ribosomal protein L25 (Molenaar 1984). In order to increase this number we cloned the BglII fragment of plasmid pBMCY138 (Bollen et al. 1982), which carries a complete L25 gene including the 5′-and 3′-flanking sequences, into the BamH1 site of the yeast multicopy vector pJDB207 (Beggs 1978; cf. Fig. 1), and transformed yeast strain AH22 with the resulting plasmid pJDB207/L25.

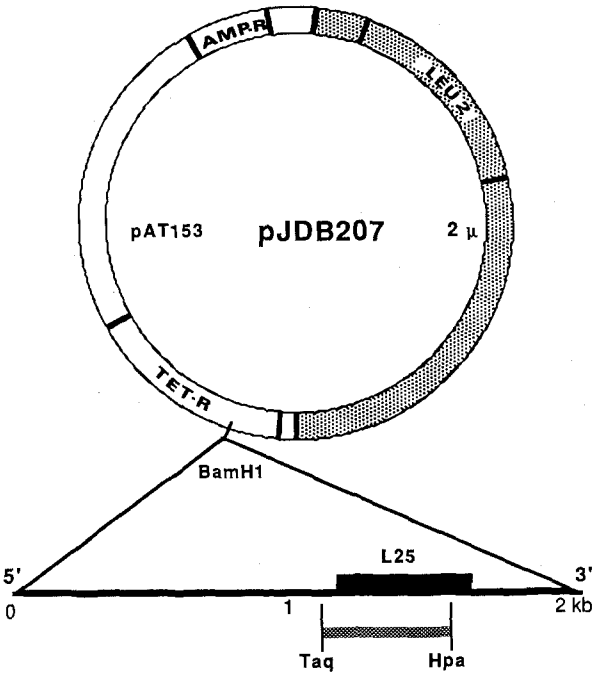

Fig. 1. Map of the recombinant plasmid pJDB207/L25. The *upper part* of the figure depicts plasmid pJDB207 (Beggs 1978) in which the yeast sequences are *shaded*. The *lower part* shows the insert derived from plasmid pBMCY138 (Bollen et al. 1982) containing the L25 gene which was cloned into the BamH1 site of the vector. The transcribed sequence is indicated by the *thick bar*. The TaqI-HpaI fragment used as a probe in Southern and Northern analyses is indicated by the *shaded bar*

The copy number of the plasmid in the transformed cells was determined by Southern hybridization (Southern 1975) using a TaqI-HpaI fragment spanning most of the L25 gene (Fig. 1) as a probe. The results, quantified by scanning the autoradiogram (Fig. 2), put the number of extra copies of the L25 gene at about 50.

The data listed in Table 1 demonstrate that this increase in the gene copy number does not result in a significantly elevated level of the corresponding protein. For this experiment total protein was isolated from a mixture of cells pulse-labelled for 3 min with [^{35}S]-methionine and cells labelled uniformly with [^{3}H]-methionine. After 2D gelectrophoresis according to Mets and Bogorad (1974) the ratio of ^{35}S to ^{3}H was determined for several r-protein spots, including the one corresponding to L25. Table 1 lists these A_i values (Gorenstein and Warner 1977) normalized to the ^{35}S/^{3}H ratio of the total protein sample. Taking into account the

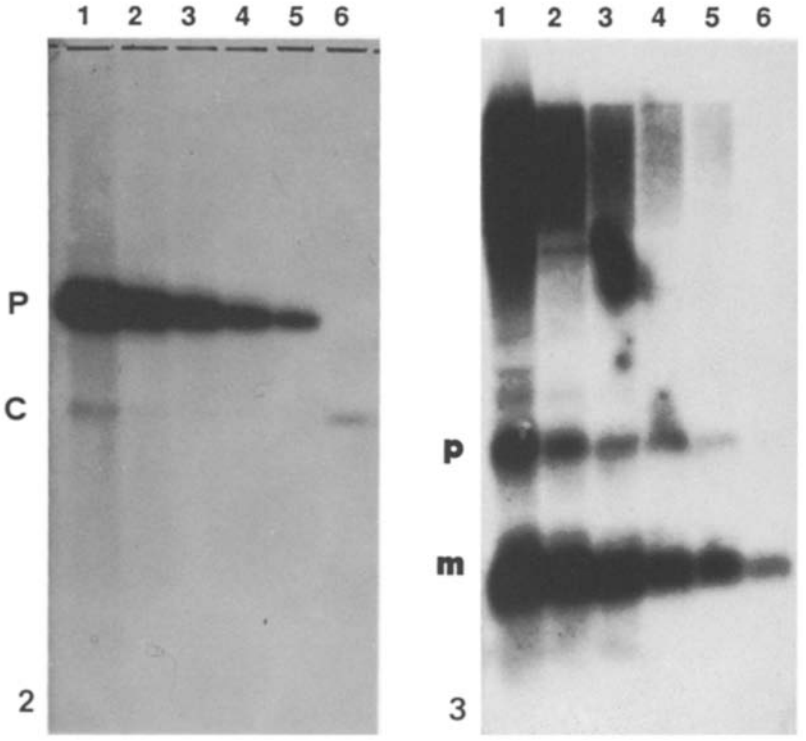

Fig. 2. Determination of the copy number of plasmid pJDB207/L25 in transformed AH22 yeast cells. DNA was isolated from the transformed cells, digested with HindIII and subjected to Southern analysis using the L25-specific probe shown in Fig. 1. In each of *lanes 2–5* half the amount of DNA present in the previous lane was applied to the gel. *Lane 6* is a control containing DNA from untransformed host cells. P and C indicate the positions of the plasmid-derived and the chromosomal signal respectively

Fig. 3. Determination of the steady state concentration of L25 transcripts in pJDB-207/L25 transformed cells. Total polyA$^+$ RNA was isolated from the transformed cells and subjected to Northern analysis using the L25-specific probe shown in Fig. 1. In *lane 1* a total of 10 μg of RNA was applied to the gel. In each of *lanes 2–5* half the amount of RNA present in the previous lane was applied. *Lane 6* is a control containing 10 μg of RNA from untransformed host cells. p and m indicate the precursor and mature L25 mRNA band respectively

Table 1. Relative rate of synthesis (A_i, [Gorenstein and Warner 1977]) of ribosomal proteins in pJDB207/L25 transformed cells (T) and cells containing the vector without the L25 insert (C). Exponentially growing cells transformed with pJDB207/L25 were labelled for the time indicated with [^{35}S]methionine as described in Materials and methods. The pulse-labelled cells were mixed with a fixed amount of control cells uniformly labelled with [^{3}H]methionine. Total protein was extracted and separated by 2D gel electrophoresis according to Mets and Bogorad (1974). After autoradiography of the dried gel several easily identifiable ribosomal protein spots were cut out and their [^{35}S]/[^{3}H] ratio determined. This ratio was corrected for the [^{35}S]/[^{3}H] ratio of the total protein to obtain the A_i values listed

Protein	3 min pulse		20 min pulse	
	T	C	T	C
L2	1.02	1.18	0.93	1.06
L3	0.78	0.92	0.88	1.00
L8	1.11	0.95	0.86	0.80
L22	0.89	1.00	1.07	0.97
L25	1.27	1.05	1.18	1.09
L29	0.98	0.73	0.97	1.03
L30	1.01	1.01	0.62	0.75

A_i values for control cells pulsed for 3 min as well as for transformants and control cells pulsed for 20 min (Table 1) we estimate the level of L25 in the transformants to be increased by at most 20%. Thus accumulation of L25, like that of other yeast r-proteins (Himmelfarb et al. 1984; Abovich et al. 1985) is strictly regulated.

In order to ascertain at which level this control is exerted we first determined the steady state amount of L25 transcripts in the transformed cells. PolyA$^+$ RNA was separated on agarose gels and the blots were hybridized with the same L25-specific probe described above. Scanning of the resulting autoradiogram (Fig. 3) showed the transformants to contain approximately 35 times the amount of (apparently mature) L25 mRNA present in control cells. We therefore conclude that regulation at the transcriptional level does not significantly contribute to the control of L25 accumulation. The somewhat lower level of L25 mRNA as compared to the number of extra gene copies may be due to a slightly increased turnover of the excess (pre-)mRNA (Warner et al. 1985).

The autoradiogram in Fig. 3 also displays a distinct band migrating at the position of precursor L25-mRNA. Since this band does not show up in the control lane it is difficult to judge whether the ratio of pre-mRNA to mature mRNA is increased in the transformants. If such modulation of splicing indeed does occur, however, again the contribution to the control of L25 accumulation can only be relatively minor.

Clearly thus, as has been shown for other yeast r-proteins (Warner et al. 1985; Abovich et al. 1985) accumulation of L25 is controlled mainly at the level of either translation or stability of the protein. We therefore examined structure, fate and function of the excess L25-mRNA.

Primer extension analysis showed the plasmid-derived L25-mRNA to display the same qualitative and quantita-

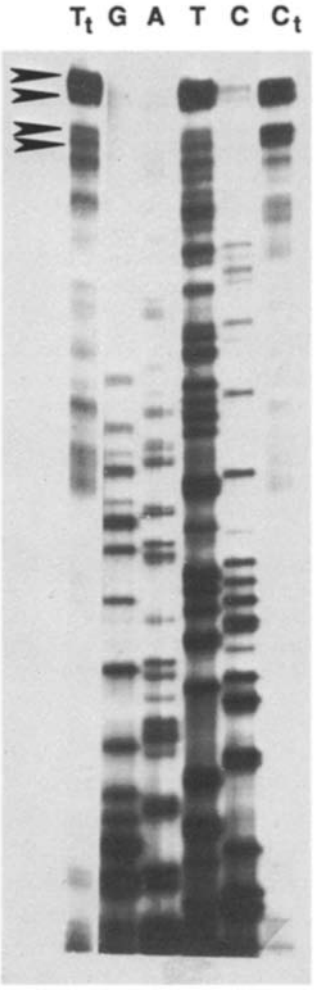

Fig. 4. Mapping of the 5′ end of the L25 transcripts isolated from pJDB207/L25 transformed cells by primer-extended sequencing. The experiment was performed as described previously using RNA isolated from the transformed cells (Leer et al. 1984b). The *lanes* marked T_t and C_t show the 5′ end mapping of L25 mRNA from transformed and control cells respectively. The *lanes* marked $A-C$ show the sequencing reactions of L25 mRNA from the transformed cells. The *arrows* indicate the major transcription initiation sites

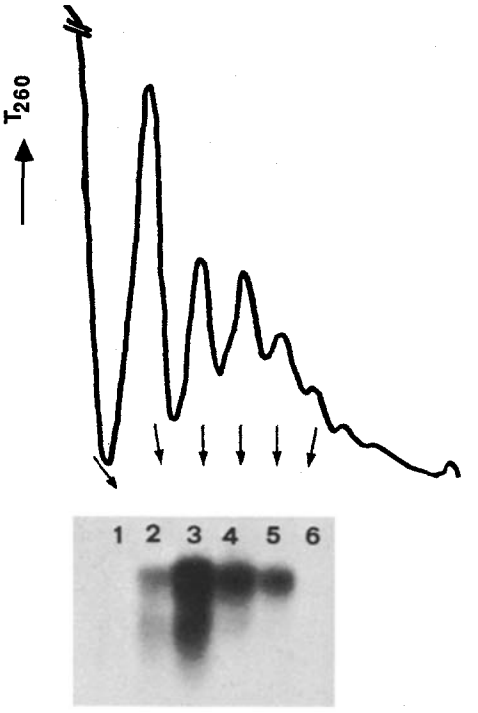

Fig. 5. Polysomal distribution of the excess L25 mRNA in pJDB207/L25 transformed cells. Polysomes were isolated as described previously (Mager and Planta 1976) and pooled as indicated. Total RNA from each fraction was subjected to Northern analysis using the L25-specific probe shown in Fig. 1. The same amount of RNA was applied in each lane

tive heterogeneity at its 5′-end as does L25-mRNA extracted from control cells (Fig. 4). Major transcription initiation sites map at positions -25 and -21 (with respect to the AUG start codon), minor sites are located at positions -11 and -8 (cf. also Leer et al. 1984b). Plasmid-derived and genome-derived L25-mRNA were also indistinguishable at their 3′-ends as demonstrated by S1 mapping experiments (data not shown).

After cell fractionation studies had shown that the excess L25-mRNA accumulates in the cytoplasm we isolated polysomes and surveyed the various size classes for the presence of L25-mRNA by Northern hybridization. Figure 5 demonstrates that all L25-mRNA present in the transformed cells is associated with polysomes. No L25-mRNA could be detected in the top fractions of the gradient. Moreover, the distribution over the various size classes in the transformed cells does not differ from that observed in control cells (data not shown). Finally, when polyA$^+$ RNA was isolated from the transformed cells and translated in vitro in the reticulocyte system the amount of L25 synthesized, assayed by immunoprecipitation, reflected the increased amount of L25-mRNA present in these cells very well (data not shown). Therefore, the excess L25-mRNA appears to be translated normally, putting the major control of L25 accumulation at the level of protein turnover.

To see whether excess L25 is produced but rapidly degraded we pulsed transformed and control cells with [^{35}S] methionine for 10–45 s and compared the incorporation of radioactivity into L25 by displaying total protein on a two-dimensional gel. The autoradiograms depicted in Fig. 6 compare the results of a 45 s pulse of transformed and control cells. It is clear that in the transformed cells L25 is overproduced in appreciable amounts. We obtained similar results with shorter pulse times. Since, as shown in Table 1, overproduction can not be detected using pulse times of 3 min or longer, the excess L25 protein is subject to very rapid turnover. From these results we conclude that the level of L25 in the trans-

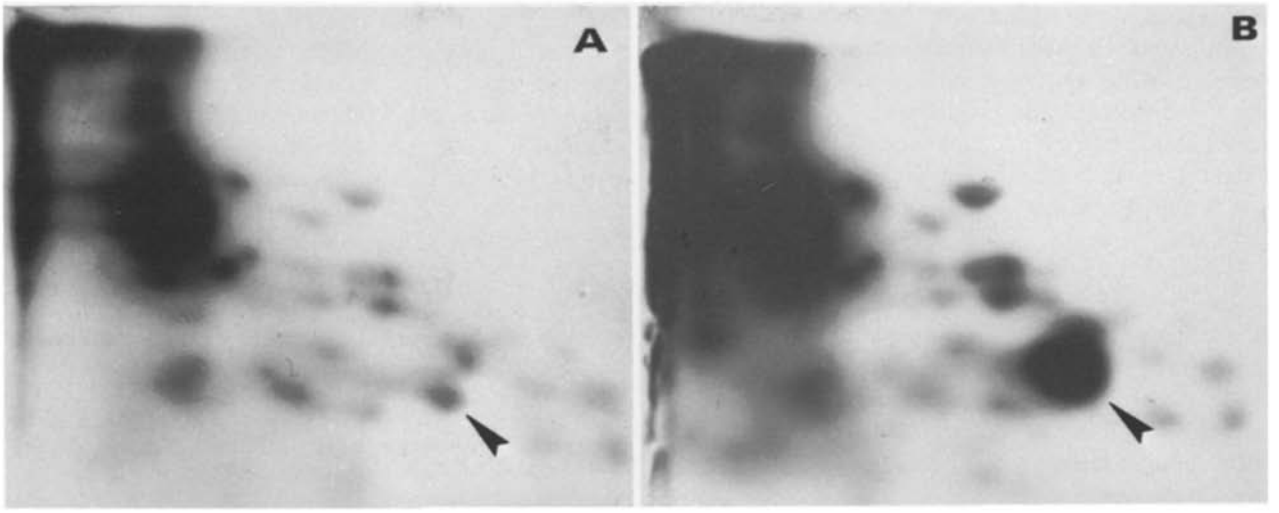

Fig. 6A, B. Overproduction of L25 protein by pJDB207/L25 transformed cells. Exponentially growing transformed and control cells were labelled with [^{35}S] methionine for 45 s as described in Materials and methods. Total protein was isolated and displayed on a 2D gel according to Mets and Bogorad (1974). The gels were dried and autoradiographed. A Protein isolated from cells transformed with pJDB207/L25. B Protein from the same number of cells transformed with the vector without the insert. The origin is in the upper left hand corner. The *arrows* indicated the position of the L25 spot

formed cells is controlled mainly, if not exclusively, by extremely rapid degradation of excess protein.

Discussion

In yeast, as well as in other organisms, the cellular levels of ribosomal constituents are controlled both on a global and an individual basis. The first type of control is apparent from the fact that the ribosome content of the cells depends on the physiological conditions (reviewed by Warner 1982; Planta and Mager 1982). Since yeast cells do not contain significant pools of free ribosomal constituents, the rate of synthesis of the set of these constituents as a whole must increase or decrease in response to, for instance, the nature of the nutrients available. This type of global regulation appears to be based principally on modulation of transcription. So far, the signal(s) mediating this control are unknown. The conserved sequence elements present in the 5′-flanking regions of most yeast r-protein genes (Teem et al. 1984; Leer et al. 1985), however, may well play a role in the coördinated response of r-protein gene transcription. These elements have a stongly activating effect on transcription (L. P. Woudt, W. H. Mayer and R. J. Planta, unpublished observations). Similar elements occurring in the flanking sequences of two genes for yeast translation factors act as binding sites for (a) protein factor(s) (Huet et al. 1985).

The individual control of the cellular level of r-proteins becomes evident when the number of genes for a particular r-protein in yeast cells is increased by transformation with a multi-copy vector. In none of the cases studied so far, including the one described in this paper, did such an increase in gene copy number cause a significant increase in the steady state level of the corresponding r-protein (Himmelfarb et al. 1984; Warner et al. 1985; Abovich et al. 1985). The regulatory mechanisms responsible for this compensation, although all operating post-transcriptionally, are variegated. Modulation of splicing, translation initiation and even protein turnover have each been suggested as the principal factor controlling accumulation of one or another r-protein (Warner et al. 1985; Abovich et al. 1985; this paper). Again, the signal(s) mediating these various modes of fine tuning have yet to be identified.

In this paper we report the results of gene dosage experiments involving the gene for yeast r-protein L25. We have previously shown L25 to be the most strongly rRNA-binding protein from the large ribosomal subunit (ElBaradi et al. 1984) and have identified its binding site on the 26S rRNA (ElBaradi et al. 1985). The existence of structural homology between part of this binding site and certain regions of L25 mRNA prompted us to ask whether accumulation of L25 might be regulated by an autogenous, (pre-)mRNA-targeted mechanism based on the same competition principle operating in bacteria.

Increasing the copy number of the L25 gene by about a factor of 50 (Fig. 2) did not result in any significant increase in the level of the protein as measured by a 3 min pulse of [^{35}S] methionine (Table 1). Therefore, the yeast cells can compensate for even this extreme gene dose (4–10 times that used in previous experiments [Warner et al. 1985; Abovich et al. 1985]).

Pulse-labelling experiments using very short ($<$ 1 min) pulses showed that at least a major part of the L25

gene dosage compensation takes place at the level of protein stability. From autoradiograms such as those displayed in Fig. 6 we estimate L25 in the transformed cells to be oversynthesized at least 10-fold relative to control cells. Since a 3 min pulse does not reveal any significant oversynthesis the excess protein must be degraded very rapidly, its half life being in the order of less than 1 min.

The rapid turnover of excess L25 might simply be the result of the inherent instability of r-proteins that are not being incorporated into (pre)ribosomal particles (Gorenstein and Warner 1977). Excess L25, transported into the nucleus, will fail to be sequestered by pre-ribosomal RNA and consequently would be degraded. Recently such an assembly-mediated modulation of turnover was proposed by Abovich et al. (1985) for yeast r-protein rp51 which displays a similarly short half life in cells containing extra copies of the gene encoding this protein. We feel, however, that this simple explanation is not applicable to L25. Assuming an elongation rate of 7 amino acid residues/sec (Waldron et al. 1974), the L25 half life is of the same order of magnitude as the translation time of the protein, which contains 142 amino acids (Leer et al. 1984b; L. P. Woudt, unpublished observations). The two values could be even closer than is apparent from the data presented here because the extent of overproduction of L25 may be an underestimate. The method used to stop the labelling probably does not result in instantaneous inhibition of degradation. Therefore, the cells seem to be able to sense overproduction of L25 much more rapidly than can be explained by the failure of excess protein to bind to precursor rRNA. The nature of the mechanism signalling L25 overproduction remains unclear. In view of the swiftness of the response, however, it is likely to be located in the cytoplasm. Moreover, the mechanism must be L25-specific since the levels of other r-proteins are not affected by the presence of extra L25 gene copies (data not shown), an observation made also in similar studies with other yeast r-proteins (Warner et al. 1985; Abovich et al. 1985).

Although the extremely rapid turnover of excess protein certainly constitutes a major contribution to the control of L25 accumulation it may not be the exclusive regulation mechanism. The discrepancy between the increase in mature mRNA concentration and the estimated oversynthesis of L25 leaves room for additional regulation at the level of translation. Neither the polysomal distribution of the excess L25 mRNA (Fig. 4) nor its efficient in vitro translation is conclusive evidence against translational regulation although the results make such a mechanism less likely. Autogenous translational regulation is made less plausible by our failure to detect any interaction between L25 and either mature L25 mRNA (isolated from the transformed cells by hybrid selection) or precursor L25 mRNA (synthesized in the SP6 system) by filter binding assays. The same technique readily demonstrated interaction of the protein with 26S rRNA (ElBaradi et al. 1985 and unpublished experiments) as well as binding of *E. coli* ribosomal protein ES4 to its own mRNA (Deckman and Draper 1985). We are presently trying to identify the mechanism involved in the rapid turnover of L25 and to settle the involvement of translational regulation in the dosage compensation of L25.

Acknowledgements. The authors are indebted to Drs. R. J. Leer, L. P. Woudt and J. J. v. d. Heuvel for valuable discussions, to Dr. R. J. Leer for supplying the pJDB207/L25 plasmid and to Dr. J. J. van den Heuvel and Mrs. M. M. C. van Raamsdonk-Duin for their expert assistance during part of the experimental work. We thank Mrs. P. G. Brink for preparing the typescript. This work was supported in part by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

References

Abovich N, Gritz L, Tung L, Rosbash M (1985) Mol Cell Biol 5:3429–3435
Beggs JD (1978) Nature (London) 275:104–109
Bollen GHPM, Mager WH, Jenneskens LW, Planta RP (1980) Europ J Biochem 105:75–80
Bollen GHPM, Molenaar CMT, Cohen LH, van Raamsdonk-Duin MMC, Mager WH, Planta RJ (1982) Gene 18:29–37
Bromley S, Hereford L, Roshbash M (1982) Mol Cell Biol 2: 1205–1211
Davis RW, Thomas M, Cameron J, St John TP, Scherer S, Padgett RA (1980) Methods Enzymol 65:404–411
Deckman IC, Draper DE (1985) Biochemistry 24:7860–7865
ElBaradi TTAL, Raué HA, deRegt VCHF, Planta RJ (1984) Europ J Biochem 144:393–400
ElBaradi TTAL, Raué HA, deRegt VCHF, Verbree EC, Planta RJ (1985) EMBO J 4:2201–2107
Gorenstein C, Warner JR (1977) Mol Gen Genet 157:327–332
Hardy SJS, Kurland CG, Voynow P, Mora G (1969) Biochemistry 8:2897–2905
Himmelfarb HJ, Vassarotti A, Friesen JD (1984) Mol Gen Genet 195:500–506
Huet J, Cottrelle P, Cool M, Vignais M-L, Thiele D, Marc C, Buhler J-M, Sentenac A, Fromageot P (1985) EMBO J 4: 3539–3547
Jacobs FA, Bird RC, Sells BH (1985) Europ J Biochem 150: 255–263
Kaltschmidt E, Wittmann MG (1970) Anal Biochem 36:401–412
Kruiswijk T, Planta RJ (1974) Mol Biol Rep 1:409–415
Kruiswijk T, Planta RJ, Krop JM (1979) Biochim Biophys Acta 517:378–389
Leer RJ, van Raamsdonk-Duin MMC, Mager WH, Planta RJ (1984a) FEBS Lett 175:371–376
Leer RJ, van Raamsdonk-Duin MMC, Hagendoorn MJM, Mager WH, Planta RJ (1984b) Nucleic Acids Res 12:6685–6700
Leer RJ, van Raamsdonk-Duin MMC, Mager WH, Planta RJ (1985) Curr Genet 9:273–277
Mager WH, Planta RJ (1976) Europ J Biochem 62:193–197

Maniatis T, Fritsch FF, Sambrook J (1982) Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Messing J (1982) In: Setlow JK, Hollaender A (eds) Genetic engineering, principles and methods, vol 4. Plenum Press, New York, pp 19–34

Mets L, Bogorad L (1974) Anal Biochem 57:200–210

Molenaar CMT (1984) PhD Thesis. Vrije Universiteit, Amsterdam

Nazar RN (1979) J Biol Chem 254:7724–7729

Nazar RN, Wildeman AG (1983) Nucleic Acids Res 11:3155–3168

Nomura M, Gourse R, Baughman G (1984) Annu Rev Biochem 53:57–117

Pierandrei-Amaldi P, Beccari E, Bozzoni I, Amaldi F (1985) Cell 42:317–323

Planta RJ, Mager WH (1982) In: Busch H, Rothblum L (eds) The cell nucleus, vol 12. Academic Press, New York London, pp 213–226

Schaap PJ, Molenaar CMT, Mager WH, Planta RJ (1984) Curr Genet 9;47–52

Shulman RW, Warner JR (1978) Mol Gen Genet 161:221–223

Southern EM (1975) J Mol Biol 98:503–517

Stoyanova BB, Hadjiolov AA (1979) Europ J Biochem 96: 349–356

Teem JL, Abovich N, Käufer NF, Schwindinger WF, Warner JR, Levy A, Woolford J, Leer RJ, van Raamsdonk-Duin MMC, Mager WH, Planta RJ, Schultz L, Friesen JD, Fried H, Rosbash M (1984) Nucleic Acids Res 12:8295–8312

Udem SA, Warner JR (1972) J Mol Biol 65:227–242

Waldron C, Jund R, Lacroute F (1974) FEBS Lett 46:11–16

Warner JR (1982) In: Strathern JN, Jones EW, Breach JR (eds) The molecular biology of the yeast *Saccharomyces cerevisiae*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 529–560

Warner JR, Mitra G, Schwindinger WF, Student M, Fried HM (1985) Mol Cell Ciol 5:1512–1521

Communicated by L. A. Grivell

Received February 20, 1986