Investigating Sequence Features of eIF3 and eIF4A Target mRNAs

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Abstract

Translation regulation is critical for maintaining cell homeostasis and its misregulation leads to diseases such as cancer. Translation initiation is highly regulated and requires several eukaryotic initiation factors (eIFs). eIF3 has been found to serve as both a scaffold in preinitiation complexes and a regulator of translation for certain mRNAs. eIF4A unwinds mRNA in preparation for recruitment to initiation complexes. Both eIF3 and eIF4A have been found to bind to and regulate translation of the *JUN* mRNA. This suggests a new mechanism for translation regulation that can also act on other mRNAs in the cell. From this analysis, we were able to identify common sequence features of eIF3 and eIF4A target mRNAs. As a whole, this study broadens the understanding of the mechanisms of translation regulation mediated by eIF3 and eIF4A, which serves as a blueprint for targeting disease.

Introduction

Figure 1. First steps of translation initiation

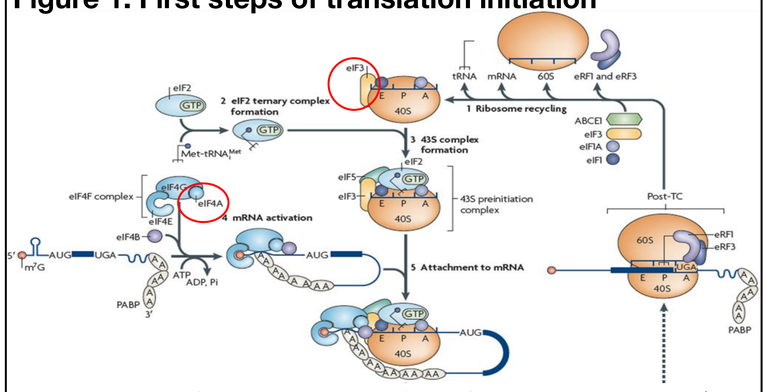

Fig. 1| Mechanism of translation initiation adapted from Jackson et al. (2010).[1] eIF3 acts as a scaffold for pre-initiation complexes and eIF4A unwinds mRNA in preparation for recruitment to initiation complexes.

Figure 2. eIF3 interacts with a subset of mRNAs and regulates translation

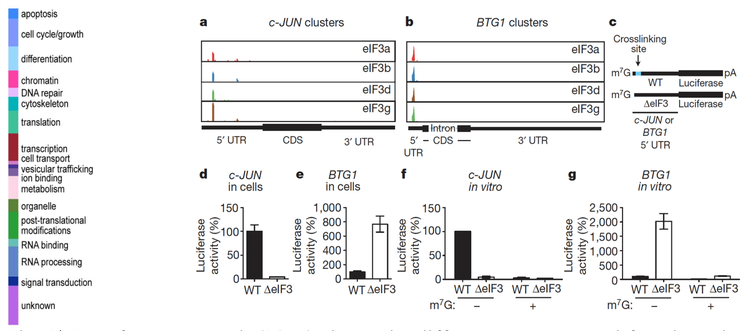

Fig. 2| Data from Lee et al. (2015) shows the different processes and functions that eIF3 target mRNAs are involved in.[2] As well, data shows that eIF3 predominantly interacts with the 5'UTR of mRNAs. Moreover, eIF3 can act as a repressor or activator in mRNAs. In the case of *JUN* it acts as an activator.

Figure 3. eIF3 binding site (PAR-CLIP site) interacts with a stem loop in *JUN*

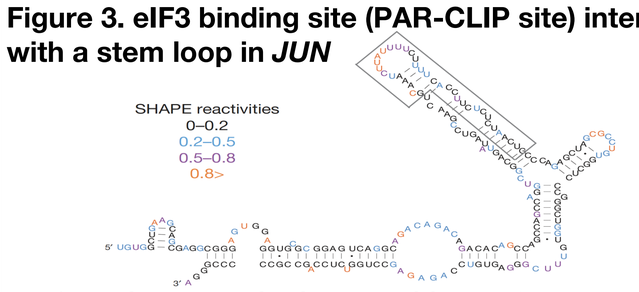

Fig. 3| Secondary structure data for ~250nt of the *JUN* 5'UTR, reported by Lee et al. (2015).[2] eIF3 interacts with this mRNA at the boxed region (PAR-CLIP site).

Figure 4. Multifactor Complex (MFC)

Fig. 4| The MFC is a translation initiation intermediate. The previously reported MFC by Asano et al. (2000) was shown to be composed of eIF1, eIF2, eIF3, eIF5 and methionyl tRNA (Met-tRNA).[3] However, the Cate Lab has found a novel human MFC (unpublished data), which was found to be composed of eIF2, eIF3, eIF4A, eIF4G and Met-tRNA. (Figure courtesy of Angélica M. González-Sánchez, created with BioRender.com).

Figure 5. eIF4A target sequences

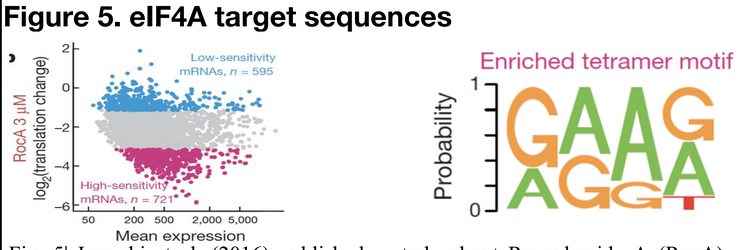

Fig. 5| Iwasaki et al. (2016) published a study about Rocaglamide A (RocA), a compound that clamps eIF4A onto specific mRNA sequences.[4] The study showed that *JUN* was highly sensitive to RocA, meaning it is an eIF4A target mRNA. They also showed that GAAG and GAAA are target sequences of eIF4A.

Objectives

- To investigate the sequence features of a select group of mRNAs that are eIF3 and eIF4A target mRNAs
- To find common sequence features between a select group of eIF3 and eIF4A target mRNAs and hypothesize a mechanism for translation regulation of these mRNAs

Methodology

Cross reference analysis:
- 116 mRNAs found to be targeted by eIF3 & eIF4A
- 37 found to interact significantly w/ eIF4A (p>0.05)
- 17 found to have highest significance w/ eIF4A (p>0.001)

Functional Analysis:
- GeneCard & UniProt: literature search on top 17
- DAVID Bioinformatics Resources database: investigate roles of all 37 significant eIF4A targets
- Subset of 21 mRNAs involved nuclear processes and RNA-binding

Analyze sequence features:
- ENSEMBL: genomic DNA sequence, 5'UTR sequence, CDS, transcript isoform info
- UCSC Genome Browser: 5'UTR sequences and secondary structures
- Using SnapGene: UCSC and ENSEMBL 5'UTR was compared
- IGV: Sequence of PAR-CLIP found

Mapped out Features:
Using SnapGene and the genomic DNA sequence the eIF3 binding site (PAR-CLIP site), annotated & unannotated 5'UTR, annotated & unannotated CDS, and eIF4A target sequences (GAAA/G) were mapped

Primers:
- Used In-Fusion Cloning Primer Design Tool to design primers for NanoLuciferase reporter constructs for future experiments

Secondary Structure Predictions:
- University of Vienna RNA Fold Web Sever & Mathews Group RNA Structure Prediction Web Server used to find secondary structure predictions of PAR-CLIP site & GAAA/G sites

Methodology

Figure 6. mRNAs chosen for further analysis

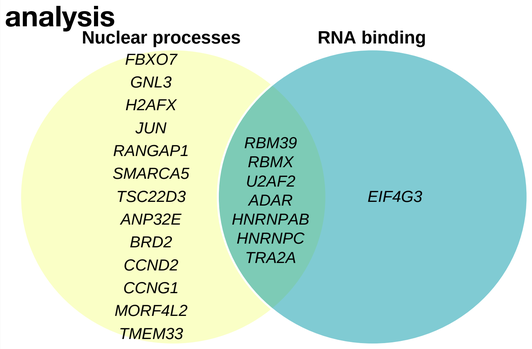

Fig. 6| Using DAVID Bioinformatics Resources Database, a subset of 21 mRNAs were found to have common functions of nuclear processes and RNA binding.

Figure 7. PAR-CLIP site length per mRNA

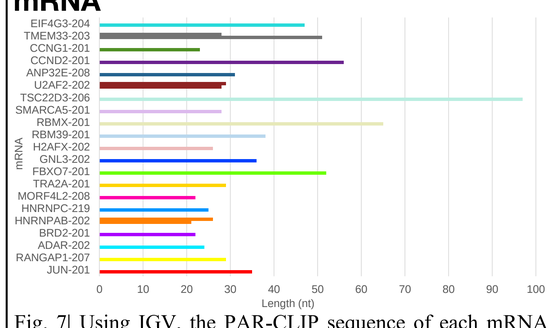

Fig. 7| Using IGV, the PAR-CLIP sequence of each mRNA was found most of PAR-CLIP sites were recorded to be ~ 30nt long.

Methodology

Figure 8. Location of the PAR-CLIP site per mRNA

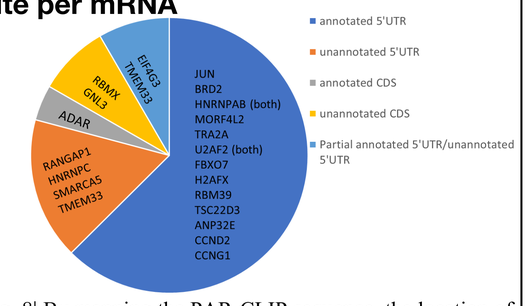

Fig. 8| By mapping the PAR-CLIP sequence, the location of the PAR-CLIP was found for each mRNA. While most were found in the annotated 5'UTR, some PAR-CLIP sites were found in other regions.

Figure 9. Quantification of eIF4A target sequences relative to the PAR-CLIP site

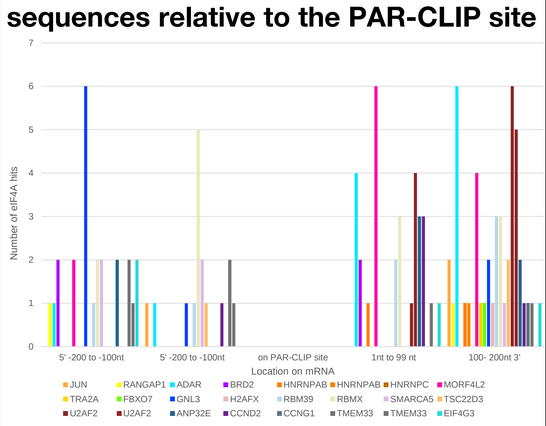

Fig. 9| The amount of GAAA/Gs were quantified in the regions -200nt 5' of the PAR-CLIP site and +200nt 3' of the PAR-CLIP site.

Figure 10. Quantification of secondary structure predictions

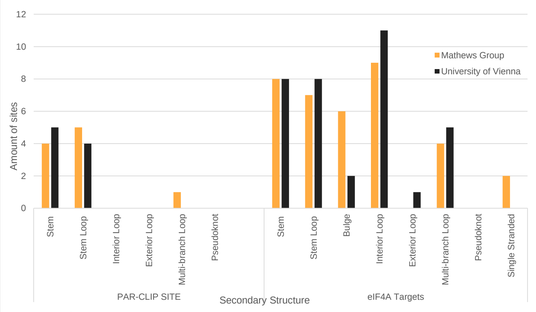

Fig. 11| Using University of Vienna RNAFold WebSever & Mathews Group RNAstructure Prediction Web Server the secondary structure was found for the PAR-CLIP site & eIF4A targets within ~ +/- 200nt of the PAR-CLIP.

Figure 11. BRD2 secondary structure

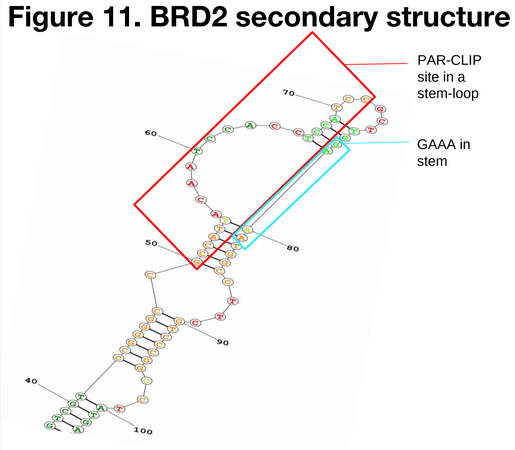

Fig. 11| Secondary structure prediction for a region of BRD2's 5'UTR. PAR-CLIP site and an eIF4A target sequence (GAAA) secondary structure prediction are shown in red and blue box respectively. The PAR-CLIP is predominantly in a stem loop while the GAAA is in a stem.

Methodology

Figure 12. eIF4A targets per region per mRNA

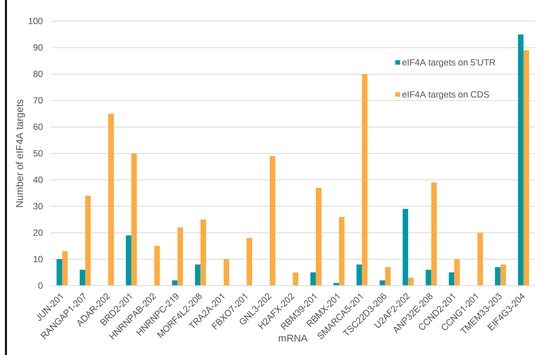

Fig. 12| The eIF4A targets (GAAA/G) were counted per region per mRNA. Most mRNAs showed a greater amount of GAAA/G sequences on the CDS opposed to the 5'UTR.

Conclusions

- The mRNAs that were both targets of eIF3 and eIF4A had many common functions including some that were previously seen in eIF3 target mRNAs
- Most of the PAR-CLIP sites were found to be ~30nt long, giving an estimate of the sequence length eIF3 binds to
- While the PAR-CLIP sites in most mRNAs were found in the 5'UTR supporting the Lee et al. (2015) data, eIF3 was seen to also interact in other regions of the mRNA
- Some regions of the 5'UTR were poorly annotated in the databases, which we have labeled as "unannotated 5'UTR" for the purposes of this study
- The CDS contains more eIF4A target sequences than the 5'UTR potentially due to the substantial difference in lengths
- None of the eIF4A target sequences were found on the PAR-CLIP site and the majority were found upstream of the PAR-CLIP site
- Most of the PAR-CLIP sites were found in stems and stem loops suggesting that eIF3 binds to highly structured areas
- The eIF4A target sequences were found in a variety of secondary structures which can be potentially explained by the fact that eIF4A scans mRNAs rather than binds to them like eIF3

References

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factors, eIF1, eIF2, eIF3, eIF5, and initiator tRNA(Met) is an important translation initiation intermediate in
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