Investigating the Effects of eIF3 Mutations on Start-Codon Recognition Reveals its Roles Across the Transcriptome

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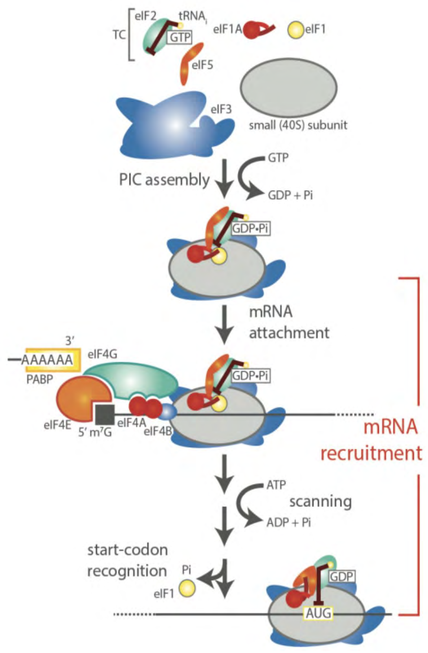
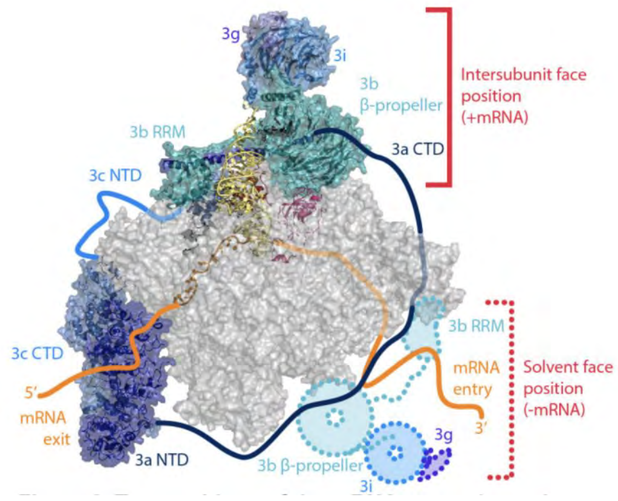
Translation Initiation is a Complex Multi-step Pathway in Eukaryotes that Regulates Gene Expression

- Translation initiation requires the formation of the pre-initiation complex (PIC), which then attaches to the 5' end of messenger RNA (mRNA) and then scans the mRNA in order to locate the start (AUG) codon.



Translation Initiation Pathway
Image Citation: Aitken Lab

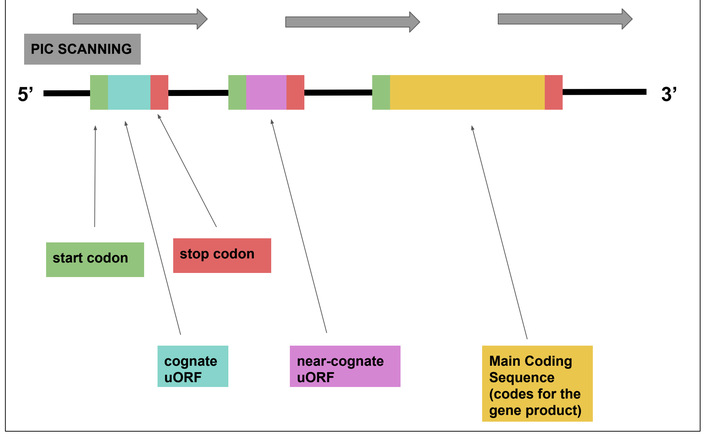
- Translation initiation involves at least twelve different eukaryotic initiation factors. Eukaryotic initiation factor 3 (eIF3) is the largest, most complex, and least understood of these initiation factors.
- eIF3 participates in each phase of translation initiation and plays a large role in mRNA attachment, scanning, and start-codon recognition.
- In *Saccharomyces cerevisiae* (budding yeast), eIF3 is made up of 5 subunits: eIF3a, eIF3b, eIF3c, eIF3g, and eIF3i. All five of these eIF3 subunits are overexpressed in different cancer cells and are causally linked to cancer development.



eIF3 is the Largest Initiation Factor in the Pre-Initiation Complex
Image Citation: Aitken Lab, Adapted from Llácer et al., bioRxiv 2018.

uORFs test the fidelity of start-codon recognition

- An upstream open reading frame (uORF) is a translatable stretch of RNA defined by a start and stop codon that is located upstream of the main coding sequence of an mRNA.
- Our focus is on how the presence of uORFs on mRNAs impact their sensitivity to mutations to eIF3.
- Cognate uORFS begin with the universal AUG start codon, whereas near-cognate uORFs begin with a start codon that differs from AUG by one nucleotide (e.g. CUG or UUG).



Comparing the Translational Efficiency of Two Mutants

- **Ribosome Profiling** locates the position of every translating ribosome on every mRNA molecule in living cells
- **mRNA Sequencing** is used to thoroughly determine the abundance of each individual mRNA

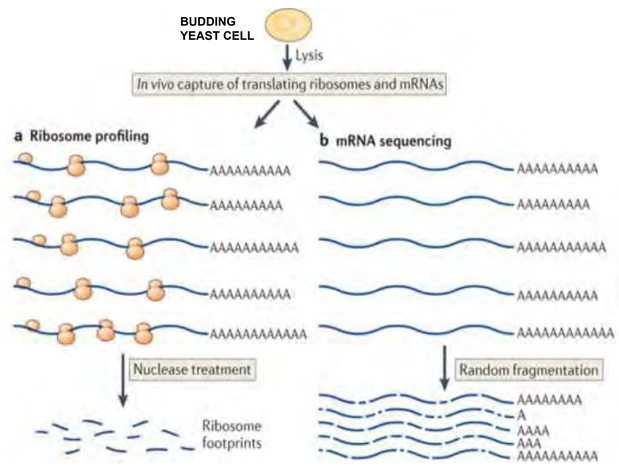


Image Citation: Adapted from Brar and Weissman, Nature Reviews 2015.

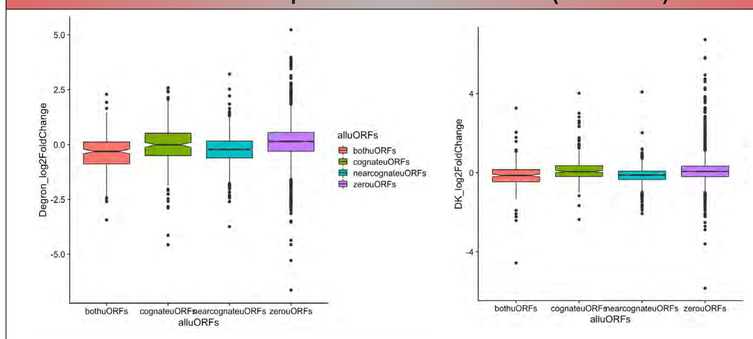
By comparing the information from these two approaches, we can calculate the **translational efficiency of individual mRNAs** and how this is impacted by mutations to eIF3.

We then investigate the features of mRNAs most sensitive to specific mutations of eIF3 to **infer the mechanistic contributions of eIF3 during translation initiation**.

DDKK Mutant Strain: disrupts the interaction of eIF3i and eIF3g with the remainder of the eIF3 complex

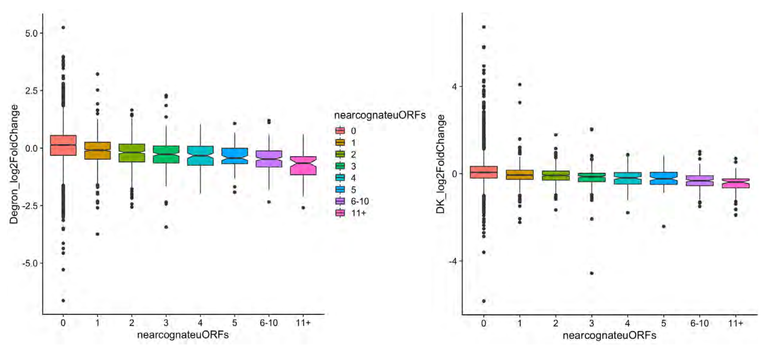
Degron Mutant Strain: disrupts the entire eIF3 complex

mRNAs containing near-cognate uORFs show greater decreases in translation in the presence of both mutants (Plots 1 + 2)



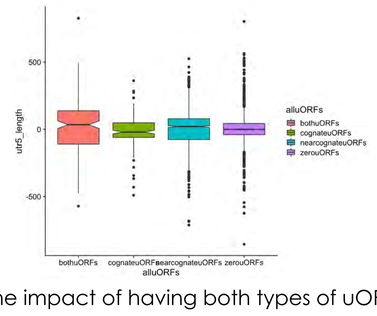
We examined the impact of having both types of uORFs, only cognate uORFS, only near-cognate uORFs, and no uORFs on the translational efficiency of the two mutant strains.

Increasing numbers of near-cognate uORFs result in decreasing mutant translational efficiency (Plots 3 + 4)



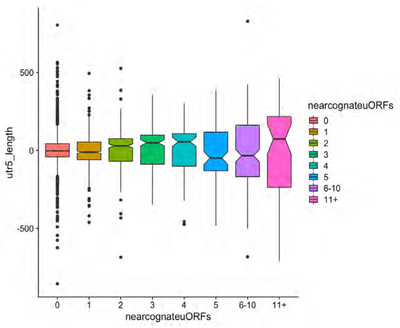
We examined the impact of the number of near-cognate uORFs on translational efficiency of the two mutant strains. As the number of near-cognate uORFs increases, the translational efficiency gradually decreases in both mutants.

Increasing 5' untranslated region (UTR) length results in an increase of near-cognate uORFS (Plot 5)



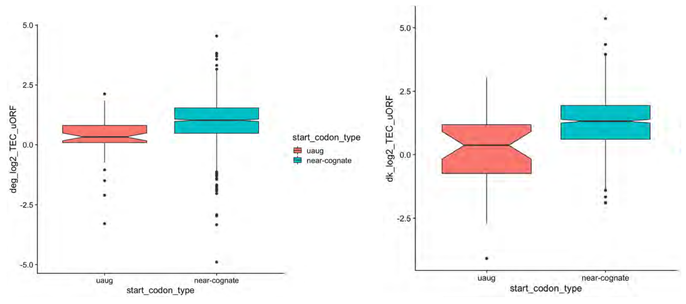
We examined the impact of having both types of uORFs, only cognate uORFS, only near-cognate uORFs, and no uORFs on the 5' untranslated region (UTR) length of the two mutant strains. The 5' UTR is distance from where the PIC is attached to the start codon. It has structured elements which the PIC resolves as it is scanning along the mRNA to ensure scanning efficiency. Compared to cognate uORFs, near-cognate uORFs are more prevalent as the length of the 5' UTR increases.

Increasing numbers of near-cognate uORFs result in increasing 5' UTR length (Plot 6)



We examined the impact of the number of near-cognate uORFs on 5' UTR length. As the number of near-cognate uORFs increased, the 5' UTR length increased as well.

Mutant translational efficiency is most impacted in near-cognate uORFs (Plots 7 + 8)



Both mutants evidently result in an increased translational efficiency for the near-cognate uORFs as compared to the cognate uORFs.

Conclusion and Future Studies

- From Plots 1, 2, 7, and 8, we observe that a mutation that disrupts the interaction of eIF3i and eIF3g with the remainder of the complex (*DDKK*) and a mutation that disrupts the entire eIF3 complex (*eIF3 degron*) both result in increased translation of near-cognate uORFs as compared to cognate uORFs.
  - This effect is most severe on mRNAs that are sensitive to both mutations.
- We also observed that increasing numbers of near-cognate uORFs result in gradually decreasing translational efficiency in both the *DDKK* and *eIF3 degron* mutants from the 3rd and 4th plots.
- In addition, we observed from the 5th plot, as the length of the 5' UTR increases, there is an increase in near-cognate uORFs. Also, in the 6th plot, we observed that as the number of near-cognate uORFs increases the length of the 5' UTR increases as well. This demonstrates a potential correlation between 5' UTR length and the number of near-cognate uORFs.

We are currently testing these effects with statistical models. **Taken together, these observations are consistent with a model in which eIF3 contributes to discrimination against near-cognate start codons, with the eIF3i and eIF3g subunits playing an important role in this discrimination.**

Further experiments must be conducted on the individual subunits of eIF3 to see how consistent these results are within the entire complex.

Ultimately, we hope to provide a mechanistic framework for understanding how disruption of translation initiation is involved in cancer.

Acknowledgments

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Please scan the following to listen to Shanya's presentation: