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Supplemental Information

An mRNA-Derived Noncoding RNA Targets and Regulates the Ribosome

Andreas Pircher, Kamilla Bakowska-Zywicka, Lukas Schneider, Marek Zywicki, and Norbert Polacek

SUPPLEMENTAL FIGURES

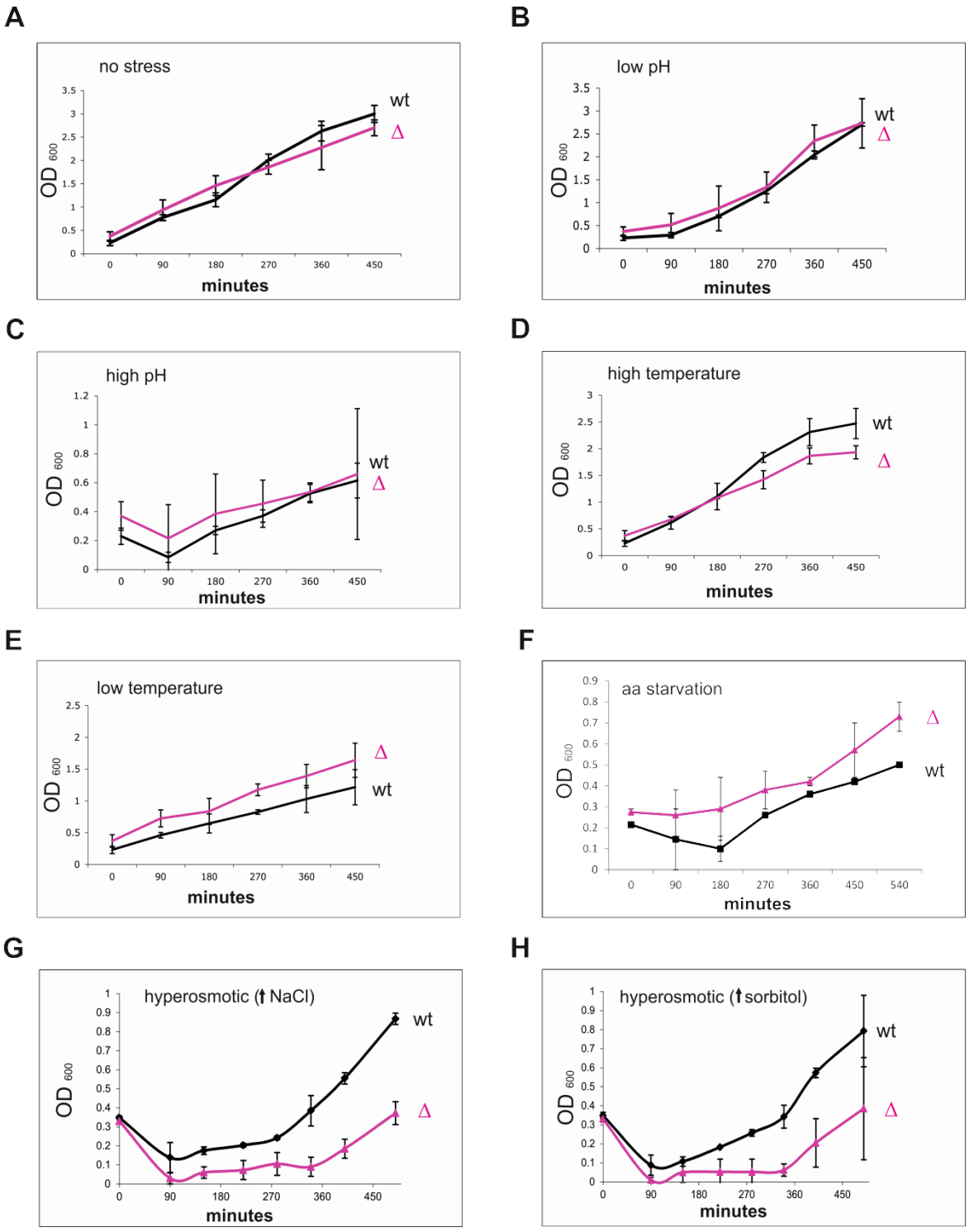

Figure S1: Growth characteristics of the *TRM10* knock-out strain under stress (related to Figure 2). Growth potential of the *TRM10* knock-out strain (*trm10Δ*; Δ) was compared to *S. cerevisiae* wild-type (wt) cells by monitoring growth at OD_{600}. No stark growth phenotype of the *trm10Δ* cells relative to the wt strain was observed in rich medium (**A**), at low pH (**B**), at elevated pH (**C**), at high (**D**) or low (**E**) temperatures, or during amino acid (aa) starvation conditions (**F**). In contrast, under hyperosmotic stress conditions (0.7 M NaCl or 1.025 M sorbitol, respectively) the *trm10Δ* cells showed a slow growth phenotype (**G, H**). Each experimental point was done in duplicates and the growth curves were repeated three times. The mean and the standard deviations are shown.

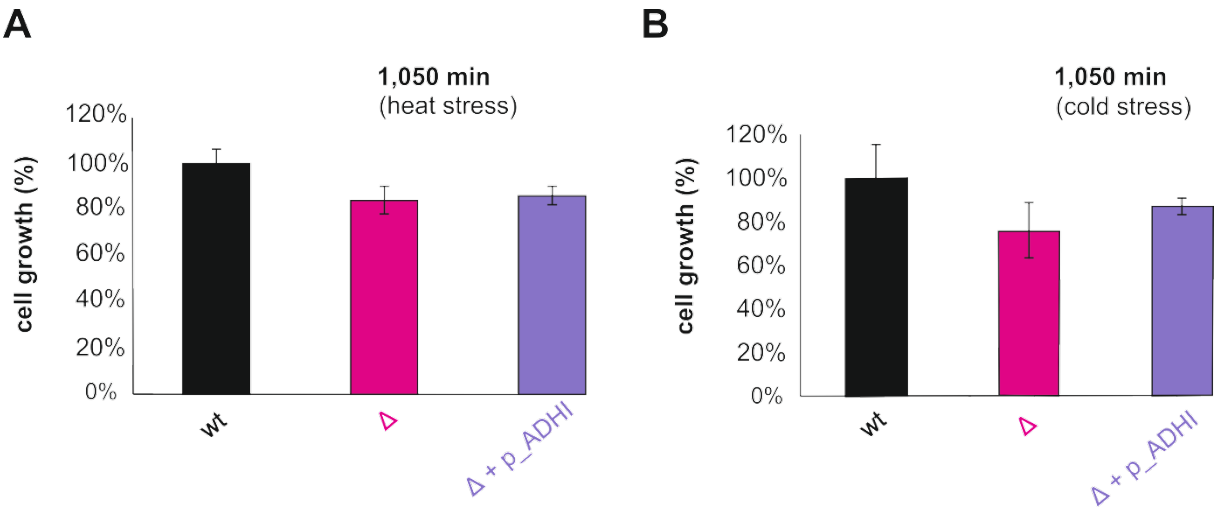

Figure S2: Temperature stress does not influence growth of the *TRM10* knock-out strain (related to Figure 2)**.** The growth after heat (**A**) or cold stress (**B**) was monitored by measuring growth at OD_{600} after 1,050 minutes after stress induction. The cell densities of stationary phase wt cultures that were re-diluted to OD_{600} 0.1 and subsequent incubated under temperature stress conditions for 1,050 minutes (black bars) were set to 100%. Under both temperature stress conditions the *trm10Δ* strain (Δ) or the *TRM10* overexpression strain (Δ + p_ADHI) grew similarly compared to the wt cells. Experiments were done in triplicates with indicated standard deviations.

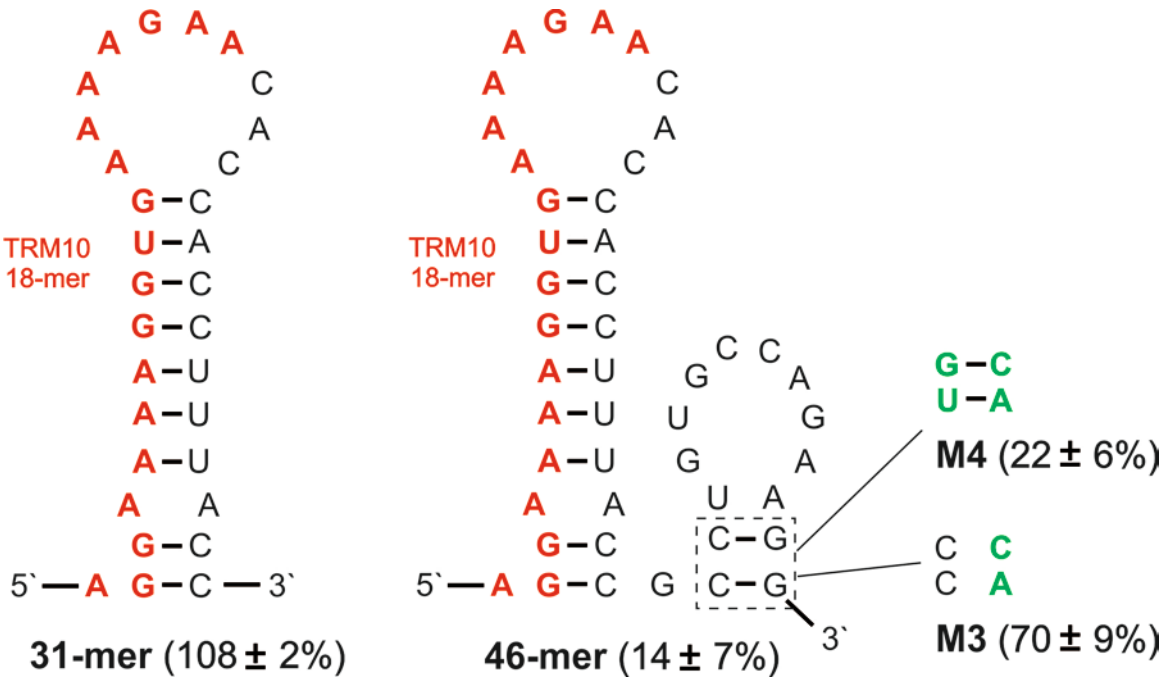

Figure S3: Effects of secondary structure context of the 18-mer sequence on translation inhibition in the metabolic labeling assay (related to Figure 4). Secondary structure predictions of various 5' parts of the *TRM10* mRNA, including or excluding the 5' untranslated region, always embedded the 18-mer sequence (red) in a stable stem-loop structure. The synthetic 31-mer RNA (left) contains solely the predicted long stem-loop while the 46-mer (right) carries in addition a predicted small 3'hairpin. The 31-mer, the 46-mer, or mutant variants thereof (M3, M4; green) were introduced into yeast spheroplast. The efficiencies of ^{35}S-Met incorporation into newly synthesized proteins in the presence of these RNA strands are given in parentheses. The ^{35}S-Met incorporation in the absence of synthetic RNA was taken as 100%. Values shown represent the mean and the standard deviation of at least three metabolic labeling experiments. *P* values were generated via a two-tailed unpaired Student`s *t* test (** $P < 0.05$). The data highlight the importance of the small downstream hairpin for possible processing of the active 18-mer fragment. Its destabilization (M3) causes severe loss of translation inhibition while its restoration, by introducing compensatory mutations (M4), almost completely recovered the inhibitory activity. We note that the signal on the northern blots in Figs. 1B,C and Fig. 2 of the main text depict a ~45 residue long putative processing intermediate which might correspond to the 46-mer RNA shown here.
Bioinformatic analyses revealed that the 18-mer sequence can be found in multiple eukaryotic species however it is always located outside of the *TRM10* paralogs, primarily in intergenic regions.

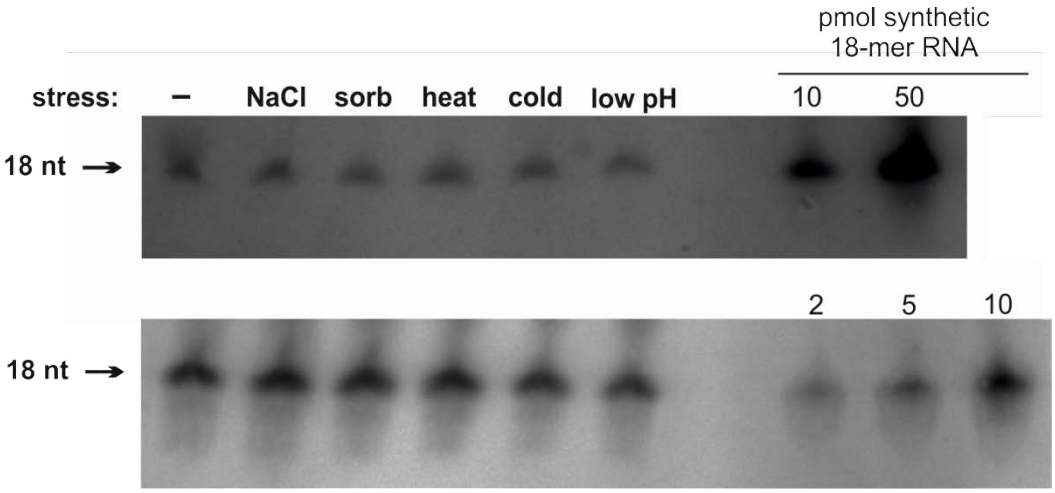

Figure S4: Quantification of the *TRM10*-derived 18-mer RNA abundance (related to Figure 1)**.** 15 µg total RNA originating from 50 ml unstressed (-) or stressed wt yeast cells (hyperosmotic stress: elevated NaCl or sorbitol (sorb); heat, cold, or low pH stress) were loaded on a denaturing polyacrylamide gel. For quantification known amounts of the synthetic 18-mer RNA (2, 5, 10, or 50 pmol) were loaded on the same polyacrylamide gel and subsequently transferred to a nitrocellulose membrane. A radiolabeled LNA antisense probe against the *TRM10* 18-mer RNA was then used for hybridization. The signal for the 18-mer RNA is indicated by an arrow. The intensities of the northern blot signals were quantified and used to calculate the amount of the cellular *TRM10* 18-mer RNA concentration. Two representative northern blots are shown (note that the lower blot is identical to the one shown in Fig. 1B of the main text). The mean value of three quantification experiments revealed ~27,000 18-mer RNA molecules per yeast cell. Considering the volume of unstressed *S. cerevisiae* cells of ~40 femtoliter (Petelenz-Kurdziel et al., 2011) gives a cellular 18-mer RNA concentration of ~1.1 µM.

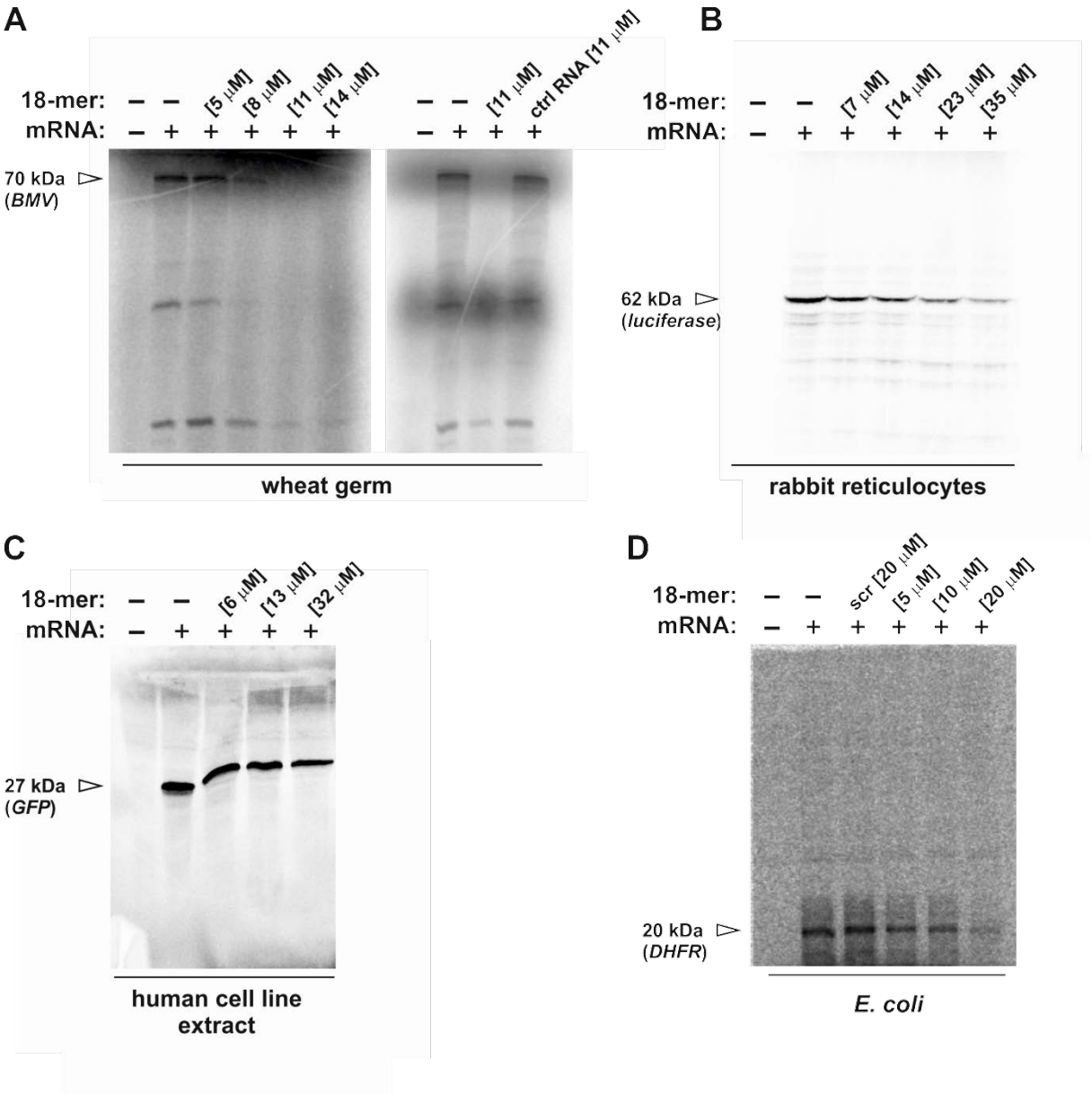

Figure S5: Effect of the yeast *TRM10* 18-mer RNA on i*n vitro* translation in different systems (related to Figure 5). **(A)** *In vitro* translation using the wheat germ system was performed either in the absence (-) or in the presence of the synthetic *TRM10* 18-mer RNA (f.c. 5 – 14 µM). The reaction is inhibited by the 18-mer RNA with an apparent IC_{50} of 7 µM. Addition of 11 µM of an unrelated 18-mer RNA oligo (5' UCCCGUCAUCACCCACCA 3') served as specificity control. **(B)** *In vitro* translation using the rabbit reticulocyte system. The reaction is inhibited by the 18-mer RNA with an IC_{50} of 14 µM. (**C)** The *in vitro* translation reaction using the human system is only mildly inhibited by the *TRM10* 18-mer RNA and the IC_{50} is > 30 µM. (**D)** The 18-mer RNA inhibits the *E. coli in vitro* translation assay with an IC_{50} of 13 µM. Addition of 20 µM (f. c.) of a scrambled 18-mer (scr) had no effect on translation efficiency. The positions of the full-length translation products in the SDS polyacrylamide gels are marked by open arrowheads. The respective protein names are given in the parentheses.

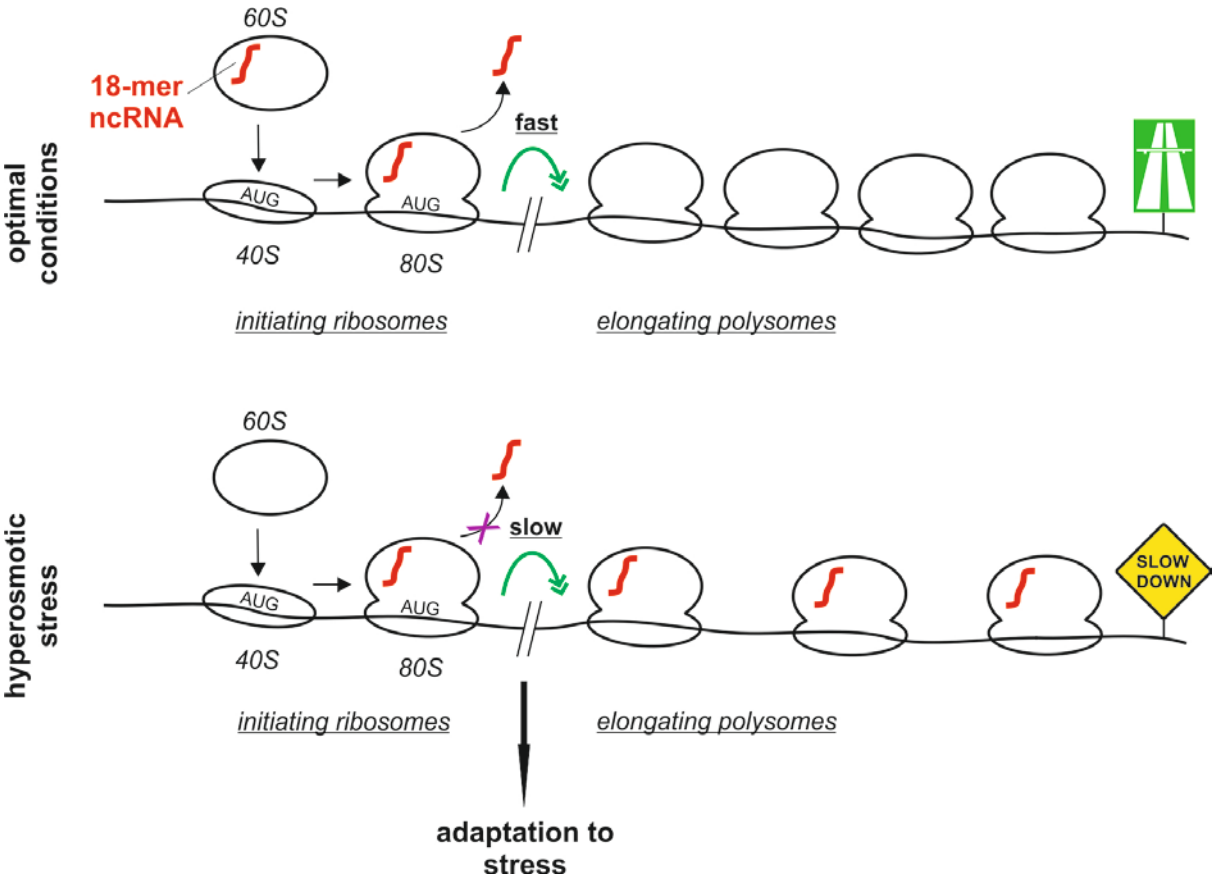

Figure S6: Proposed model of 18-mer RNA-dependent translation inhibition and stress adaptation (related to Figures 1-6)**.** The presented data are compatible with the following model: Under optimal growth conditions the 18-mer RNA (red) associates with 60S subunits and 80S ribosomes during the initiation phase of protein synthesis. Upon rapid entering the elongation phase (green arrow), the 18-mer dissociates and the ribosome can effectively translate mRNA yielding dense polysomes. During hyperosmotic stress induction the initiating ribosome cannot escape the initiation phase fast which results in an increased monosome/polysome ratio (see Figure 2A of the main text). Ribosomes with bound 18-mer ncRNA only enter slowly into the elongation phase yielding a sparse polysome population. These salt-stressed polysomes do not dissociate the 18-mer RNA. This results in an almost quantitative relocation of the 18-mer RNA from the initiating into the elongating pool of ribosomes under hyperosmotic stress (see Figure 2A of the main text). These events attenuate global protein synthesis and consequently cell metabolism thus allowing cellular stress-adaptation programs to kick in. This temporal 18-mer ncRNA-depending down regulation of protein synthesis during the onset of salt stress is crucial for subsequent efficient growth at hyperosmotic conditions.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Yeast strains, growth conditions and constructs. BY4742 (*MATalpha, his3-Δ1, leu2-Δ0, lys2-Δ0, ura3-Δ0*) wild-type (wt) cells and all mutant cells derived from this strain were grown in Sc-Leu medium (yeast synthetic complete drop out medium- Leu; *Sigma Aldrich*) at 30°C. BY4742 and the *TRM10* knock-out strain (*trm10Δ*; obtained from the Yeast MATalpha Collection; *Open BioSystems*) were transformed with the empty pRS415 plasmid or pRS415 carrying the cloned *TRM10* gene under its endogenous promoter, respectively. The start codon mutant (ATG was changed to the stop codon TGA) as well as the synonymous codon mutants M1 and M2 were introduced into the cloned *TRM10* gene by PCR site-directed mutagenesis. Cell growth was monitored under different stress conditions: cold shock (18 °C), heat shock (38 °C), low pH (by adding HCl to a final pH 4.0), high pH (adding Tris/Cl pH 8.5 to a final pH 8.0), amino acid starvation(Gasch et al., 2000), sugar starvation (in Sc-medium without any galactose addition), hypo-osmotic conditions (Gasch et al., 2000), or hyper-osmotic conditions (by adding NaCl to a f.c. of 0.7 M or by adding sorbitol to a f.c. of 1.025 M). For overexpression of the *TRM10* locus, the *TRM10* gene (as an untranslatable CTG start-codon construct) was cloned under the control of the strong yeast promoter ADHI (yielding the plasmid p_ADHI), and transformed into *trm10Δ*.

Primer extension analysis. To monitor the methylation status of m^1G_9 of $tRNA^{Gly}$, primer extension analysis was performed as previously described (Jackman et al., 2003; Polacek and Barta, 1998). As template total RNA was isolated using the MasterPure™ Yeast RNA Purification Kit (*Epicentre*) according to the manufacturer`s instructions. The reverse transcription reaction (final volume: 5 µl) was stopped by the addition of 7 µl loading buffer (60% formamide, 0.1% bromphenol blue, 0.1% xylene cyanol). An aliquot of the reactions was run in 1 x TBE at 10 W constant power on 10% polyacrylamide gels and visualized by using a PhosphorImager (FLA-3000; *Fuji* Photo Film).

Metabolic labeling. Yeast spheroplasts were prepared from a 50 ml wt culture with an OD_{600} of 0.6 by the addition of 350 U of zymolyase (*Zymo Research*) at 30°C for 25-30 min as previously described (Russell et al., 1991). Spheroplasts were resuspended in 2 ml STC (1 M sorbitol, 10 mM Tris/HCl pH 7.5, 10 mM $CaCl_2$) and DMSO was added to a final concentration of 15%. For one reaction 100 µl spheroplasts were combined with 10 pmol synthetic 18-mer RNA (or 3'-extended variants thereof) (*Microsynth*) and electroporated using the *BioRad* Micro Pulser (1,500 V, 25 µlF, 200 Ω). The scrambled 18-mer strand (5-AAGUGAAGAAGGAAGAAA-3') or 18-mer RNAs containing point mutations (M1: 5'-AGGAAAAAGUUAAAAGAA-3'; M2: 5'-AGGAGAAAGUUAAAAGAA-3') served as

specificity controls. For a translation inhibition control 7.5 µg/µl cycloheximide was added to the spheroplasts. Subsequent to electroporation 1 ml YPD/1 M sorbitol was added and the reaction transferred into a 1.5 ml eppendorf tube. Under these conditions we were able to introduce ~200,000 molecules of the synthetic 18-mer RNA per spheroplast. This quantification was done by following the signal of a ^{32}P-end-labeled 18-mer RNA. Yeast spheroplasts were incubated in 1 ml YPD/1M sorbitol after electroporation with the ^{32}P-end-labeled 18-mer. Spheroplasts were pelleted at 4,000 rpm (in a table-top Eppendorf centrifuge) and washed with YPD/1M sorbitol. This pelleting/washing procedure was performed in total two times. Finally the fraction of introduced ^{32}P-labled 18-mer RNA was determined by measuring the pellet and the corresponding supernatant fractions by liquid scintillation counting. For one reaction 450 µl of the sample was incubated at 30°C for 15 min before the addition of 1 µl ^{35}S-methionine (1,000 Ci/mmol, 10 mCi/ml) and the incubation was continued for 1 h. Labeled proteins were precipitated by adding 1 ml 20% (f.c.) TCA and incubated at 95°C for 20 min followed by filtration through a glass-fiber filter and quantified by liquid scintillation counting. For SDS PAGE analysis of labeled proteins, the spheroplasts were collected by centrifugation at 4,000 rpm and the cell pellet was resuspended in 20 µl 2x Laemmli buffer (125 mM Tris/HCl pH 6.8, 20% glycerine, 4% SDS, 10% 2-mercaptoethanol, 0.02% bromphenol blue). Samples were heated at 95°C for 3 min and loaded on a 10% SDS PAGE. ^{35}S-labeled proteins were then visualized via a PhosphorImager (FLA-3000; *Fuji Photo Film*) and analyzed with the Aida Image Analyzer program.

***In vitro* translation.** For *S. cerevisiae* S30 extract preparation 1.5 l yeast culture was grown to a final OD_{600} of 1.5 at 30°C in YPD medium and collected at 4,000 rpm. The cell pellet was resuspended and washed three times in 15 ml ribobuffer (30 mM HEPES/KOH pH 7.5, 100 mM KOAc, 2 mM $Mg(OAc)_2$, 2 mM DTT, 0.1 mM PMSF, 8.5% mannitol). The cell pellet was frozen in liquid nitrogen and ground three times in a pre-cooled mortar. The resulting powder was resuspended in 9 ml ribobuffer with one additional TM complete tablet (cOmplete mini, *Roche*) and subjected to an additional centrifugation step (30,000 x g, 7 min at 4°C). The resulting S30 supernatant was aliquoted and snap-frozen in liquid nitrogen. *In vitro* translation reactions were terminated by mixing them with an equal volume of 2 x Laemmli buffer, heated at 95°C for 3 min and loaded on a 10 % SDS PAGE. Protein bands were visualized by using a PhosphorImager (FLA-3000; *Fuji Photo Film*) and quantified with the densitometric program Aida Image Analyzer. *In vitro* translation reactions in the wheat germ (*Promega*), rabbit reticulocyte (*Promega*), human (*Pierce Human In Vitro Protein Expression Kit; Fisher Scientific*), or *E. coli* (*New England BioLabs*) systems were performed according to the manufacturer`s instructions.

Polysome profiling. For polysome profiling 1.5 liters of *S. cerevisiae* cells were grown to mid-exponential phase (OD_{600} 0.8), pelleted by centrifugation in the presence of 100 µg/ml cycloheximide. The cell pellet was frozen in liquid nitrogen and lysed with acid washed glass beads in lysis buffer (20 mM HEPES-KOH, pH 7.4, 100 mM KOAc, 2 mM $MgOAc_2$, 0.5 mM DTT, 1 mM PMSF (100 mM), 100µg/ml cycloheximide) using a FastPrep (*MP Biomedicals*) three times at 5 m/sec. Supernatant was transferred to a new tube and centrifuged at 12,000 rpm for 10 minutes. Always 150 A_{260} units of this lysate were layered on top of a 10-40% sucrose gradient, prepared in buffer containing 20 mM HEPES-KOH, pH 7.4, 100 mM KOAc, 2 mM $MgOAc_2$, 0.5 mM DTT, and 100 µg/ml cycloheximide and centrifuged for 5 hours at 25,00 0 rpm in an SW28 swing-out rotor. Polysomal, monosomal and subunit fractions were isolated by emptying the gradient using a peristaltic pump and simultaneously monitoring the absorbance at 254 nm. To remove polysomes from mRNAs and to dissociate the 80S ribosomes, the polysome gradient analysis was performed as above but in the presence of 10 mM EDTA (Del Prete et al., 2007).

Northern blot analyses. To analyze the cellular distribution of the 18-mer RNA, we blotted RNA extracted from the ribosome-containing pellet fraction (P100) and the corresponding supernatant fraction (S100) of a 100,000 x g centrifugation. Therefore S30 extract was centrifuged at 33,000 rpm at 4°C for 15 hours using a Ti-60 rotor. From the resulting supernatant (S100) RNA was precipitated by adding 2.5 vol. ethanol. The pelleted RNA was further subjected to PCI extraction and subsequently ethanol precipitated. The ribosome pellet (P100) was resuspended in Buffer B (2 mM $Mg(OAc)_2$, 100 mM KOAc, 20 mM HEPES, pH 7.4, 0.1 mM PMSF, 1 mM DTT, 20% glycerol) and the RNA PCI extracted and subsequently precipitated with ethanol. Both RNA pellets (from S100 and P100) were resuspended in equal volumes of H_2O and equal portions were loaded on an 8% denaturating polyacrylamide gel and subsequently electro-blotted onto nylon membranes. Finally the membrane was hybridized to a ^{32}P 5`end labeled LNA probe (*Exiqon*) complementary to the *TRM10* 18-mer RNA sequence.

SUPPLEMENTAL REFERENCES

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