

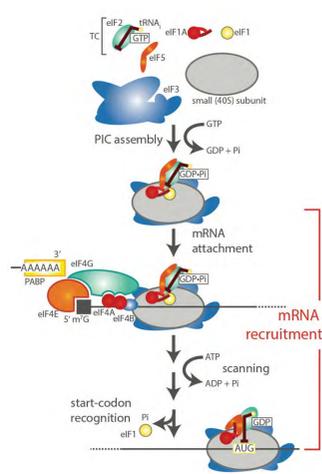
Investigating the efficacy of a recombinant system to study the role of eIF3 in translation initiation

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Translation initiation is the most regulated, rate-limiting step in protein synthesis

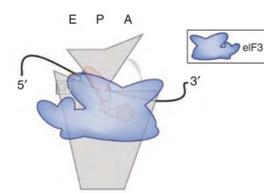


Translation initiation is the first step in protein translation, starting with the formation of the pre-initiation complex (PIC). In eukaryotes, the PIC consists of twelve essential initiation factors (eIFs) that latch onto the 40S ribosomal subunit.

Once the PIC is formed, the PIC binds to the 5' end of mRNA to begin scanning for the start codon (AUG). Eventually, the start codon is recognized and translation elongation can begin.

Figure adapted from Aitken Lab grant: The Role of the mRNA Entry Channel Arm of eIF3 in mRNA Recruitment

eIF3 is the largest and most complex initiation factor



Aitken, C.E. & Lorsch, J.R. (2012)

eIF3 consists of multiple subunits and physically surrounds the PIC, allowing it to potentially contribute to every step of translation initiation.

Mutations to eIF3 may lead to aberrant levels of protein translation

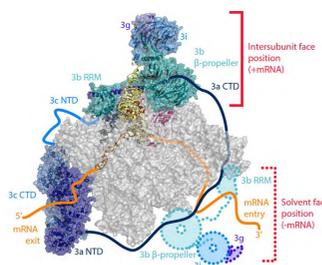
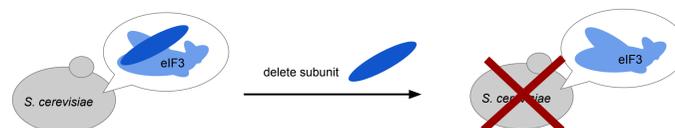


Figure adapted from Aitken Lab grant: The Role of the mRNA Entry Channel Arm of eIF3 in mRNA Recruitment and Ullrich et al. (2018).

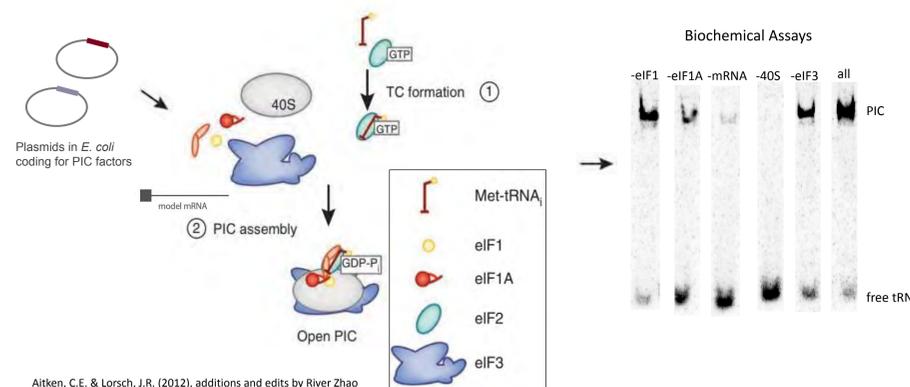
The mRNA entry and exit channels appear to play important roles in mRNA attachment/recruitment, scanning, and start-codon recognition. eIF3's subunit eIF3a connects the mRNA entry and exit channels and may assist in proper communication between the channels.

Therefore, mutations to eIF3 may cause significant irregularities in translation initiation, possibly leading to the development of human cancers.

Creating an *in vitro* recombinant system is necessary to study lethal eIF3 variants



In our model system, budding yeast, or *Saccharomyces cerevisiae*, eIF3 is essential for translation. Mutations or deletions of eIF3 and its subunits tend to be lethal, which prevents us from investigating eIF3 variants.



Aitken, C.E. & Lorsch, J.R. (2012), additions and edits by River Zhao

To circumvent this obstacle, we use an *in vitro* recombinant system.

Tracking Methionyl-tRNA (Met-tRNA) that has been radioactively labelled with ³⁵S allows us to quantify PIC formation and mRNA recruitment through biochemical assays. We hope to show that the activity of the recombinant system is indistinguishable from that of the system purified endogenously.

Employing a gel shift assay allows us to analyze levels PIC association and activity

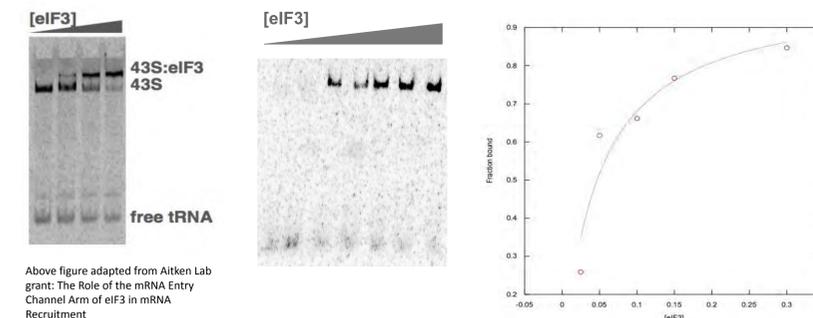
A gel shift assay allows us to analyze the efficacy of our recombinant system. Differences in molecular weight cause heavier complexes to form bands higher in the gel.

- "TC only" lane: the ternary complex (TC) only, which includes eIF2, tRNA and GDPNP.
- "-eIF3" lane: the entire 48S complex, which includes the TC, 40S ribosomes, eIF1, eIF1A, and mRNA.
- "+eIF3" lane: the 48S complex and eIF3.

Increased PIC formation and the presence of a gel shift would indicate that our recombinant system was functioning. Running an eIF3 titration would then allow us to determine the experimental K_D and compare it to the K_D of the native system, which serves as our benchmark.



PIC formation in the recombinant system appears similar to the native system

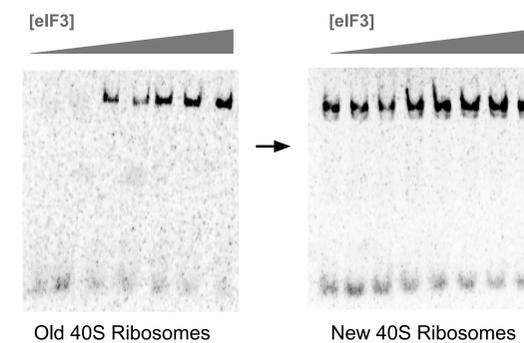


Above figure adapted from Aitken Lab grant: The Role of the mRNA Entry Channel Arm of eIF3 in mRNA Recruitment

Titrating eIF3 yields an enzymatic Michaelis-Menten curve with an approximate dissociation constant (K_D) of 46 nM. While the expected K_D of 38 nM corroborates our findings, we did not achieve the previously seen gel shift between the -eIF3 and +eIF3 lanes (Aitken et. al 2016).

Further development of the recombinant system is necessary to improve our knowledge of eIF3

We have been attempting to isolate any issues with our reactants by forming the PIC in the absence of various factors. We were able to determine that our initial working stock of 40S ribosomes prevented proper PIC formation.



A functioning recombinant system will allow us to pursue future research on eIF3 mutants. Understanding the effects of eIF3 mutations on levels of PIC formation, levels of mRNA recruitment, and rate of mRNA recruitment may then allow us to characterize the overall role of eIF3. This could draw a potential link between eIF3 variants and aberrant levels of protein translation, and therefore to the development of human cancers.

References

1. Aitken C.E., et al. (2016). Eukaryotic translation initiation factor 3 plays distinct roles at the mRNA entry and exit channels of the ribosomal preinitiation complex. *eLife*, 5, e20934. <https://doi.org/10.7554/eLife.20934>.
2. Aitken, C.E. & Lorsch, J. (2012). A mechanistic overview of translation initiation in eukaryotes. *Natural Structure & Molecular Biology* 19, 568-576. <https://doi.org/10.1038/nsmb.2303>.

Acknowledgements

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Audio Code

