Polysome-seq as a Measure of Translational Profile from Deoxyhypusine Synthase Mutant in *Saccharomyces cerevisiae*

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Abstract. The profile of proteins observed in a cell is characterized by the control of gene expression, which has several regulation points acting individually or in concert, such as epigenetic, transcriptional, translational, post-transcriptional or post-translational modification. Copulating the total mRNA data and mRNAs actively translated can facilitate the identification of the key regulatory points of gene expression. Here, we analyze the transcriptional and translational profiles of the deoxyhypusine synthase mutant *dys1-1* in yeast. This enzyme is involved in the post-translational modification of translation factor eIF5A, which has an important role in the elongation translational process. This work presents gene expression data from the total mRNA levels and the polysomally-loaded mRNAs for the *Saccharomyces cerevisiae DYS1* and *dys1-1* strains, based on RNA-seq and Polysome-seq. Our results showed that for this mutant, most of the changes in the transcripts forwarded for translation are due to transcriptional control; and, to solve translation problems, cell responds with positive regulation of ribosome biogenesis. Besides, polysome-seq as a tool to study translation profiles is useful to understand gene expression changes.

Keywords: eIF5A · Gene regulation · Ribosome biogenesis

1 Introduction

Protein synthesis consists of decoding the messenger RNA. This process is catalyzed by ribosomes and mediated by translation factors. The regulation of the repertoire of proteins expressed in a cell is determined by the selective control of gene expression by several cellular mechanisms, such as epigenetic, transcriptional, translational, post-transcriptional or post-translational modification [4,18,21].

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The eukaryotic translation elongation factor 5A (eIF5A - ortholog elongation factor P (EF-P) of bacteria) is a highly conserved protein in eukaryotes and archaea [5,7,19]. In addition, eIF5A is essential for cell viability in all tested eukaryotes [3,20].

eIF5A undergoes a post-translational modification which leads to hypusine biosynthesis, called hypusination. This process is irreversible and involves two enzymatic steps. In the first one, a deoxyhypusine synthase catalyzes the modification of a specific lysine residue (K51 in *Saccharomyces cerevisiae*) to a hypusine in a spermidine-dependent manner. In the second one, it occurs a hydroxylation by deoxyhypusine hydroxylase with molecular oxygen as the source. Both enzymes are also evolutionarily conserved [1,15]. Hypusinated eIF5A is described to aid in the efficiency of peptide binding of motifs that tend to induce ribosomes stalling and also assists with translational termination [22]. In this study, by measuring the total mRNAs of cells (transcriptome) and the polysomally-loaded mRNAs (translatome) for the yeast deoxyhypusine synthase mutant *dys1-1* and its wild-type counterpart [9], we obtained a picture of overall relationship between the two changes for the majority of genes. Polysome-seq can explain the regulation of post-transcriptional gene expression, as a reliable measure for a translational profiling study, showing the mRNA recruited for translation. We show that the majority of statistically significant differences at RNA-seq level correspond to similar differences at Polysome-seq level, suggesting that, in most transcripts for this mutant, changes in translation are due to a transcriptional control and ribosome biogenesis is the main response to translational problems.

2 Materials and Methods

2.1 Strain and Growth Conditions

Saccharomyces cerevisiae strains SVL613 (MATa leu2 trp1 ura3 his3 dys1::HIS3 [DYS1/TRP1/CEN - pSV520]) and SVL614 (MATa leu2 trp1 ura3 his3 dys1::HIS3 [dys1 W75R T118A A147T /TRP1/CEN - pSV730]), *DYS1* and *dys1-1*, respectively, were used to RNA highthroughput experiments. Cells were grown under previously described conditions [9].

2.2 Polysome Profilling

For the polysome profiling assay, cell extracts from *DYS1* and *dys1-1* strains were prepared as described in [9]. Briefly, the cell cultures were grown to mid-log phase ($OD600\,\text{nm} = 0.6$) and cross-linked with 1% formaldehyde for 1 h in ice bath. 15 A260 nm units of cell lysates were layered onto 10–50% (w/w) sucrose gradients and centrifuged for 3 h (39.000 rpm at 4 °C in Beckman SW41-Ti rotor). The absorbance at 254 nm of gradient fractionation was continuously measured. Fractions corresponding to mRNA populations bound by 3 ribosomes were pooled and stored at −80 °C for future RNA isolation.

2.3 RNA Isolation

For total RNA isolation, *DYS1* and *dys1-1* strains were grown in exponential phase an OD600 0.6. Cultures were centrifuged and cell pellets were stored at −80 °C. Cell lysis was conducted with zymolyase and total RNA was extracted using the RNeasy mini kit (cat. number 74104, Qiagen). The polysome-associated RNA from pooled fractions was extracted using TRIzol® Reagent, following the manufacturer's protocol (cat. n 15596026, ThermoFisher Scientific). Both total RNA and polysome-associated RNA were quantified using a NanoDrop 2000 Spectrophotometer (ThermoFisher) and the integrity was verified by electrophoresis gel on 2100 Bioanalyzer equipment (Agilent, Santa Clara, CA), using a High Sensitivity Total RNA Analysis Chip.

2.4 Library Preparation and Sequencing

Library preparation and sequencing (RNA-seq) for total and polysome-associated RNA were conducted by Life Sciences Core Facility (LaCTAD) from State University of Campinas (UNICAMP). Three biological replicates for transcriptome analysis (RNA-seq of total RNA) or translatome analysis (RNA-seq of polysome-associated RNA) from *DYS1* and *dys1-1* strains were carried out according to the manufacturer's guidelines for TrueSeq kit (catalog number RS-1222001, Illumina) by selection of mRNA by poly-A tail. These 12 libraries were sequenced for 51 cycles paired-end on a Illumina HiSeq 2500 platform.

2.5 RNA-seq Data Analysis

The public server (usegalaxy.org/) was used to process the highthroughput data. FASTQ files had their quality checked by the FastQC tool (Galaxy Version 0.72). TrimGalore! (Galaxy Tool Version: 0.4.3.1 + galaxy1) was used to remove reads with Phred quality score <25 and adapter strings. Files were mapped against a *S. cerevisiae* non-coding RNA (ncRNA) sequence file (downloads.yeastgenome.org/sequence/S288C_reference/rna/archive/rna_coding_R64-1-1_20110203.fasta.gz), by Bowtie software (Galaxy Tool Version: 1.1.2) with the parameters –v 2 –y –a –m 1 –best –strata –S –p 4. The mapping and quantification of reads was performed by Stringtie software (Galaxy Tool Version: 1.3.4) with standard parameters. Only genes in which the median read count of the three replicates was larger than 10 in all conditions (*dys1-1* and *DYS1* strain, for RNA-seq and for Ribo-Seq from polysome-profiling) were kept. The filtered table of counts contained data for 5.334 genes. Count of reads was converted into RPM (reads per million).

2.6 Ribo-Seq and Protein Abundance Comparative Analysis

We used the table of counts converted in $\log_2$RPM to compare the relative abundance of total or polysome-bound mRNAs in wild-type strain between two published ribosome profiling data: RPM normalized data from Ribo-Seq [23] and Ribo-Seq from polysome-profiling [10]; and protein abundance estimation [6].

2.7 Differential Expression Analysis

Non-normalized RNA-seq count tables were used as input in anota2seq (ver. 1.2.0; datatype = "RNA-seq", normalize = TRUE, transformation = "TMM-log2") and normalized using Trimmed Mean of M-values (TMM). Changes in translational efficiencies were assessed using the *anota2seqAnalyze* function. We applied e*anota2seqSelSigGenes* function to identify differentially expressed genes, separately for RNA-seq and polysome-profiling RNA-seq data and analysis of partial variance for identification of gene expression modes from both profiles. Significance was determined using an adjusted p-value limit of 0.05.

2.8 Enrichment of Gene Ontology and Enrichment Analysis of Transcription Factors

For the regulatory gene groups, we performed gene ontology (GO) analysis with terms of biological process to determine whether specific biological functions were enriched using Yeastmine database [8]. Fisher's exact test was used to test for statistically significant differences, and the Holm-Bonferroni correction test procedure to adjust for the effects of multiple tests [2]. GO terms were considered significant when FDR <0.05. Gene lists obtained via the statistical differential from transcriptome profile were submitted to the PSCAN (v1.5, http://159.149.160.88/pscan/) online tool.

3 Results and Discussion

3.1 RNA-seq and Polysome-Seq Experiments in *DYS1* and *dys1-1* Strains

We conducted transcriptional and translational profiling (Fig. 1A) for *S. cerevisiae dys1-1* strain and its wild-type counterpart. The number of RNA-seq reads mapping to a gene was used to quantify the relative abundance of the transcript, whereas the Polysome-seq provided a quantification of the translatome (Table 1).

Table 1. Number of mapped reads for each sample

Profile	$DYS1_1$	$DYS1_2$	$DYS1_3$	$dys1\text{-}1_1$	$dys1\text{-}1_2$	$dys1\text{-}1_3$
Transcriptional	55801619	34587137	116149587	30329854	45292306	51380070
Translational	1214644	1221375	1070191	6150284	1936203	6720246

After filtering out non-expressed genes (see Methods), the table of read counts per gene contained data for 5,334 *S. cerevisiae* annotated ORFs. Both transcriptional and translational profiles results were highly reproducible among biological replicates for each strain (Fig. 1B and 1C) (Table 2 and 3).

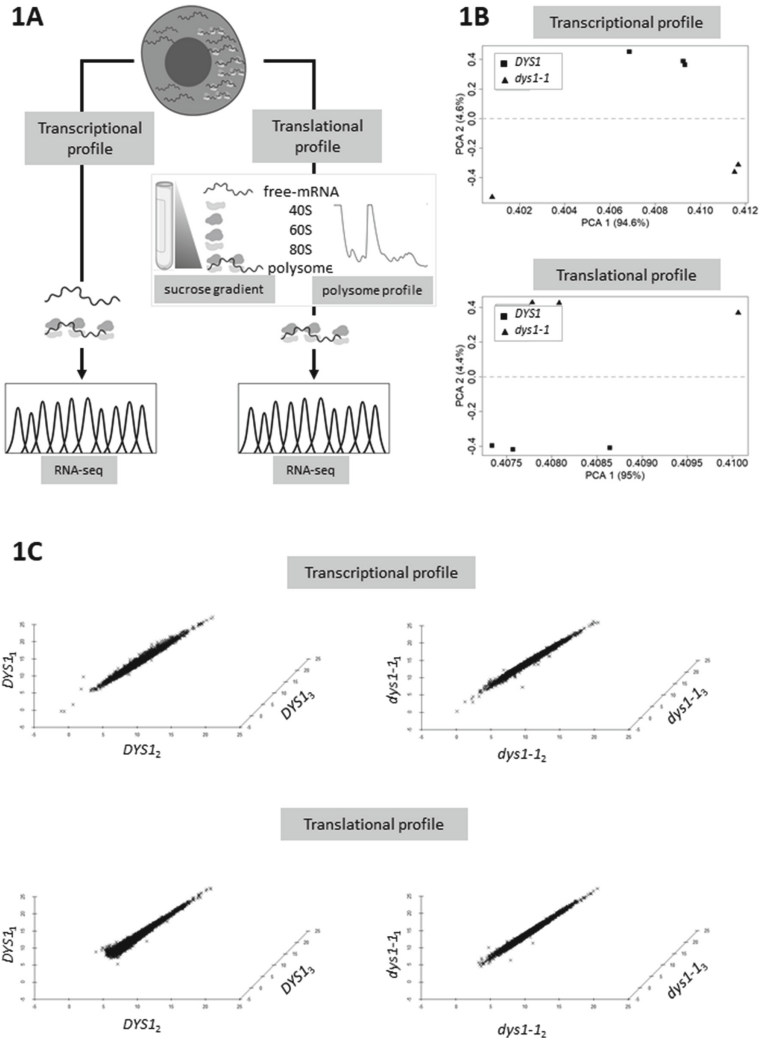

Fig. 1. (A) Experimental approaches for studying the transcribed and recruited mRNAs for translation. Transcriptional profile: the total RNA is extracted, the mRNAs are separated and subjected to large-scale sequencing. Translational profile: extracts are separated by ultracentrifugation through sucrose gradient which is then fractionated while its absorbance is continuously monitored at 254 nm (A254), allowing the separation of free RNA, the 40S and 60S ribosomal subunits, the 80S monosomes and the polysomes. The RNA is isolated from individualized gradient fractions and pooled for further large-scale analysis. (B) Principal Component Analysis indicating the distribution of replicates in the plan. Three biological replicates independent of the *DYS1* and *dys1-1* strains are represented in the distribution graphs along two main components, from the normalized RPM values of the genes sequenced by RNA-seq of each profile. (C) Linear correlation between replicates of $\log_2$RPM values of genes sequenced by RNA-seq. The linear correlation of the $\log_2$RPM values of experimental replicates for the transcriptional profile varied between 0.94 and 0.98 whereas for the translational profile this value varied between 0.98 and 0.99.

Table 2. Pearsons correlation values for $\log_2$RPM values from transcriptional profile for each replicate

	$DYS1_1$	$DYS1_2$	$DYS1_3$	$dys1\text{-}1_1$	$dys1\text{-}1_2$	$dys1\text{-}1_3$
$DYS1_2$	0.973	1				
$DYS1_3$	0.983	0.992	1			
$dys1\text{-}1_1$	0.940	0.929	0.935	1		
$dys1\text{-}1_2$	0.935	0.916	0.922	0.995	1	
$dys1\text{-}1_3$	0.925	0.906	0.914	0.986	0.990	1

Table 3. Pearsons correlation values for $\log_2$RPM values from translational profile for each replicate

	$DYS1_1$	$DYS1_2$	$DYS1_3$	$dys1\text{-}1_1$	$dys1\text{-}1_2$	$dys1\text{-}1_3$
$DYS1_2$	0.973	1				
$DYS1_3$	0.968	0.973	1			
$dys1\text{-}1_1$	0.845	0.853	0.847	1		
$dys1\text{-}1_2$	0.845	0.855	0.854	0.986	1	
$dys1\text{-}1_3$	0.854	0.867	0.865	0.989	0.988	1

3.2 Polysome-seq as a Measure for Translational Profile

One technique aimed for studying the composition of mRNAs recruited for translation by large-scale analysis is the polysome profiling, which segregates mRNAs associated with polysomes from ribosome-free mRNAs, associated with RNA-seq (Fig. 1A). In addition to Polysome-seq, Ribo-seq methodology, or ribosome profiling, is based on the sequencing of ribosome-protected fragment (RPF) mRNAs [12]. We observed high Pearson correlations with the $\log_2$RPM wild-type data from this study to ribosome profiling wild-type data available in the literature [10,23] and (Fig. 2A and 2B).

Next, we compared the wild-type strain quantification of gene expression by RNA-seq and Polysome-seq to published proteomic data [6]. The correlation and coefficient of determination from translatome (Polysome-seq) to the proteome normalized abundances (Fig. 2C) was higher than the transcriptome measurements (Fig. 2D), indicating that this former quantification of gene expression provides a more accurate picture of protein abundance, since translation is regulated by (1) translation rate, (2) translation rate modulation, (3) modulation of a protein's half-life, (4) protein synthesis delay, (5) protein transport [17,18]. So Polysome-seq allows a better understanding of regulatory mechanisms that involves post-transcriptional gene expression programs [11,13], as regulation via tuning transcript levels alone [16], resulting in a profile of selected mRNAs recruited for translation.

3.3 Yeast Hypusination Mutant *dys1-1* Responds Transcriptionally for Gene Regulation

We first calculated the gene expression level fold change (FC) between the two strains using RNA-seq and Polysome-seq data separately and we observed similar numbers of differentially expressed genes (DEGs) for both profiles - 2432 and 2826 DEGs for transcriptional and translational level, respectively - (Fig. 3A and 3B), however, Polysome-seq data had a higher variance than RNA-Seq data for the significant $\log_2$FC distribution (Fig. 3C), a consistent result for a mutant involved with a translational factor.

To establish the relationship between mRNA and polysome-associated mRNA changes when comparing *DYS1* and *dys1-1*, we categorized DEGs into gene expression modes by computing analysis of partial variance with transcriptome and translatome (Fig. 3D): (1) Homodirectional DEGs, significantly change in both profiles in a concordant way, indicating a transcriptional regulation; (2) Polysome-only DEGs, up or down polysome-associated mRNA with no significant changes in mRNA levels, a result of translation regulatory mode; (3) Transcriptome only DEGs, differences in mRNA levels not followed by a significant change in polysome-associated mRNA, a result of buffering regulatory mode; (4) Antidirectional DEGs, significantly change in both profiles but antidirectional ways. Most DEGs (67%) showed a coupled significant change, i. e., genes with significant homodirectional change in both the transcriptome and the translatome (Fig. 3E). This result is in accordance with the fact that under stress conditions, differential expressed proteins correlated strongly with the corresponding mRNA level, indicating that transcriptional control seems to be the major driver behind changes in protein levels [14].

Transcriptionally regulated genes were significantly enriched for Gene Ontology (GO) biological process terms as "maturation of SSU-rRNA" (GO:0030490), "transposition" (GO:0032196), "RNA modification" (GO:0009451) (Table 4) and Transcription Factors (TF) as Tod6, Dot6 and Stb3 (Table 5). Additionally, BUD27, the gene that encodes a protein which impacts the homeostasis of the ribosome biogenesis process by regulating the activity of the three RNA polymerases [17], is classified as an homodirectional gene and upregulated in both profiles. Taking together, these results revealed a cell response to ribosome biogenesis, a high-energy consumption process that requires stringent regulation to ensure proper ribosome production to deal with cell growth and protein synthesis in different environmental and metabolic situations [17].

The results of this study illustrate the use of Polysome-seq as a measurement of mRNAs recruited for translation. We identified for a deoxyhypusine synthase mutant *dys1-1*, a protein involved in translation, a pattern of gene expression control that is transcription dependent and upregulation of ribosome synthesis is one of the cell responses to translation impairment.

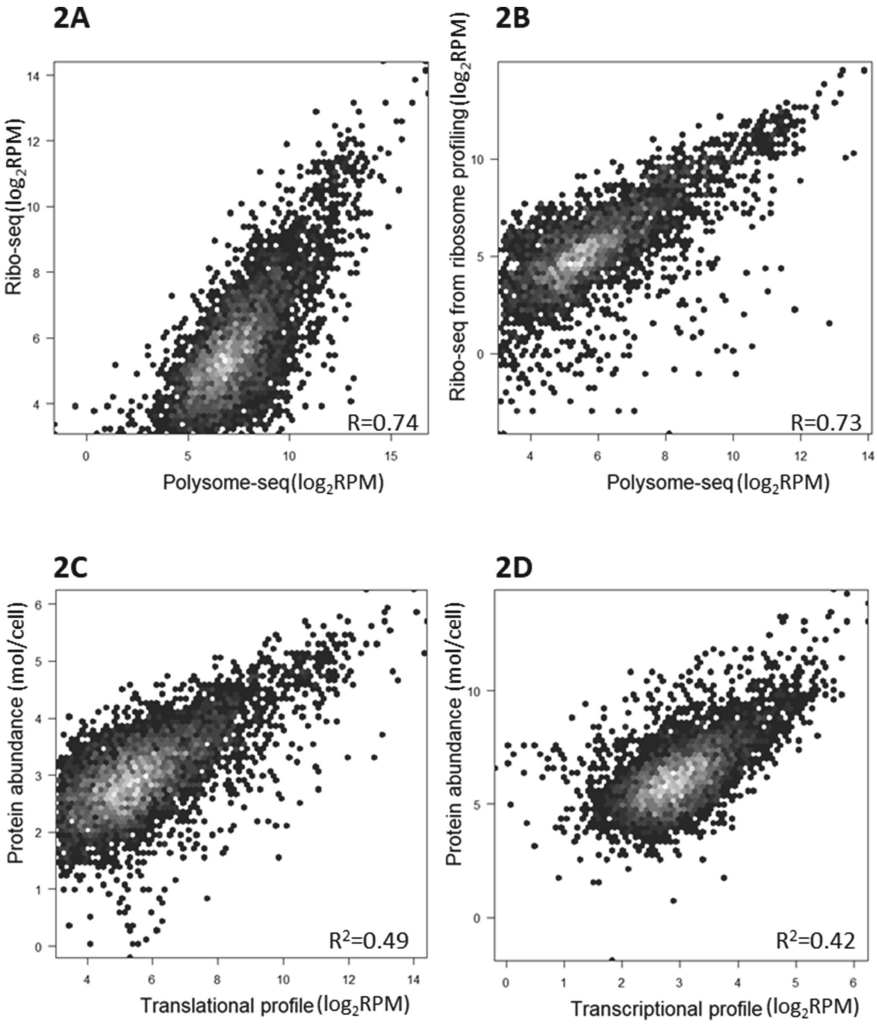

Fig. 2. Polysome-seq correlates satisfactorily to Ribo-seq data and is a good predictor of protein abundance. (A) Correlation between the translational profile ($\log_2$RPM) of this study and the translational profile of obtained by Ribo-seq ($\log_2$RPM) [23]. (B) Correlation between the translational profile of this study ($\log_2$RPM) and the translational profile of obtained by a combination of polysomal profile followed by Ribo-seq ($\log_2$RPM) [6]. C) Distribution between protein abundance (molecules per cell) and the translational profile ($\log_2$RPM) of this study. D) Distribution between protein abundance (molecules per cell) and the transcriptional profile ($\log_2$RPM) of this study. Protein abundance data are indicated in molecules per cell according to [6].

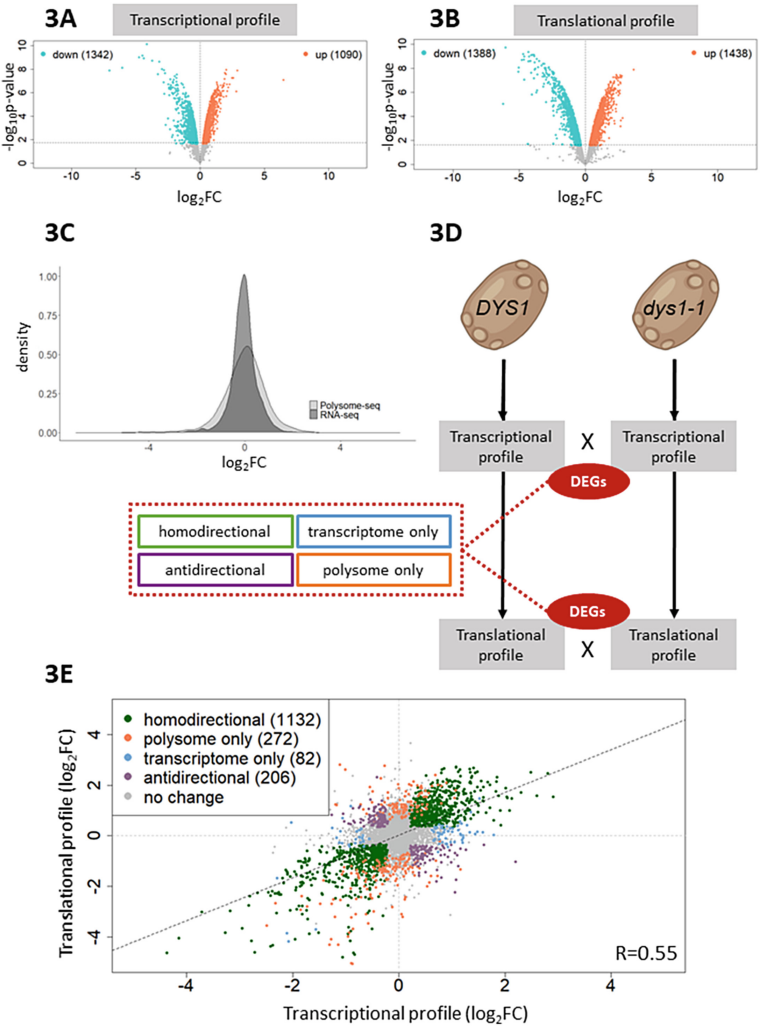

Fig. 3. Volcano plot of the distribution of the transcripts differentially expressed in the transcriptional profile (A) and translational profile (B). The values of $-\log_1 0$ p-value were plotted according to the differencial expression between *DYS1* and *dys1-1* ($\log_2$ fold change). Downregulated genes are highlighted in blue (left), upregulated genes, in orange (right); dashed horizontal line indicates an adjusted p-value of 0.05. (C) Distribution of gene expression fold change (FC) values. FC was calculated as the ratio between the number of reads in *dys1-1* and *DYS1* strains. We took the average number of reads per gene among the replicates. (D) Scheme of differential expression analysis between the transcriptional and translational profile of the *dys1-1* mutant. Genes classified as differentially expressed were called transcriptome only (blue), polysome only (orange), antidirectional (purple) - significantly opposite variations between transcriptional and translational profiles - and homodirectional (green) - variations significantly converging between both profiles. (E) Distribution of the $\log_2$ fold change of the transcriptional and translational profile. Genes showing statistical differences between *dys1-1* and *DYS1* were simultaneously compared in the two profiles. Categories are defined in 3D. (Color figure online)

Table 4. Gene Ontology (GO) analysis of transcriptionally regulated mRNAs from *dys1-1* mutant as determined by anota2seq

Analysis by Anota2seq - "Homodirectional"				
Term ID	Description	$\log_1 0$ p-value	Dispensability	N genes
GO:0030490	Maturation of SSU-rRNA	−3.000.000	0.00	53
GO:0032196	Transposition	−53.792	0.03	42
GO:0032197	Transposition, RNA-mediated	−34.237	0.12	40
GO:0006278	RNA-dependent DNA biosynthetic process	−76.021	0.24	39
GO:0090305	Nucleic acid phosphodiester bond hydrolysis	−61.694	0.28	98
GO:0001510	RNA methylation	−45.888	0.29	24
GO:0006396	RNA processing	−64.134	0.37	175
GO:0009451	RNA modification	−27.911	0.40	43
GO:0034660	ncRNA metabolic process	−28.050	0.48	166
GO:0000966	RNA 5'-end processing	−62.684	0.48	28

Table 5. Transcriptional factor (TF) enrichment analysis of differentially expressed genes in the transcriptional profile from *dys1-1* mutant as determined by anota2seq

MatrixID	Matrix name	p-value
MA 0350.1	TOD6	1,92E−23
MA 0351.1	DOT6	1,72E−21
MA 0390.1	STB3	1,14E−05
MA 0378.1	SFP1	4,49E−03
MA 0398.1	SUM1	4,64E−02
MA 0345.1	NHP6A	1,12E−01
MA 0346.1	NHP6B	2,73E−01
MA 0386.1	SPT15	0.000184904
MA 0418.1	YAP6	0.000859765
MA 0435.1	YPR015C	0.00121852

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References

1. de Almeida, O.P., et al.: Hypusine modification of the ribosome-binding protein eIF5A, a target for new anti-inflammatory drugs: understanding the action of the inhibitor GC7 on a murine macrophage cell line. Curr. pharm. Des. **20**(2), 284–92 (2014)
2. Benjamini, Y., Yekutieli, D.: The control of the false discovery rate in multiple testing under dependency. Ann. Stat. (2001). https://doi.org/10.1214/aos/1013699998
3. Buskirk, A.R., Green, R.: Ribosome pausing, arrest and rescue in bacteria and eukaryotes. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. **372**, 20160183 (2017). https://doi.org/10.1098/rstb.2016.0183
4. Chassé, H., Boulben, S., Costache, V., Cormier, P., Morales, J.: Analysis of translation using polysome profiling. Nucleic Acids Res. (2017). https://doi.org/10.1093/nar/gkw907
5. Chen, K.Y., Liu, A.Y.: Biochemistry and function of hypusine formation on eukaryotic initiation factor 5A. NeuroSignals (1997). https://doi.org/10.1159/000109115
6. Csárdi, G., Franks, A., Choi, D.S., Airoldi, E.M., Drummond, D.A.: Accounting for experimental noise reveals that mRNA levels, amplified by post-transcriptional processes, largely determine steady-state protein levels in yeast. PLoS Genet. **11**(5), e1005206 (2015). https://doi.org/10.1371/journal.pgen.1005206
7. Dever, T.E., Ivanov, I.P.: Roles of polyamines in translation. J. Biol. Chem. **293**(48), 18719–18729 (2018). https://doi.org/10.1074/jbc.TM118.003338. http://www.jbc.org/
8. Engel, S.R., et al.: The reference genome sequence of saccharomyces cerevisiae: then and now. G3: Genes Genomes Genetics **4**(3), 389–398 (2014). https://doi.org/10.1534/g3.113.008995
9. Galvão, F.C., Rossi, D., Silveira, W.D.S., Valentini, S.R., Zanelli, C.F.: The deoxyhypusine synthase mutant dys1-1 reveals the association of eIF5A and Asc1 with cell wall integrity. Plos One **8**(4), e60140 (2013). https://doi.org/10.1371/journal.pone.0060140
10. Heyer, E.E., Moore, M.J.: Redefining the translational status of 80S monosomes. Cell **164**(4), 757–769 (2016). https://doi.org/10.1016/j.cell.2016.01.003
11. Ingolia, N.T.: Ribosome profiling: new views of translation, from single codons to genome scale. Nat. Rev. Genet. **15**, 205–213 (2014). https://doi.org/10.1038/nrg3645
12. Ingolia, N.T., Ghaemmaghami, S., Newman, J.R., Weissman, J.S.: Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science **324**(5924), 218–223 (2009). https://doi.org/10.1126/science.1168978
13. Jin, H.Y., Xiao, C.: An integrated polysome profiling and ribosome profiling method to investigate in vivo translatome. In: Methods in Molecular Biology, vol. 1712, pp. 1–18. Humana Press Inc. (2018). https://doi.org/10.1007/978-1-4939-7514-31
14. Lahtvee, P.J., et al.: Absolute quantification of protein and mRNA abundances demonstrate variability in gene-specific translation efficiency in yeast. Cell Syst. **4**(5), 495–504 (2017). https://doi.org/10.1016/J.CELS.2017.03.003. https://www.sciencedirect.com/science/article/pii/S2405471217300881#mmc4
15. Landau, G., Bercovich, Z., Park, M.H., Kahana, C.: The role of polyamines in supporting growth of mammalian cells is mediated through their requirement for translation initiation and elongation. J. Biol. Chem. **285**(17), 12474–12481 (2010). https://doi.org/10.1074/jbc.M110.106419

16. Liu, Y., Beyer, A., Aebersold, R.: On the dependency of cellular protein levels on mRNA abundance. Cell **165**, 535–550 (2016). https://doi.org/10.1016/j.cell.2016.03.014
17. Martínez-Fernández, V., et al.: Prefoldin-like Bud27 influences the transcription of ribosomal components and ribosome biogenesis in Saccharomyces cerevisiae. RNA (2020). https://doi.org/10.1261/rna.075507.120
18. Piccirillo, C.A., Bjur, E., Topisirovic, I., Sonenberg, N., Larsson, O.: Translational control of immune responses: from transcripts to translatomes. Nat. Immunol. **15**(6), 503–511 (2014). https://doi.org/10.1038/ni.2891. http://www.nature.com/doifinder/10.1038/ni.2891
19. Rossi, D., Kuroshu, R., Zanelli, C.F., Valentini, S.R.: eIF5A and EF-P: two unique translation factors are now traveling the same road. Wiley Interdiscip. Rev.: RNA **5**(2), 209–222 (2014). https://doi.org/10.1002/wrna.1211
20. Schnier, J., Schwelberger, H.G., Smit-McBride, Z., Kang, H.A., Hershey, J.W.: Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast Saccharomyces cerevisiae. Mol. Cell. Biol. (1991). https://doi.org/10.1128/MCB.11.6.3105
21. Schuller, A.P., Green, R.: Roadblocks and resolutions in eukaryotic translation. Nat. Rev. Mol. Cell Biol. **19**(8), 526–541 (2018). https://doi.org/10.1038/s41580-018-0011-4. http://www.nature.com/articles/s41580-018-0011-4
22. Schuller, A.P., Wu, C.C.C., Dever, T.E., Buskirk, A.R., Green, R.: eif5a functions globally in translation elongation and termination. Mol. Cell **66**(2), 194–205 (2017). https://doi.org/10.1016/j.molcel.2017.03.003
23. Sen, N.D., Zhou, F., Ingolia, N.T., Hinnebusch, A.G.: Genome-wide analysis of translational efficiency reveals distinct but overlapping functions of yeast DEAD-box RNA helicases Ded1 and eIF4A. Genome Res. **25**(8), 1196–1205 (2015). https://doi.org/10.1101/gr.191601.115