High-resolution ribosome profiling reveals translational selectivity in the bovine blastocyst

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Introduction

Transcriptomic analyses of early mammalian embryos from multiple species have been comprehensively conducted in the last decade. However, the mRNAs detected from overall transcriptomic profile of an embryo or a single cell does not necessarily represent their functional status, as there is a gap between the overall transcriptome and the mRNAs that are actively translated. Ribosome profiling has been developed to infer the translational status of a specific mRNA species and so analyze the genome-wide translatome. However, the broad application of ribosome profiling has been slowed by its complexity and the difficulty of adapting it to low-input samples, e.g. embryos. In this study, we developed an ultra-low-input ribosome profiling protocol optimized for mammalian embryos and systematically analyzed both polysome- and nonpolysome-bound mRNA profiles of in vitro produced bovine blastocysts.

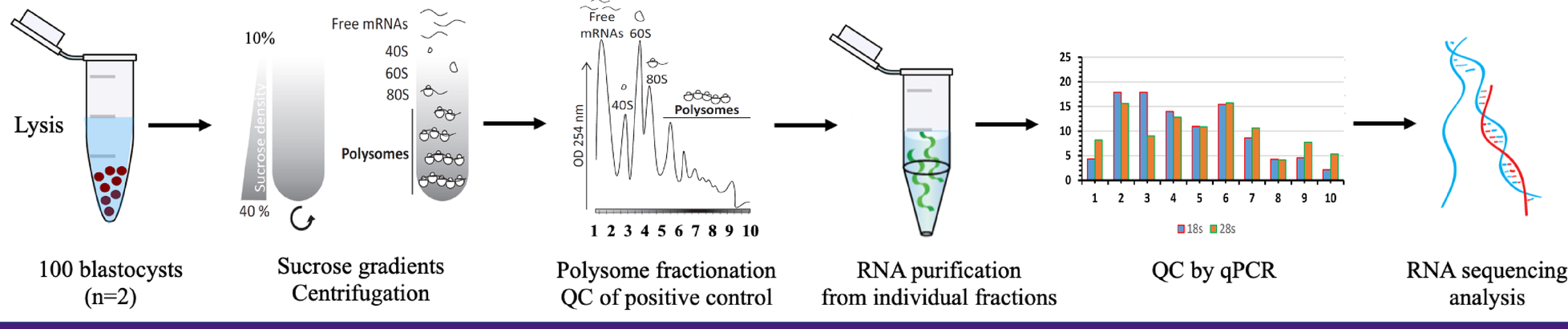
Materials and Methods

Embryo production: In vitro produced bovine embryos were cultured to blastocyst stage, treated with 100 ng/μL cycloheximide for 10 mins and then snap frozen in storage buffer.

Polysome fraction: Ten equal fractions were collected by means of sucrose density gradient and ultracentrifugation of lysates from 100 pooled blastocysts (n=2), and subsequently subjected to RNA isolation and RNA sequencing analysis (**Figure 1**).

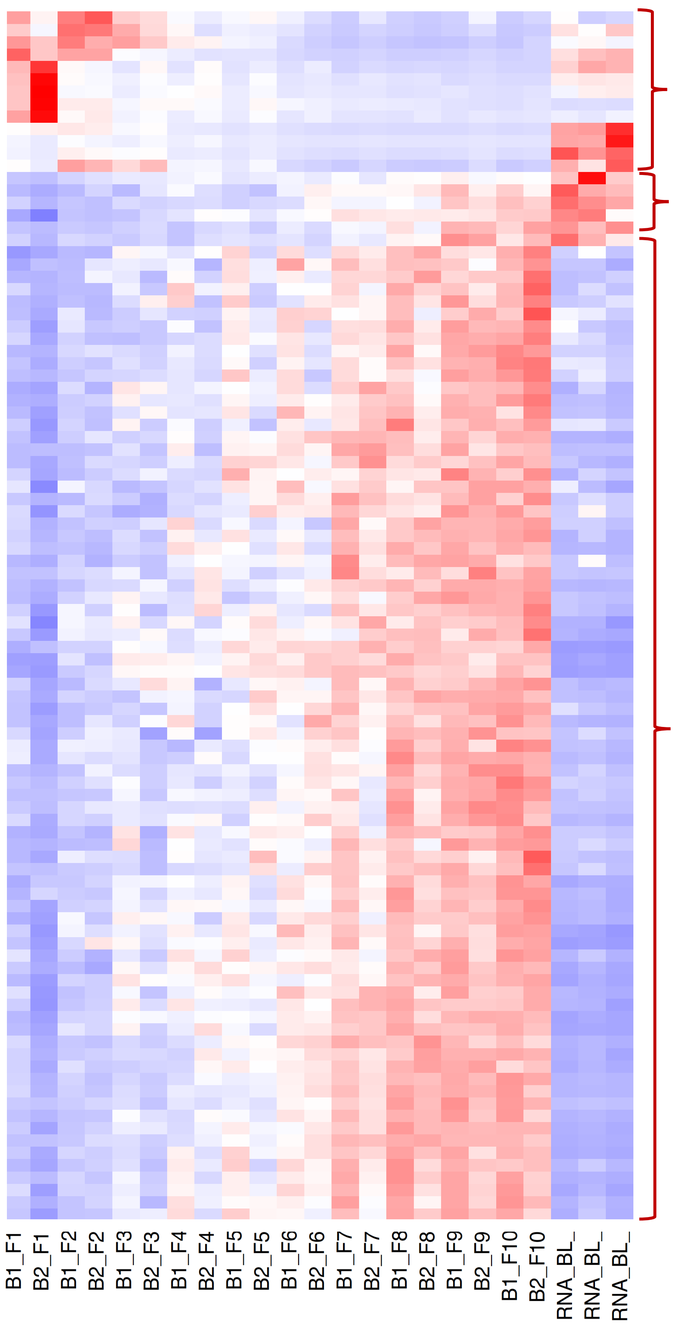
Immunofluorescence staining: Validation of novel active translatomic datasets.

Figure 1. Overview of procedures of embryo collection and the isolation of polysome mRNAs.



Results

I. Clustering of polysome fractions based on gene expression profiles



Inactive translating mRNAs that are highly expressed in blastocysts
e.g. COX2, COX3, ATP6, etc.

Translation of the highly expressed, yet developmentally essential genes

e.g. RPL36AL and RPL11, etc

"Bona Fide" active translating mRNAs

Transcriptional factors: POU5F1, ESRRB, etc.

Regulatory enzymes: JMJD7, ALKBH4, ALKBH7, METTL26, etc.

Figure 2. Heatmap representing the comparison of transcriptional profiles between each polysome fraction and whole transcriptome. 275 genes upregulated in higher fraction, i.e. F7-10; 13 genes downregulated in lower fraction, i.e. F1-6.

II. Clustering of polysome fractions based on KEGG pathways

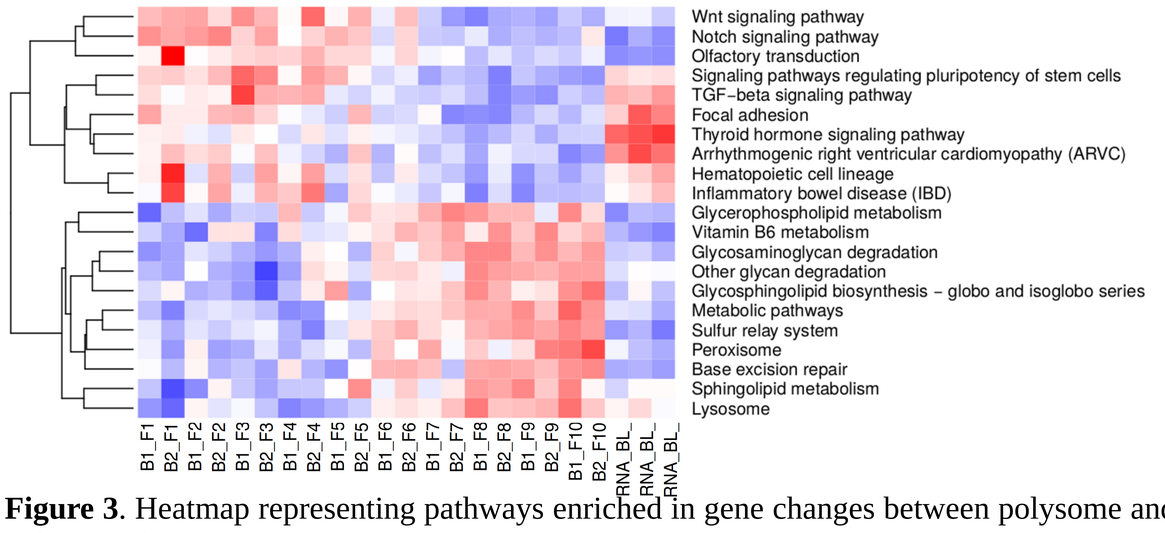


Figure 3. Heatmap representing pathways enriched in gene changes between polysome and non-polysome fractions.

III. Pathways analysis of the 275 genes that are enirched in higher fraction (F7-10)

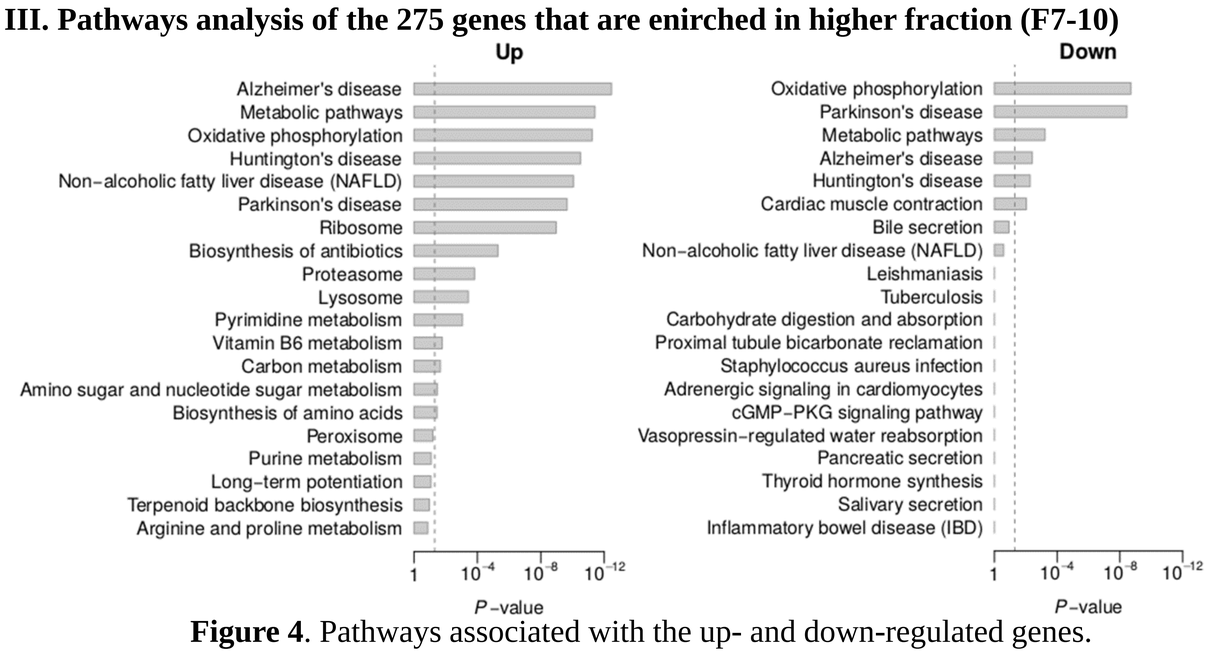


Figure 4. Pathways associated with the up- and down-regulated genes.

IV. Immunofluorescence staining of the active translating mRNAs (JMJD7)

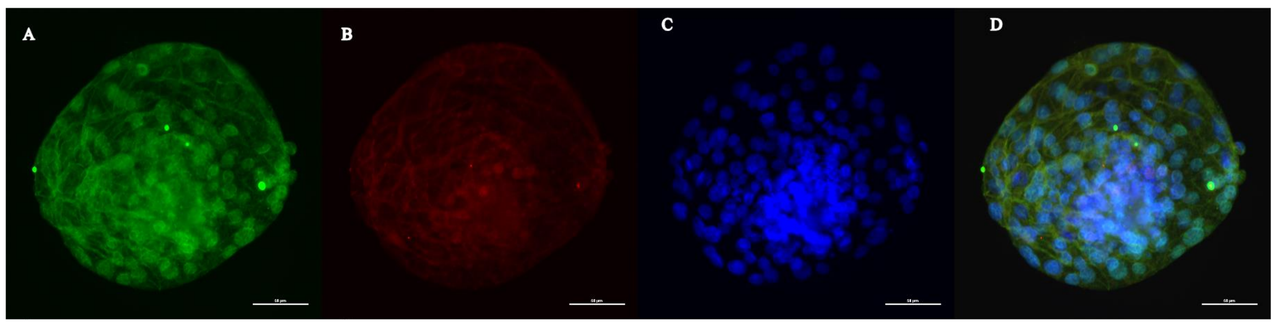


Figure 5. Immunofluorescence staining of JMJD7. Blastocyst was stained against JMJD7 (A); NANOG, as marker of inner cell mass (B); DAPI (C); Merge (D).

Conclusions

- **Identified "Bona Fide" active translating mRNAs in bovine blastocyst, the selective translating these mRNAs suggests they are essential for the function of bovine embryo implantation.**
- **Revealed the translation of the highly expressed, and developmentally essential pathways in the bovine blastocyst.**
- **The low-input ribosome profiling protocol and the data presented here set an example and open future avenues for detailed ribosome-fraction based translatome analyses to reveal novel cellular/embryonic functional regulators beyond transcriptomic data.**

Acknowledgments

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