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Modulation of protein synthesis and degradation maintains proteostasis during yeast growth at different temperatures

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ABSTRACT

To understand how cells regulate each step in the flow of gene expression is one of the most fundamental goals in molecular biology. In this work, we have investigated several protein turnover-related steps in the context of gene expression regulation in response to changes in external temperature in model yeast *Saccharomyces cerevisiae*. We have found that the regulation of protein homeostasis is stricter than mRNA homeostasis. Although global translation and protein degradation rates are found to increase with temperature, the increase of the catalytic activity of ribosomes is higher than the global translation rate suggesting that yeast cells adapt the amount of translational machinery to the constraints imposed by kinetics in order to minimize energy costs. Even though the transcriptional machinery is subjected to the same constraints, we observed interesting differences between transcription and translation, which may be related to the different energy costs of the two processes as well as the differential functions of mRNAs and proteins.

1. Introduction

Gene expression consists of the conversion of genetic information, stored as DNA, into functional molecules, represented mainly as proteins. RNA, however, has an important place in the central dogma of molecular biology which may be due to its primitive origin that precedes both DNA and proteins in evolution [1]. Regulation of gene expression has been shown to occur mostly at the mRNA level [2,3,4], possibly due to both its central role within the flow of genetic information and its chemical instability. Consequently, proteins, despite being the ultimate goal of gene expression and performing most of the structural and catalytic functions in the cell, are generally poorly regulated at the synthesis and degradation levels [2]. Therefore, it appears that the majority of gene expression regulation in eukaryotic cells is focused on controlling mRNA concentrations ([mRNA]), and that protein synthesis is subsequently dependent on variations in [mRNA] with less specific translational regulation.

To date, the global transcription and translation strategies utilized by eukaryotic cells to survive environmental changes have been poorly studied. This is largely due to the existence of many individual parameters that can be altered by external conditions and the variations in physiological control employed by cells in response to them. Transcription (synthesis) rate (SR) can be measured as the density of active RNA polymerase (RNA pol) molecules on genes multiplied by their elongation speed (Fig. 1). Similarly, translation rate (TLR) depends on the speed and density of active elongating ribosomes and on [mRNA] that, in turn, depends on the equilibrium between SR and mRNA stability (RS). Finally, cellular protein concentration, similarly, depends on the equilibrium between TLR and protein stability (PS). It is generally assumed that cells have homeostatic control for most molecules, including mRNAs and proteins [5,6,7,8], which have been termed ribostasis [9] and proteostasis [10] [11] respectively.

Deciphering the strategies that cells employ to sustain ribostasis, proteostasis and their interplay is the key to fully understand how cells evolved to adapt and thrive in the changing environment [12]. One of the most common environmental variations is external temperature, which imposes physicochemical constrains on the behavior of cellular molecules. Transcription and translation processes consist of the

Abbreviations: RNA pol, RNA polymerase; NTP, nucleotide triphosphate; SR, mRNA synthesis rate; TLR, translation rate; O.D., optical density; ORF, open reading frame; DR, mRNA degradation rate; qPCR, quantitative PCR; [mRNA], mRNA concentration; poly-A, polyadenylated; SD, synthetic defined medium; Met, methionine; Q_{10}, increase in reaction rate when temperature increases 10 °C; IR, initiation rate

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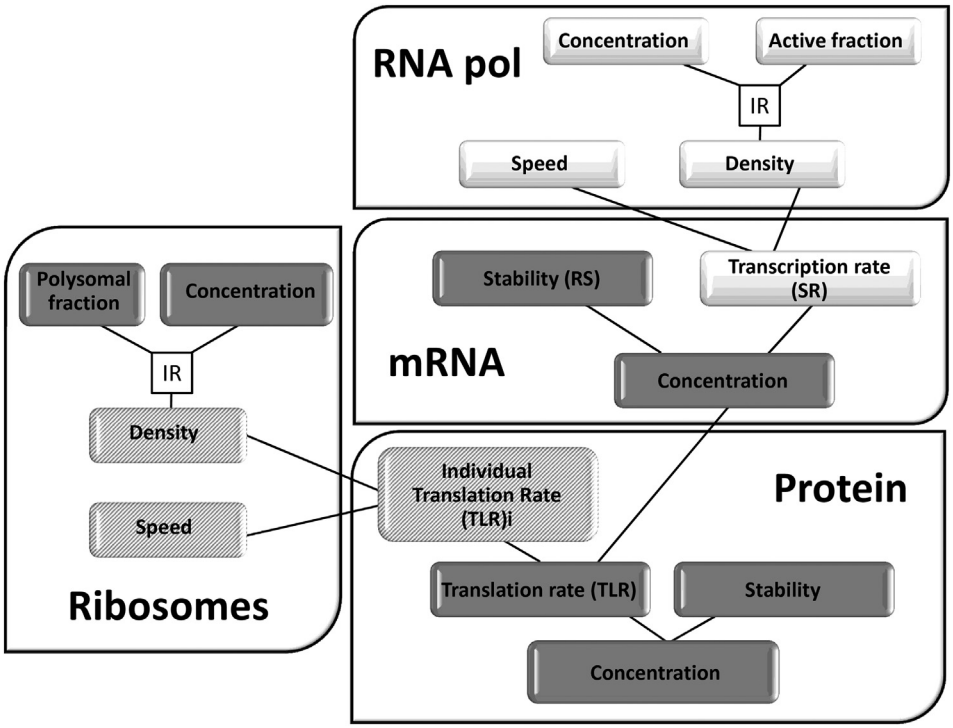

Fig. 1. Schematic representation of the experimental parameters that determine mRNA and protein equilibrium in *S. cerevisiae*. Equilibrium exists between various parameters of the gene expression flow mediated by strict regulations at the levels of transcription, translation, [mRNAs] and [proteins]. By acting on one or more of these parameters, a cell is able to maintain intracellular equilibrium when faced with environmental variations such as temperature (see main text for details). Parameters measured in a previous study [17] are colored in white, those calculated experimentally in this study are in dark gray and those inferred mathematically from these studies are colored in pale gray. In all cases the values used in these studies refer to total or average values for all parameters. IR: initiation rate.

assemblage of a series of chemical reactions, which are governed by thermodynamic and kinetic laws that rely, among other factors, on temperature. The velocity of enzymatic reactions increases with temperature according to the Arrhenius dependence of the catalytic constant [13,14]. The rate of increase of most biological reactions occurs with a temperature coefficient (Q_{10}) of ~2 to 3 for every 10 °C. For enzyme-catalyzed reactions, another factor that can influence on reaction rate is the thermostability of the enzyme [14]. With respect to the control of translation, it should also be considered that lower temperatures stabilize mRNA secondary structures, which in turn may have an inhibitory effect in translation. This effect has been shown in bacteria [15] and proposed for yeast cells at 15 °C [16]. These physicochemical constraints have influenced cellular evolution to drive the acquisition of gene expression mechanisms that are optimally adapted to not only the regular growth temperature but also to its fluctuations in a limited range for each organism.

In a previous study, we described the dependence of transcription on temperature in the single cell model organism *Saccharomyces cerevisiae* [17]. In the wild, this yeast grows in a relatively broad temperature range (from 10 to 39 °C [18,19]), as such that regulatory mechanisms have evolved to allow the cells to survive and adapt to potential external temperature variations. We found that RNA polymerase II (RNA pol II) in this yeast species elongates from 0.67 to 1.31 kb/min in the temperature range between 23 and 37 °C. We observed a change in RNA pol II density that is approximately reciprocal to its speed. This limits the variation of SR over the range of growth temperatures studied. Nonetheless, we also observed a 25% change in SR within the optimal yeast growth temperature range (30–37 °C), which suggested an alteration in the RNA pol II initiation rate, corresponding to changes in the total amount of this enzyme. It is important to remark that within our study we exclusively investigated global parameters, namely total SR and total amount of RNA pol II, as well as global average RNA pol II density and elongation speed. This was because the objective of that study was not to describe differences between genes or gene categories but to understand the adaptation strategy used by the cell to regulate global transcription in response to changes in growth temperature.

In the present study, we investigated ribostasis and proteostasis in *S. cerevisiae* cells grown at different temperatures. We show that global [mRNA] is homeostatically controlled within a certain range and that the homeostatic control of protein concentration is stricter and maintained by compensatory effects occurring at the levels of protein synthesis and stability. Ribosome specific activity increases with temperature, as predicted by kinetic laws; however the increase in global protein synthesis is less due to the reduction in ribosome concentration. A similar limited increase is observed in protein degradation. Our results suggest that although temperature imposes similar constraints upon mRNA and protein synthesis and degradation machineries, the adaptation utilized by the cell for each process is distinct, possibly due to the higher energetic cost of translation compared to transcription. This study provides the first overview of the global homeostatic response of a eukaryotic cell to environmental changes.

2. Materials & methods

2.1. Yeast strains, media and growth conditions

S. cerevisiae GYLR-4B (*Mat α, his3Δ1, lys2Δ, ura3Δ, met15Δ, pGAL-YLR454w::URA3, trp1Δ::hisG*) was used for the indicated experiments described in Supplementary Fig. S1 and in Supplementary Table I. For all experiments related to protein biosynthesis/degradation, the yeast strain BQS252 (*Mat a, ura3–52, GAL2*) was used. Both strains have an S288c genetic background. GYLR-4B yeast cells were grown in liquid YPD (2% glucose, 2% peptone, 1% yeast extract) and BQS252 cells were grown in either YPD or synthetic minimal medium containing uracil (SD + ura: yeast nitrogen base without amino acids 0.67%, 2% glucose, 20 μg/mL uracil). Experimental assays were performed with cells

grown for at least seven generations until O.D.$_{600}$ 0.3 at the indicated temperatures.

The use of two different strains was necessary to compare previously published data related to transcription (done in GYLR-4B, [17,20]) with new data related to translation. To perform the protein biosynthesis and stability assays, cells needed to be grown in a medium lacking methionine (Met). This avoids the dilution of the radioactively-labeled Met with Met present in the medium, maximizing incorporation efficiency. Since the GYLR-4B (*met15Δ*) is not suitable for growth in minimal media lacking Met, we therefore used the isogenic *MET15* yeast strain (BQS252). Although the strain change may introduce some noise in the comparison, the behavior of the two strains in their growth media was very similar with regard to the growth temperature changes (see Fig. 3B,D) which allows the general comparison of transcription and translation processes as a function of the external temperature. Moreover, some of the analyses were done in both strains ([RNA], [mRNA] and [protein] determinations) and showed similar profiles.

To determine growth rate, BQS252 and GYLR-4B cells were grown in liquid SD + ura and YPD, respectively, at the corresponding temperatures from O.D.$_{600}$ ~ 0.05 to mid-late log phase. The rate of exponential growth was calculated as the time in minutes required for the O.D.$_{600}$ to double.

2.2. RNA extraction and poly-A RNA measurement

To determine RNA amount, total RNA was extracted using phenol:-chloroform extraction as described in Garre et al. [21] in biological triplicates. Serial dilutions of total RNA were then spotted on a Nylon membrane (*Nytran SPC*, *GE* Healthcare) and hybridized with specific oligo d(T)$_{40}$ probes terminally labeled with Polynucleotide Kinase (Roche). Membranes were exposed to an Imaging plate (*BAS-MP*, Fujifilm) and scanned with Fujifilm *FLA3000 Phosphorimager*. Signal intensity of the spots was quantified with *Image Gauge 4.0*.

2.3. mRNA half-life determination

For the determination of global average mRNA stability in BQS252 strain at different temperatures we followed the protocol described in [23]. Briefly, cells were grown in SD-ura medium to O.D.$_{600}$ ~ 0.3 and subjected to thiolutin treatment and samples were collected as indicated above. An RNA dot-blot was then hybridized with labeled oligo d(T)$_{40}$ and quantified as described above.

For the determination of the individual mRNA half-lives of 6 genes used in this study (*ACT1*, *RPL17*, *PHO88*, *RPL25*, *RPL5*, *RPB6*), we followed the protocol described in [22]. Briefly, GYLR-4B cells were grown at either 28 or 37 °C in YPD media and transcriptional shut-off was performed by treating the cells with the RNA pol II inhibitor thiolutin (5 μg/mL), and samples were collected at 5, 12, 25, 45, 60, 90, and 120 min following the addition of thiolutin.

2.4. Polysome fractionation from yeast cells

For the analysis of active ribosomes by polysome fractioning of cell extracts, we followed the protocol previously described in [24] using BQS252 cells grown in YPD at the indicated temperatures.

2.5. Translation rate measurements and global protein stability determination by radioactive amino acid incorporation

All translation activity experiments were performed using BQS252 grown in SD + ura media in the absence of methionine following the method described in [25] with slight changes. Briefly, cells were grown in SD + ura medium to O.D.$_{600}$ 0.3–0.4 at 23, 30, 34 and 37 °C. Each experiment was performed in triplicates as follows: approximately 1.4×10^7 cells (1.5 mL) were radioactively pulsed with 16.5 μL of a 1/20 dilution of *Easy tag express protein labeling mix* (^{35}S-L-methionine and ^{35}S-L-cysteine) stock solution (PerkinElmer) for 10 min in agitation at 650 rpm at the corresponding experimental temperatures. Ice cold solution containing cycloheximide (35 μg/mL; Sigma), L-methionine (133 μM) and L-cysteine (44 μM) was rapidly added to the culture to subsequently block the incorporation reaction. To determine total cellular intake of radioactivity, 165 μL of cell suspension was spotted onto a 2.5 cm glass fiber filter and washed three times with cold water. After washing, glass filters were dried in an aerated heater and submerged in 5 mL of scintillation solution (*Normascint Cocktail #22*, Scharlau) for radioactive counting using a *TriCarb 2810 TR liquid scintillation counter* (PerkinElmer). In parallel, protein incorporation of radioactive amino acids was determined by transferring another 165 μL of cell suspension aliquot to a new 2 mL eppendorf tube containing 165 μL of ice-cold 25% TCA. After incubation in ice bath for at least 30 min, all the liquid content was spotted into a 2.5 cm glass fiber filter that was washed once with cold 10% TCA and once with cold 96% ethanol. Glass filters were then dried and processed as described above for radioactive counting. Translation rate (TLR) was calculated as the percentage of TCA-precipitable radioactivity expressed as a fraction of the total intake of radioactivity by the cells (time 0 being the addition of cycloheximide).

To determine protein stability, we measured TCA-precipitable radioactivity at 20, 40 and 60 min after adding cycloheximide. Briefly, at each time point, 165 μL of cell suspension was transferred to a new 2 mL eppendorf tube containing 165 μL of ice-cold 25% TCA. After 30 min on ice, total intake and TCA-precipitable radioactivity were determined as above. To determine protein stability, the $\log_2$ value of the percentage of TCA-precipitable radioactivity after adding cycloheximide was plotted against time (minutes) and protein stability was calculated as the inverse of the slope of the line of best fit.

2.6. Protein quantification

BQS252 cells were grown in SD + ura medium to O.D.$_{600}$ ~ 0.3–0.4 at their respective temperatures in biological triplicates and cell number and median cell volume were then measured in a *Coulter-counter* device. To prepare protein extracts, 20 mL of culture were harvested by centrifugation and resuspended in lysis buffer (25 mM HEPES, 150 mM NaCl, 5 mM EDTA, 1% Triton X100) containing *Complete Mini Protease Inhibitor Cocktail Tablets* (Roche). Cells were then mechanically broken with 500 μL of glass beads in FastPrep (two cycles of 20 s at intensity 6500 rpm) and a 50 μL aliquot of the supernatant was precipitated overnight at 4 °C in 25% TCA. Next, pellets were washed once with acetone and then resuspended in TEAB buffer (8 M Urea, 0.5 M Triethylamonium, pH 8.5). Protein concentration was determined using *Bradford Assay* (Bio-Rad) following manufacturer's instructions.

3. Results

3.1. Reductions in total RNA and mRNA concentrations at higher temperatures are maintained within homeostatic levels

During exponential growth in yeast, mRNA levels have been shown to be approximately at a steady state [26]. The steady state is a dynamic situation in which mRNA synthesis (SR) and degradation rates (DR) are equal, maintaining a stable number of mRNA molecules within the cell. Our previous study indicated that the SR is relatively constant within the optimal range of growth temperatures, but shows a certain decrease (25%) at 37 °C [17]. In theory, changes in SR in response to different temperature can be equilibrated with changes in DR or may, alternatively, have a net impact on the [mRNA] in the cell.

To evaluate whether DR equilibrates the SR variations, we analyzed the global relative stability of polyadenylated (poly-A) RNA at three different temperatures: 23, 30 and 37 °C (Fig. 2). The results clearly show that there is a decrease in global mRNA stability of 60% in yeast

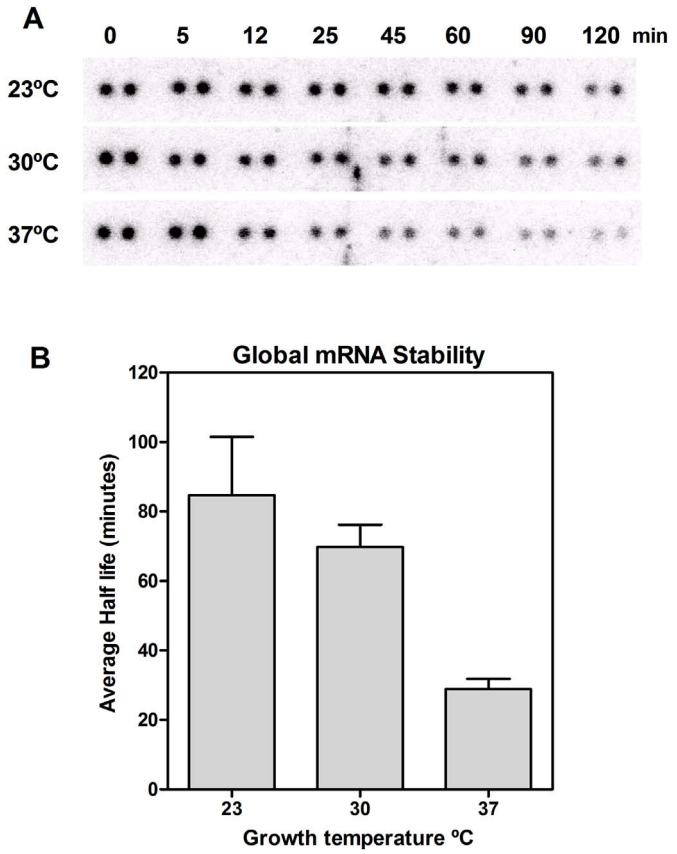

Fig. 2. Analysis of global poly(A) mRNA stability at different temperatures. A) Dot-blot analysis of a thiolutin transcription shut-off. BQS252 yeast cells grown at the indicated temperatures were incubated in the presence of thiolutin 5 μg/mL and RNA was extracted from samples taken at the indicated times. Three different RNA amounts were spotted in duplicate onto Nylon filter and hybridized with ^{32}P-labeled oligo d(T)$_{40}$. Only one concentration from a representative experiment is shown. B) The signal intensity of the spots was calculated and represented in $\log_2$ scale as a function of time (see supplementary Fig. S1). The average and standard deviation of three biological replicates is shown.

strain BQS252 with an increase in temperature (37 °C vs 23) We also analyzed the individual relative stability of 6 representative mRNAs in the GYLR-4B yeast strain at two different growth temperatures, 28 and 37 °C (Supplementary Fig. S1) and observed, on average, a 50% decrease in mRNA stability at 37 °C compared to 28 °C.

All these results suggest that [mRNA] is decreased at 37 °C. In order to confirm this, we determined the overall RNA and mRNA amounts present in GYLR-4B cells growing at 23, 30, 34 and 37 °C. RNA was extracted from identical cell number aliquots grown to exponential phase in YPD at each temperature. The total RNA obtained from this type of extractions is mainly composed of rRNA and tRNA molecules (80% and 15% of the total RNA population, respectively (see [27]). Since rRNA represents the majority of total quantified RNA, these measurements additionally serve as good estimates of the cellular ribosome concentration. In parallel, we determined the fraction of poly-A mRNA contained in total RNA as an estimator of mRNA amount within the cell. The relative mRNA value obtained was then multiplied by the measured total RNA amount per cell to obtain the absolute total mRNA per cell. Total amounts were corrected by relative median cell volume (see supplementary Table S1) to obtain concentrations of both total [RNA] and total [mRNA] in yeast cells. We observed that [RNA] in GYLR-4B cells remained relatively constant over the usual range of growth temperatures (23–30 °C) used for laboratory cultures of *S. cerevisiae* [19], but there was a decrease for both [RNA] and [mRNA] at higher temperatures (reaching 50% at 37 °C) and also for [mRNA] at 23 °C (Fig. 3A). Given that growth rate at the different temperatures can have an effect on mRNA synthesis and degradation rates [6] we determined the growth rates for GYLR-4B strain at various temperatures (Fig. 3B). We observed a significantly reduced growth rate at 23 °C compared to 30 °C, which corresponds to a reduction in SR [6]. Interestingly, although the SR, [mRNA] and mRNA stability are reduced at 37 °C with respect to 30 °C, cellular growth rate remains constant (± 10%), suggesting the existence of additional homeostatic mechanisms to maintain an optimal growth rate. We repeated the RNA and mRNA measurements in the isogenic strain BQS252, the same strain used to determine protein synthesis and degradation (shown below), and observed (Fig. 2C, D) changes in [RNA], [mRNA] and growth rates in response to different growth temperatures parallel to that of GYLR-4B. In the comparison of 30 °C to 37 °C we observed a decrease of both RNA concentrations of 25–30%, while the growth rate remains constant.

In summary, our results suggest that global [mRNA] at temperatures higher than 30 °C decreases due to a combination of reduced transcription and increased degradation. Regardless, the variation in [mRNA] remains restricted to a range of ± 30–50%, which is the deviation range within ribostatic levels in concordance to previous data [7] [28]. Importantly, the level of ribosomes ([RNA]) also decreases in parallel to [mRNA] at 37 °C, despite the growth rate being remain constant within the experimental temperatures.

3.2. Protein concentration is maintained within homeostatic levels but translation rate increases with growth temperature

Despite its importance in gene expression, mRNA is a transient molecule in the gene expression process (Fig. 1). The importance of the maintenance of protein production at an adequate rate precedes that of mRNA, as ultimately, proteins dictate the survival and proliferation of the cells. Hence, the concentration of proteins is the main parameter that needs to be adjusted when changes in cell physiology are required [29,30]. In the previous sections it was observed that the [mRNA] is reduced in cells grown at 37 °C compared to those grown at 30 °C (Fig. 3A, C; Table S1) whereas growth rate remains similar at both temperatures (Fig. 3B, D). These results suggest that the cell utilizes homeostatic mechanisms to adjust its protein levels (concentration) in order to sustain a similar growth rate at both 37 °C and 30 °C. To analyze protein homeostasis regarding growth temperature, we first quantified total protein content for identical number of BQS252 yeast cells which was then represented as protein concentrations by correction of data with the median cell volume ratios (Fig. 4A and Table S1). Results show that overall protein concentration remains constant with the change in growth temperature. Similar results were obtained for the GYLR-4B strain [17].

Despite the proteostasis observed, synthesis and/or degradation rates could still be affected by a change in growth temperature. In fact, although it is assumed that protein turnover should increase at 37 °C compared to 30 °C, no global synthesis or stability measurements have been performed at different temperatures in yeast.

Therefore, we aimed to analyze global protein production and degradation at different temperatures. First, to measure translation rates (TLR) in cells growing at the different temperatures tested (23, 30, 34 and 37 °C), we utilized a protein synthesis assay in which new proteins are synthesized with ^{35}S-labeled amino acids (see M & M). The TLR was represented as the percentage of TCA-precipitable radioactivity as previously described [25]. We observed that the TLR increases with growth temperature (Fig. 4B). This could be explained as a passive consequence of the enhanced enzymatic activity at higher temperatures, however it is lower than what is predicted by the Q_{10} rule (1.7 fold instead of the predicted 2.8–4 fold) when comparing 23 to 37 °C (Fig. 4B).

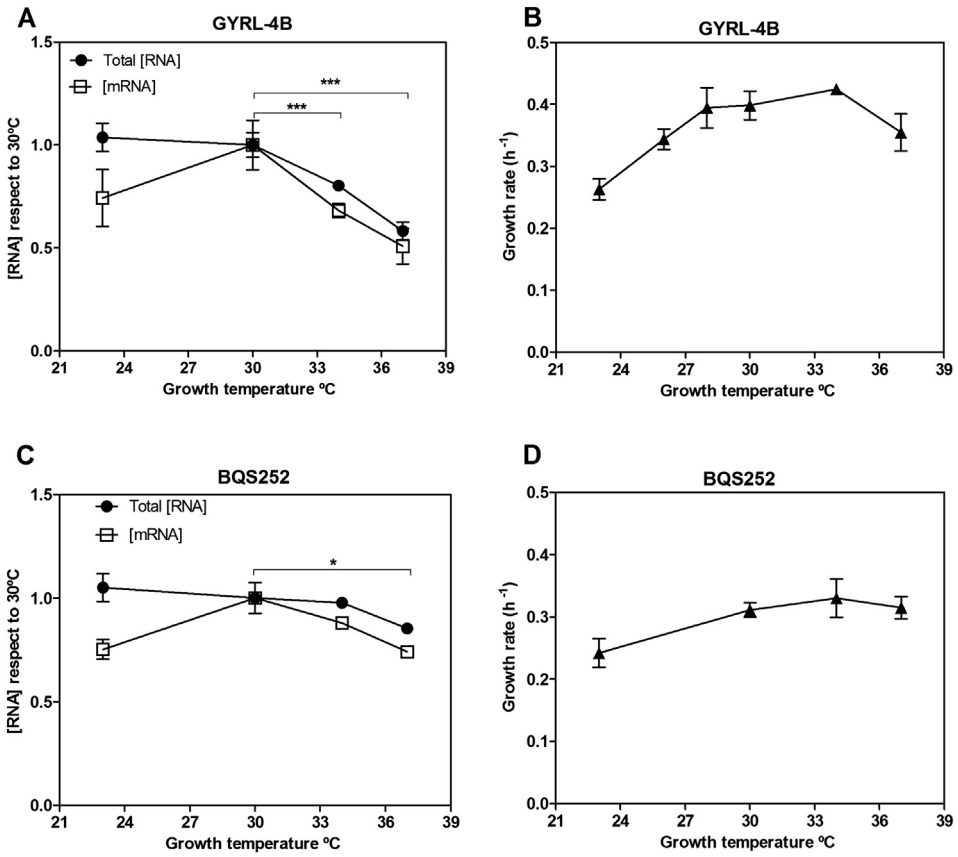

Fig. 3. Total RNA and mRNA concentrations and cellular growth rates at different temperatures. A, C) Total [RNA] and [mRNA] were determined as described in Materials and Methods in the yeast strains GYLR-4B and BQS252 cultivated in YPD or SD + ura, respectively, at the indicated growth temperatures. Data are represented as fold change of values obtained with respect to those at 30 ˚C. Graphs represent the mean and standard deviation of at least three independent experiments of each assay. Statistical comparison of the results was performed by using an analysis of variance (ANOVA) and subsequent post hoc comparisons were made by using a Tukey multiple comparison test. *P*-values were significant at the < 0.05 (*) or < 0.001 (***) level. B, D) Growth rates of *S. cerevisiae* strains GYLR-4B and BQS252 determined at different growth temperatures show similar changes. BQS252 cells were grown in SD + ura and GYLR-4B cells in YPD media to exponential phase at the indicated temperatures for a minimum of 7 generations. The GR was calculated as time in hours required for the OD_{600} to double.

3.3. The proportion of elongating ribosomes slightly decreases with temperature

Global translation rate is, on the other hand, dependent on the amount of the translation machinery (ribosomes) and/or the mRNA content present in the cells. However, changes in mRNA content are unlikely to be influential since it has been shown that mRNA molecules are in excess compared to the total ribosome content [31,32,33]. Thus, assuming that ribosomes are a limiting factor [34], their concentration would be a key parameter to determine translation. As previously mentioned, the total [RNA] serves as reflection of the ribosome content, as rRNA (~80%) and tRNA (15%) molecules account for the large majority of total RNA and rRNA and tRNA concentrations vary in parallel [27]. As shown in Fig. 3A, the total [RNA], as well as [mRNA], per cell remains relatively constant within the normal yeast growth temperature range and decreases at 37 ˚C. Hence, the observed increase in TLR at higher temperatures is accompanied by a decrease in ribosome concentration at 37 ˚C.

It is possible that, although it has been shown that yeast ribosomes are mostly (85%) bound to mRNAs [32,34], there is a percentage of free subunits due to the recycling process [35,36] that may change with temperature. In order to investigate this, we performed polysome analyses at three growth temperatures, 23, 30 and 37 ˚C. As it can be seen in Table S1 and Fig. S2 there is a slight decrease in the proportion of active ribosomes (polysomal fraction) with temperature increase. Therefore, this suggests that the reduced number of active ribosomes (polysomal fraction multiplied by [RNA], see Fig. 5) may account for the discrepancy between measured TLR, which was observed to be lower, and predicted TLR. In fact, the ribosome specific activity (calculated as the TLR relative to the fraction of active ribosomes, Fig. 4A) shows a good concordance with the theoretical Arrhenius plot (see Supplementary Fig. S3) with an increase of 2.2 fold, similar to the predicted Q_{10} when comparing 37 to 23 ˚C.

3.4. Protein stability decreases with growth temperature

Our results show that cellular protein concentration is maintained at homeostatic levels at different growth temperatures although there is an increase in translation rate (TLR) with temperature. These results suggest that a decrease in protein stability with temperature could compensate for the increase in TLR. To study the dependence of global protein stability on temperature, we developed an assay to calculate global protein decay based on the rate of degradation of ^{35}S-radioactivity in the TCA-precipitable fraction (see Materials & methods). It can be seen in Figs. 4B & 5B that, indeed, global average protein stability decreases with growth temperature. The observed decrease in protein stability could be explained as a passive consequence of the proteolytic activity at higher temperatures, although it is lower than what is predicted by the kinetic laws (1.85 fold comparing 23 to 37 ˚C, Fig. 4B). The relative decrease in protein stability is proportionate to the increase in TLR (see above). Therefore, the estimated output in protein concentration obtained by multiplying the effects of synthesis and degradation is in good accordance with experimentally determined total protein concentration (Fig. 5B).

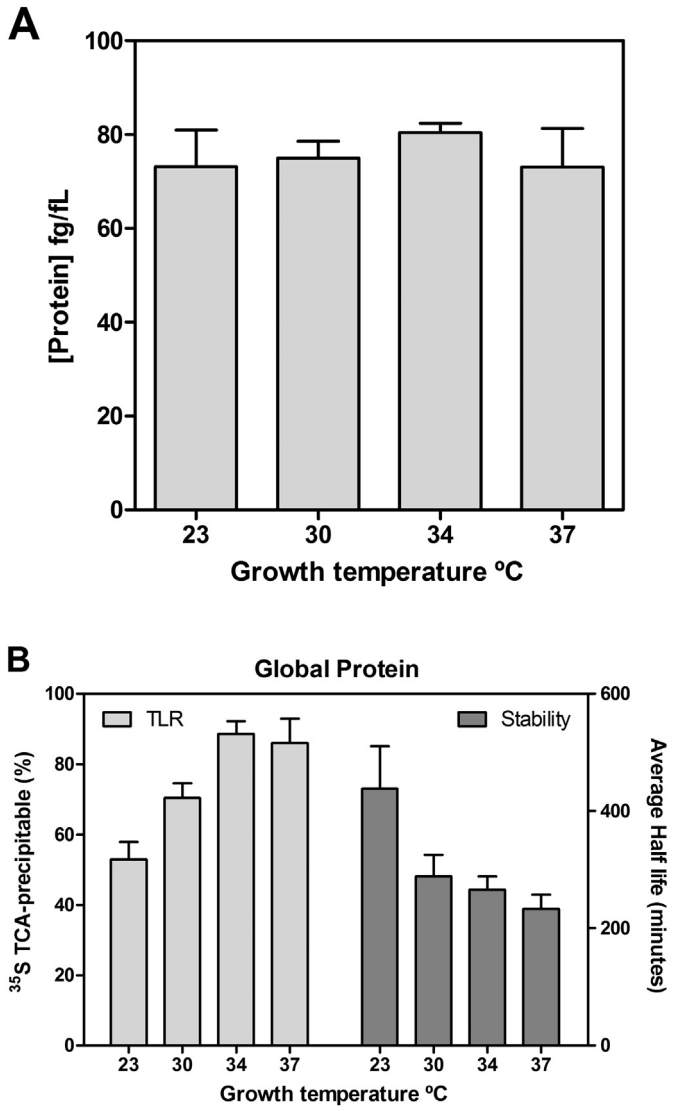

Fig. 4. Experimental determination of protein parameters at different growth temperatures. A) Experimentally determined protein concentrations in yeast cells growing at various temperatures. Protein amount in identical number of BQS252 cells was determined as described in Materials & methods and divided by cell volumes (see Table S1) to determine total protein concentration in yeast cells grown at different temperatures. B) Estimation of global translation rate (TLR) and global protein stability in BQS252 yeast strain at different growth temperatures as described in Materials & methods. Left part (pale gray bars) shows global TLR and right part (dark gray bars) show average protein half-life. For TLR determination identical number of BQS252 cells was used and data were normalized by cell volumes (see Table S1). Each experiment was performed, at least, three times and values are expressed as mean ± standard deviation.

These results confirm that, despite changes that temperature imposes on the activities of ribosomal and proteolytic machineries, yeast cells actively maintain proteostasis within a very narrow range by regulating the activities of both machineries.

4. Discussion

In the present study we have analyzed the interdependence between growth temperature and gene expression parameters in the model yeast species *S. cerevisiae*. In particular, we have studied how protein synthesis is affected by changes in external temperature. The comparison of current results with a previous study focused on transcription [17] casts light onto the similarities and differences between the processes regulating mRNA and protein cellular concentrations, which

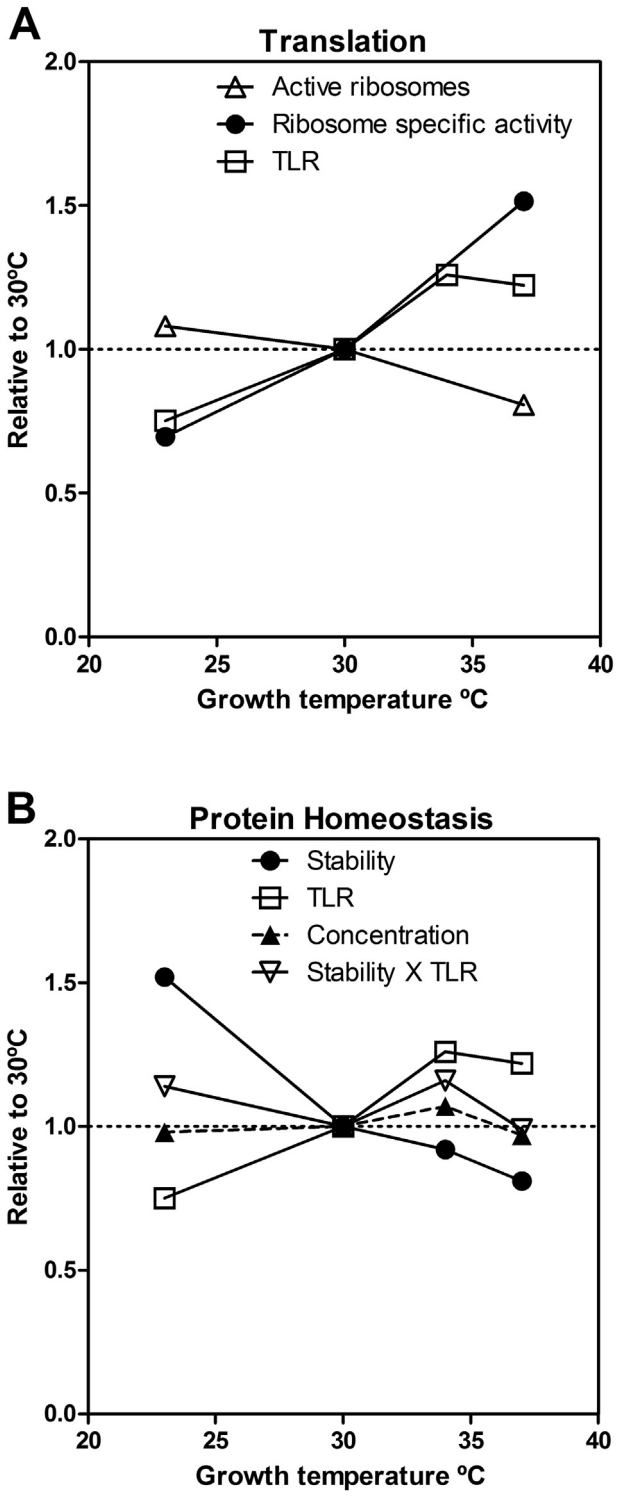

Fig. 5. Yeast cellular proteostasis. A) Relative changes in active ribosome content, polysomal fraction and ribosome specific activity were determined at different growth temperatures using the BQS252 strain. Values obtained at 30 °C are taken arbitrarily as 1. Relative ribosome content per cell is obtained from the data of total [RNA] in Fig. 3, in which [rRNA] is directly proportional to total [RNA]. Relative amount of active ribosomes (white triangles) is calculated as a fraction of ribosomes present in polysomes multiplied by relative ribosome content. Ribosome specific activity is calculated as the TLR (Fig. 4) relative to the active ribosome content per cell. B) Relative changes in global protein synthesis, stability and concentration was determined for BQS252 strain. Values obtained at 30 °C are taken arbitrarily as 1. Protein concentrations obtained experimentally are represented as black triangles. Estimated protein concentration, as the product of stability (black circles) multiplied by TLR (white squares) is represented as white triangles. All these data come from triplicate experiments shown in Figs. 3 & 4 where statistics is given.

paints a global landscape of the strategies employed by eukaryotic cells to cope with temperature changes.

Since the physicochemical dependence of enzymatic reactions with temperature unavoidably occurs, the cell needs to utilize regulatory mechanisms in order to maintain optimal energy costs, as well as the required ribostasis and proteostasis. As found in this study, in accordance with others studying different external variables [5], homeostasis of mRNA concentration is maintained within a relative wide range of ± 40% of the optimal concentration. Protein concentration, however, seems to be more finely adjusted, displaying a narrower range of variation [8,31]. The stricter proteostasis observed is reasonable since protein is the final goal of gene expression and its production is energetically more costly to the cell [12,27,33], mainly due to the much higher number of proteins present per cell compared to the number of mRNA molecules [37].

Although lower temperatures have been used in our studies, the temperature range where GR remains relatively constant (30–37 °C) is of particular interest in order to eliminate this variable that affects protein synthesis [29,35] and gene transcription [6,38]. In the context of constant GR, an increase in temperature from 30 °C to 37 °C would theoretically cause a general increase of enzymatic activities [14,19] [18], including RNA pol II [17] and ribosome activities (Fig. 6). For instance, we calculate the ribosome specific activity from the total TLR and the proportion of elongating ribosomes (Fig. 5A), and we show that ribosome activity indeed increases with temperature in a degree close to the Q_{10} prediction (1.5 fold from 30 to 37 °C). However, the exact degree of global increase for both translation and protein degradation is difficult to envision as these processes are composed of multiple interconnected steps that can be subjected to regulation. In fact, total TLR only increases 1.2 fold from 30 to 37 °C (Fig. 4B), less than the ribosome specific activity, due to the reduction in the concentration of active ribosomes (Figs. 3 & S2).

On the other hand, the reduction in ribosome content is paralleled by a reduction in total [mRNA], keeping the ribosome/mRNA ratio almost constant. This is a remarkable observation as it is known that in standard laboratory growth conditions for yeast (usually 28–30 °C), ribosomes are saturated by mRNA [31,32,39]. A reduction in ribosomes seems to be logical as the energy cost of producing ribosomes is a large part of the actual translational cost [12,27]. A recent proteomic study [40] has found that ribosomal proteins (RP) are specifically decreased at 37 °C with regard to 30 °C, which supports our results of ribosome reduction in a context of global proteostasis. In that study it has been suggested that the specific reduction in the synthesis of RP could be due to a decrease in certain thiolated tRNAs that are necessary to translate transcripts enriched in certain codons, such as the RP and ribosome biogenesis mRNAs. This, together with a reduction in RP mRNA levels, would explain the reduction in RP synthesis.

Yet, the reduction in [mRNA] is not so easily explained as an increased excess of total mRNA with regard to ribosomes will not affect TLR, at least for exponentially growing cells [12]. Nevertheless, in spite of the proportionally minimal impact on total energy expenditure that mRNA synthesis represents with regard the cost of translation, it could be beneficial to save this unnecessary overhead. Moreover, the parallel decrease in ribosomes and mRNA (Fig. 3A) ensures that ribosome density is constant. This could be important as it has been recently proposed that optimal ribosome density (calculated to be half of the theoretical maximum) optimizes TLR [41]. Thus, the decrease in [mRNA] may be explained by at least two independent reasons.

The comparison between the general transcription and translation responses to temperature increase from 30 to 37 °C, indicates that both processes are accelerated at the molecular level in accordance to Arrhenius law ([17] and Fig. S3). This is seen by the increase in speed

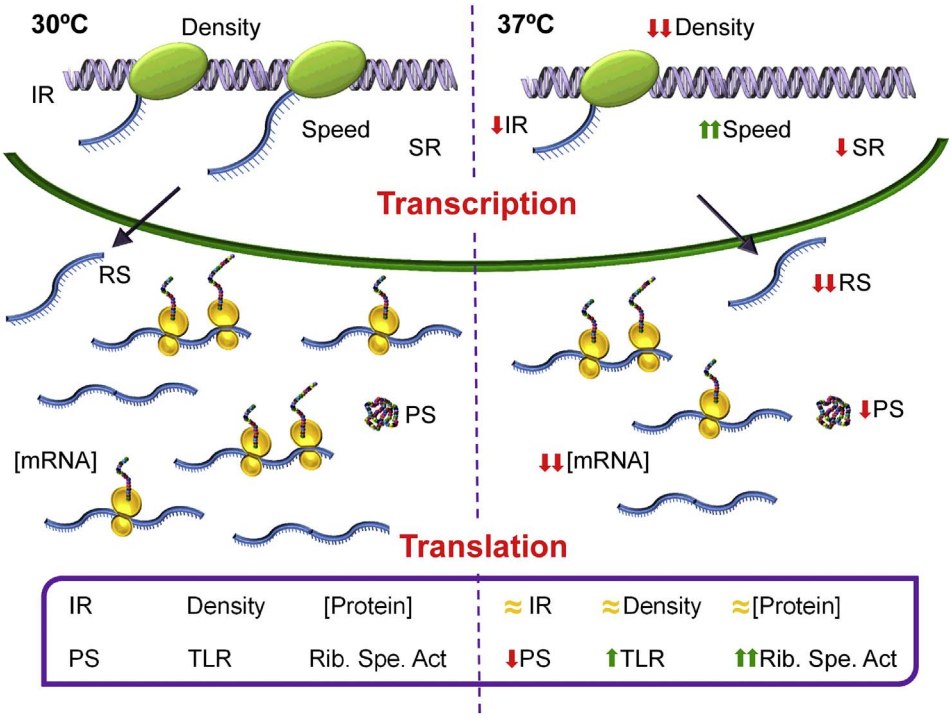

Fig. 6. Model of cellular homeostasis at the transcriptional and translational levels at 30 °C and 37 °C. At the yeast optimal temperature for growth, 30 °C, cellular processes are at equilibrium. At this temperature the synthesis rate (SR), a product of initiation rates (IR) and elongation speed of RNA pol II (green ovals), is at the optimal. However, at 37 °C the reduction of RNA pol II density and the increase on their speed, partially compensate each other to produce a small decrease in SR, which should be a consequence of a slight reduction in transcription initiation rate (IR) as previously described [17]. In the cytoplasm, the SR reduction and the reduced mRNA stability (RS) at 37 °C lead to a reduction in [mRNA]. A reduction in number of ribosomes occurs and it is proportional to that of [mRNA] maintaining an approximately constant ribosome density. Increased ribosome specific activity, that fits Arrhenius law (Fig. S2) and Q_{10} rule, is partially compensated by the reduced elongating ribosome content (Fig. 5A) provoking a limited total TLR increase. Increase in IR per mRNA and elongation speed can be inferred from the increased ribosome specific activity and constant density. However, total IR is not affected because of the reduced number of translating polysomes. Decreased protein stability (PS) compensates the small increase in TLR keeping protein concentration [protein] almost constant. Double arrows mean variation of around 50%, single arrows indicate variations of around 20–25% and ≈ marks variations < 10%. Positive changes are depicted in green and negative changes in red. See main text for further discussion.

of RNA pol II [17] and by the increase in ribosome specific activity (Fig. 5). The yeast cell, however, compensates thermodynamic increases by reducing concentration of transcriptional [17] and translational (this study) machineries although with different outputs. In transcription we detected a slight decrease of 25% in SR, as RNA pol II levels (both total and elongating molecules) decrease significantly with increasing temperatures indicating a decrease in transcription initiation [17]. Interestingly, RNA pol II protein decreases despite no change was observed for global average protein concentration with increase in temperature ([17] and Fig. 5B), indicating the presence of interdependent mechanisms to control the mRNA transcriptional machinery level in response to temperature while maintaining global protein homeostasis. In translation, as mentioned previously, ribosome specific activity is also compensated by a significant reduction in ribosome content. Due to the parallel reduction in mRNAs, translation initiation per individual mRNA is not affected (Fig. 6), contrarily to transcription initiation. Finally, total TLR slightly increases which is paralleled by a similar moderate increase in protein degradation, suggesting the existence of compensatory mechanisms in proteasomal activity to ultimately maintain proteostasis. In fact it has been shown that there is an increase in protein aggregation at 37 °C compared to 30 °C [40] which suggests increased protein turnover. However, the differential presence of HSP104-dependent aggregates at 37 °C has been discarded to be a cause of the accelerated aging of yeast cells [42]. Thus, the mechanisms of ribostasis and proteostasis should have evolved to cope with a wide number of physiological constraints that are, on the other hand, conditioned by them.

In all, our work sheds some light onto the growing knowledge of the fundamental basis of cellular function and adaptation. We show how cells rigorously maintain ribostasis and proteostasis in response to external changes through the employment of a variety of mechanisms. Hence, it is possible to envision how physiological constraints condition each cell to evolve the coping mechanisms to maintain cellular homeostasis.

Transparency document

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Appendix A. Supplementary data

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