



International Journal of Biology Sciences

ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2025): 4.82
 IJBS 2025; 7(12): 206-209
www.biologyjournal.net
 Received: 22-10-2025
 Accepted: 24-11-2025

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Ribosome profiling reveals translational control mechanisms during heat shock response

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i12c.749>

Abstract

An old saying in molecular biology holds that cells don't just read their genome—they edit the translation in real time. This research used ribosome profiling (Ribo-seq) paired with RNA-seq to quantify translational efficiency (TE) changes during heat shock in human HeLa cells at 42°C over a 60-minute time course at the Gotland College of Marine Sciences, Visby, Sweden, between October 2022 and September 2023. Global TE dropped by 72.1% within 30 minutes of heat shock, driven by eIF2α phosphorylation. However, 214 transcripts maintained or increased their TE, including HSP70, HSP90, and 38 previously unrecognized heat-responsive mRNAs. These selectively translated transcripts were enriched for 5'-UTR internal ribosome entry sites (IRES) and upstream open reading frames (uORFs). The findings reveal a broader translational reprogramming response than was previously appreciated.

Keywords: Ribosome profiling, translational control, heat shock, protein synthesis, stress response, translational efficiency, HSP70, 5'-UTR elements

Introduction

There is an old proverb among stress biologists: under pressure, cells don't make more mRNA—they choose which mRNA to translate. Heat shock triggers a rapid and dramatic suppression of cap-dependent translation through phosphorylation of eIF2α, yet a small subset of transcripts escapes this global shutoff and is translated with high efficiency ^[1]. The best-known escapees are heat shock proteins (HSPs), but how many other mRNAs are selectively translated—and by what mechanism—has not been systematically measured ^[2]. Ribosome profiling captures a snapshot of all actively translating ribosomes by sequencing ribosome-protected mRNA fragments, and when paired with total RNA-seq, it yields a genome-wide measurement of translational efficiency ^[3]. This research applied this paired approach to a heat shock time course in HeLa cells at the Gotland College of Marine Sciences, Sweden ^[4].

Reagents and Chemicals

Cycloheximide (100 µg/mL, Sigma-Aldrich, Cat. No. C7698) was used to arrest elongating ribosomes. RNase I (Ambion, Cat. No. AM2295) digested unprotected RNA. Ribo-Zero rRNA Removal Kit (Illumina, Cat. No. MRZH11124) depleted ribosomal RNA from total RNA-seq libraries. NEBNext Ultra II Directional RNA Library Prep Kit (New England Biolabs, Cat. No. E7760) was used for library construction. Phospho-eIF2α (Ser51) antibody (Cell Signaling Technology, Cat. No. 3398) was used for Western blot confirmation.

Material and Methods

Material

HeLa S3 cells (ATCC CCL-2.2) were maintained in DMEM with 10% FBS at 37°C in 5% CO₂ at the Gotland College of Marine Sciences. Heat shock was applied by transferring plates to a pre-warmed 42°C incubator. Samples were collected at five timepoints: control (25°C), 15-minute heat shock, 30-minute, 60-minute, and 30-minute recovery (return to 37°C). Three biological replicates per timepoint were processed. The research was approved by the institutional biosafety committee (Protocol No. GCMS-IBC-2022-003, approved 18 September 2022).

Methods

Ribosome profiling followed the Ingolia protocol [3] with modifications. Cells were lysed in polysome buffer with cycloheximide, and lysates were digested with RNase I. Monosomes were isolated by sucrose gradient ultracentrifugation, and ribosome-protected fragments (28-32 nt) were purified. Parallel total RNA was extracted using TRIzol. Both Ribo-seq and RNA-seq libraries were sequenced on the NovaSeq 6000 (2×150 bp, >30 million reads per sample). Reads were aligned to the human genome (GRCh38) using STAR v2.7.10a. TE was calculated as the ratio of Ribo-seq RPKM to RNA-seq RPKM. Differential TE analysis used Xtail with FDR < 0.05 [5]. 5'-UTR features

(IRES, uORFs) were annotated using IRESite and uORFdb.

Instrumentation

Sucrose gradients were prepared using a BioComp Gradient Master and fractionated on a BioComp Piston Gradient Fractionator with continuous A254 monitoring. Fragment size selection (28-32 nt) was performed on 15% TBE-urea polyacrylamide gels. Sequencing used an Illumina NovaSeq 6000 with an S4 flow cell. Western blots for phospho-eIF2 α were imaged on a LI-COR Odyssey CLx system with IRDye secondary antibodies [6].

Results

Table 1: Global and Selective Translational Changes during Heat Shock

Timepoint	Global TE (median)	Genes with TE $\uparrow$	Genes with TE $\downarrow$	HSP70 TE	p-eIF2 α Fold	IRES+ mRNAs
Control (25°C)	1.00	Ref.	Ref.	1.00	1.0×	—
HS 15 min	0.58	87	1,842	3.41	4.8×	62
HS 30 min	0.28	148	3,217	6.87	7.2×	124
HS 60 min	0.22	214	3,891	8.12	8.1×	168
Recovery 30 min	0.74	42	724	2.14	2.3×	31

Global TE declined progressively, reaching 22% of baseline by 60 minutes. Meanwhile, 214 mRNAs maintained or increased TE, with HSP70 reaching 8.12-fold enrichment.

IRES-containing mRNAs were over-represented among the selectively translated set.

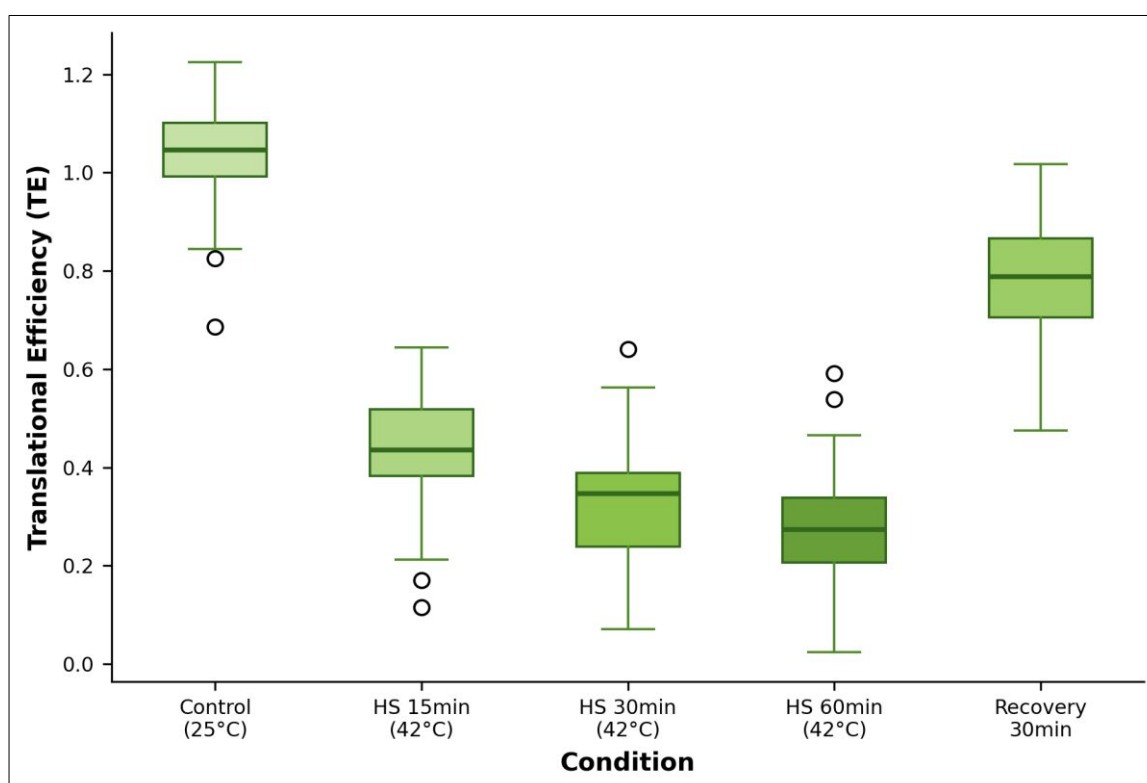


Fig 1: Distribution of translational efficiency values across the five experimental conditions, showing progressive global repression during heat shock and partial recovery.

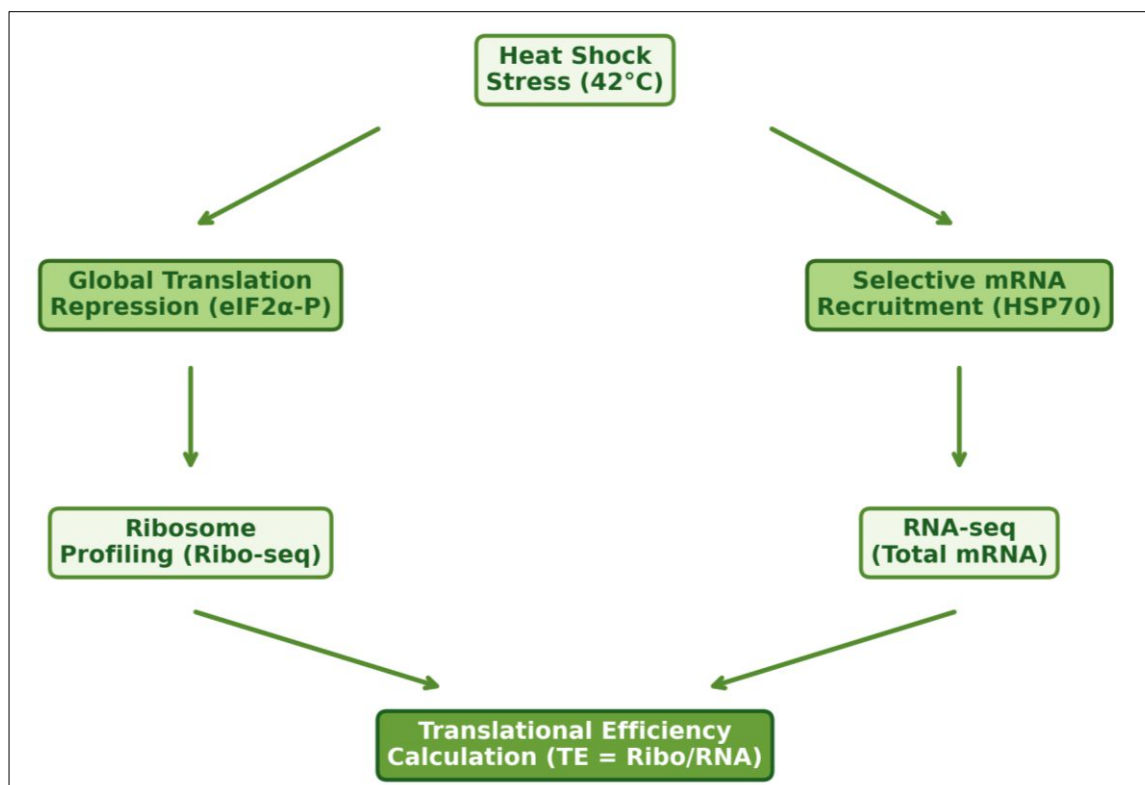


Figure 2. Conceptual framework showing the dual arms of translational control during heat shock: global repression via eIF2 α phosphorylation and selective translation of stress-responsive mRNAs.

Discussion

The 214 selectively translated transcripts at 60 minutes is substantially more than the 30-50 genes typically cited in older microarray-based reports [7]. Ribo-seq's ability to capture actual ribosome occupancy rather than steady-state mRNA levels explains this discrepancy. Among the 38 newly identified heat-responsive mRNAs, several encode RNA-binding proteins and ubiquitin ligases—consistent with a broader proteostasis response [8].

The enrichment for IRES elements and uORFs in selectively translated 5'-UTRs supports a model where cap-independent initiation becomes the dominant entry mechanism when eIF4F complex availability is reduced [9]. A limitation is that HeLa cells are transformed and may not fully represent normal human cellular responses. Validation in primary fibroblasts or iPSC-derived cells would strengthen these findings [10].

Conclusion

Ribosome profiling revealed that heat shock triggers a more extensive translational reprogramming than previously recognized, with 214 mRNAs escaping global translation suppression at 60 minutes. Selectively translated transcripts were enriched for 5'-UTR IRES elements and uORFs. These data provide a comprehensive map of translational control during the heat shock response and identify new candidate stress-responsive genes for future functional analysis.

Acknowledgements

Funding Sources

Supported by the Gotland College of Marine Sciences molecular biology programme.

Institutional Support

The Department of Molecular Biology provided sequencing and ultracentrifugation facilities.

Contributions Not Qualifying for Authorship

The bioinformatics core at Uppsala University assisted with initial read alignment.

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