



ANALYSIS OF THE TRANSCRIPTIONAL AND TRANSLATIONAL PROFILE OF AGRP NEURONS

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Introduction

- Agouti-related peptide (Agrp) neurons are localized in the arcuate nucleus of the hypothalamus.
- Agrp neurons are important for energy balance and feeding behavior regulation.
- The Agrp gene expression profile upon food deprivation (FD) is not well known.
- Utilizing single-cell RNA-Seq (scRNA-Seq) it is possible to isolate single cells and to sequence the whole set of mRNAs in a cell (transcriptome).
- Utilizing RiboTag animals, it is possible to isolate the mRNAs associated with ribosomes, the ones actively being translated.
- This project aims to analyze and compare the transcriptome and translome of Agrp neurons upon FD.

Methods

Translatome: Transcripts actively being translated were captured by immunoprecipitation. RiboTag animals were crossed with Agrp-Cre animals labeled with hemagglutinin (HA) in the *Rpl22* ribosomal gene. These animals allow specifically the immunoprecipitation of Agrp neuron labeled ribosomes. Such ribosomes were isolated and their attached mRNAs were purified for RNA-Seq. The translome upon FD was obtained and then its associated biological processes were characterized.

Transcriptome: The scRNA-Seq datasets were obtained from the Gene Expression Omnibus (GEO) database. We identified Agrp cells selecting the ones that had raw counts for the *Agrp* gene and also the ones with the highest batch corrected values for the *Agrp* gene. We obtained the Agrp transcriptome upon FD using an enrichment analysis and we then characterized its associated biological processes.

Results

- The RiboTag data sequencing quality and alignment analysis were successful, showing a high number of uniquely mapped reads.
- 1022 genes were differentially expressed upon FD. After filtering, 529 genes were considered translome.
- It was associated with the circadian regulation of gene expression process and pathways like leptin signaling in obesity and endoplasmic reticulum (ER) stress.
- The Drop-Seq data presented good results for the sequencing quality and alignment analysis, with a high number of uniquely mapped reads.
- 1190 Agrp cells were identified using the two approaches.
- Using an enrichment test, 1005 genes were found to be impacted by FD. Such genes relate to synaptic plasticity and pathways associated with synaptogenesis and glutamate signaling.
- The Agrp neurons transcriptome and translome shared 59 genes (**Figure 2**). They were associated with ER stress and leptin signaling.

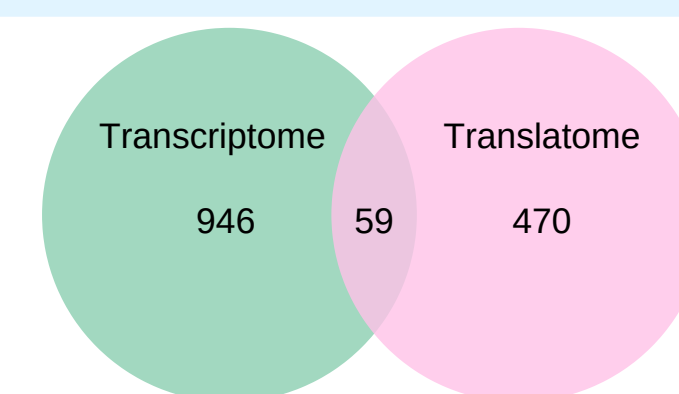


Figure 2: Venn diagram comparing Transcriptome and Translatome genes.

Discussion

- The literature shows that a negative energy balance can induce spine formation in Agrp neurons, and glutamate receptors are important for such plasticity. That indicates that, at the transcriptomic level, the response to FD could be associated with facilitation in the Agrp activation by synaptic plasticity.
- For the translational level, the observed processes relate to evidence in the literature showing the relationship of FD, the circadian clock in the Agrp neurons and feeding behavior regulation. Evidence indicates that FD can lead to ER stress and leptin signaling is involved. It has been demonstrated that ER stress in the hypothalamus of lean mice leads to Agrp resistance. This shows that the translome is related to more direct changes, at the protein level.
- The presence of processes associated with ER stress and leptin signaling for shared genes indicates that FD can impact the sensibility to leptin in both transcriptome and translome levels.

Conclusions

- We observed a distinction at the transcriptomic and translomic level in the processes that were affected by FD in Agrp neurons (**Figure 3**).

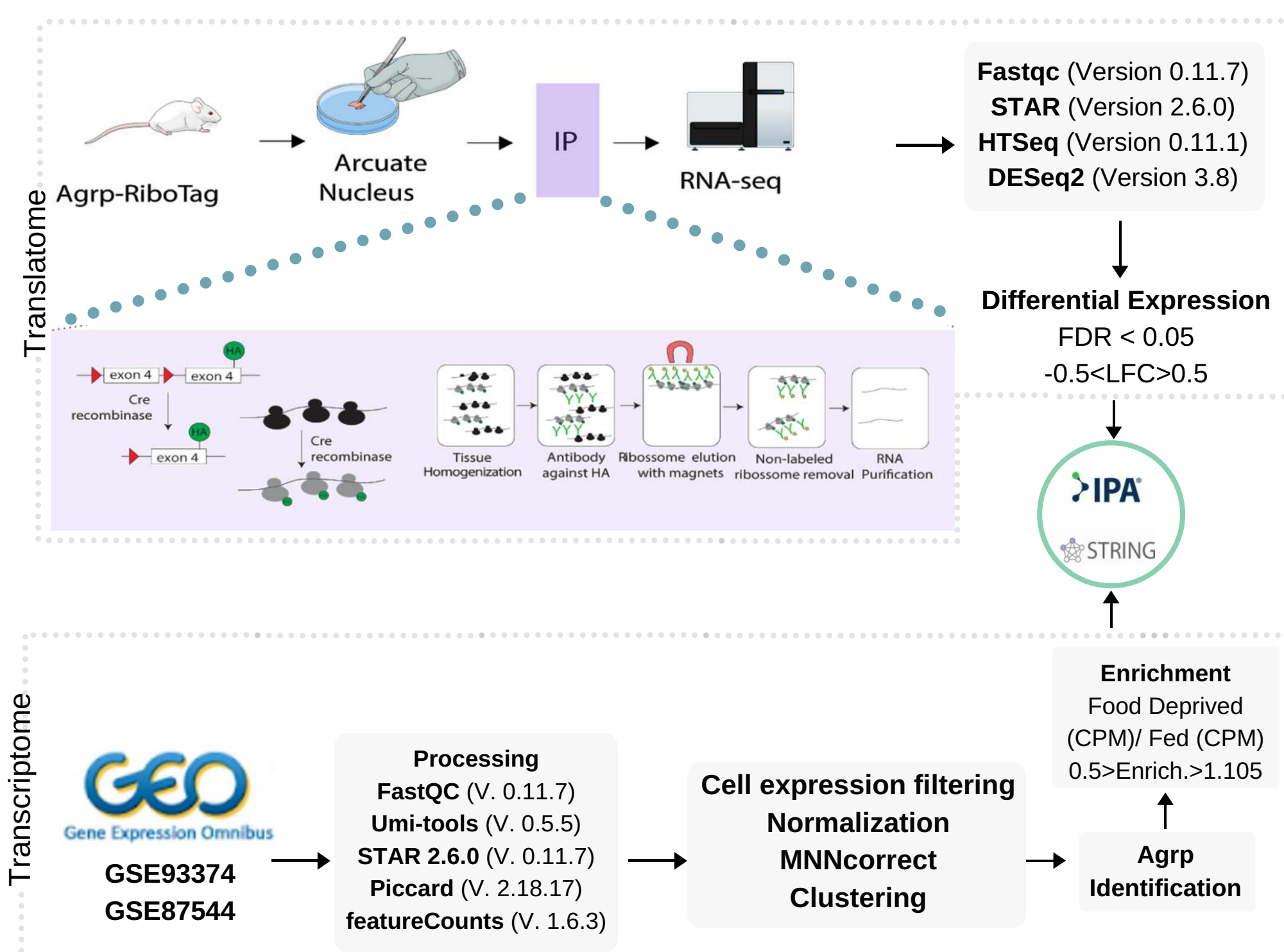


Figure 1: Data obtaintment and processing.

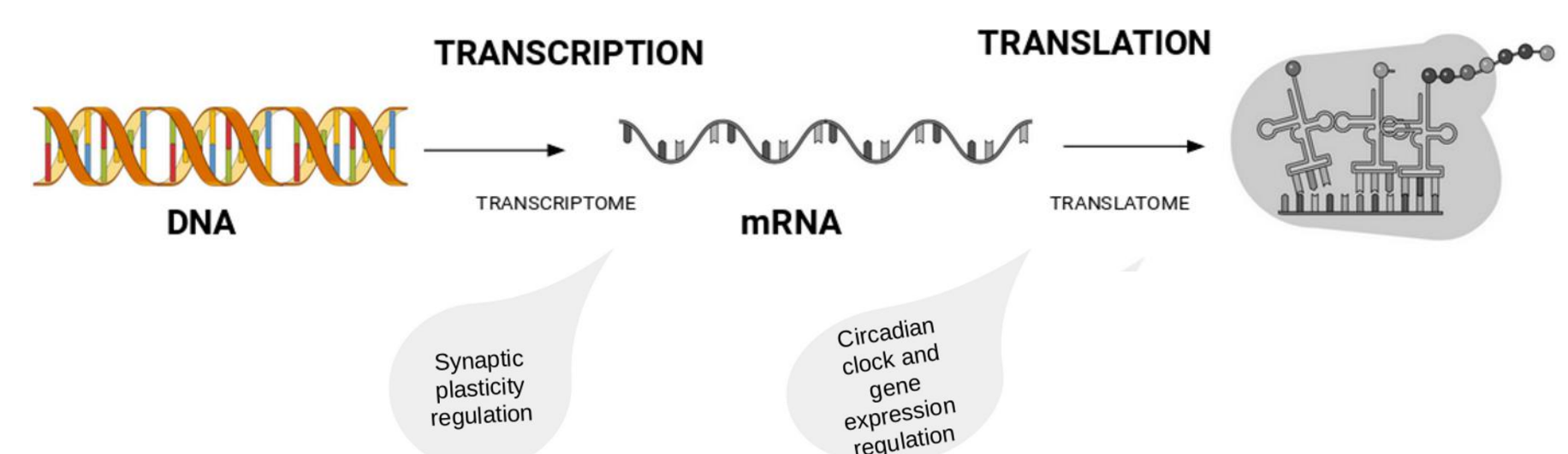


Figure 3: Summary of the biological processes associated with the Transcriptome and Translatome upon FD.