UNDERGRADUATE RESEARCH SUMMER INSTITUTE 2021

Exploring the Potential Role of eIF3d in Translational Regulation through Ribosome Profiling

Areeba Zaheer ’23, Joshua Kim ’23, and Colin Echeverría Aitken, PhD
Biochemistry Program, Vassar College, Poughkeepsie, NY, 12604

The Central Dogma

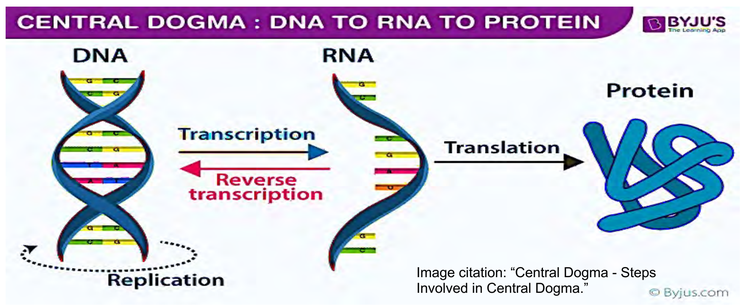
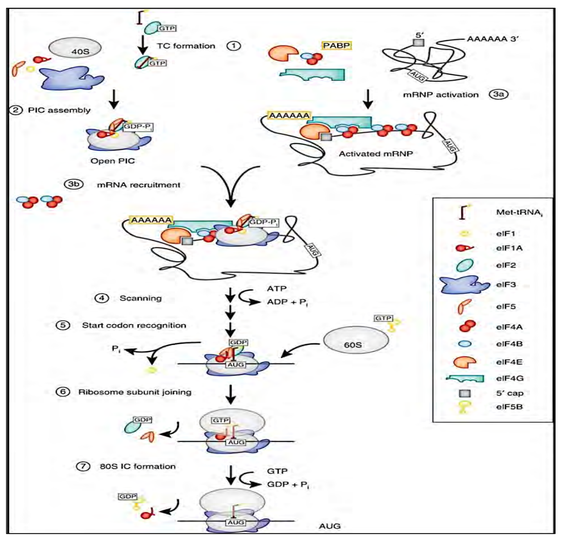


Image citation: “Central Dogma - Steps Involved in Central Dogma.”

Translation initiation is a highly regulated multi-step process



- Translation initiation is the **rate-limiting, most regulated** step of translation and sets the reading frame for protein synthesis.
- It is characterized by the formation of the **pre-initiation complex (PIC)**, which binds to one end of the mRNA to begin scanning for the start codon.
- Once it identifies the start codon, the ribosome is fully assembled.

Image Citation: (Aitken and Lorsch 2012)

eIF3 is the largest initiation factor and has an emerging role in translational regulation

- eIF3 promotes **PIC assembly** and is a key component of **mRNA recruitment** to the 40S subunit.
- Initiation is mediated by at least 12 **eukaryotic initiation factors** (eIFs), of which eIF3 is the **largest** and **most complex**.

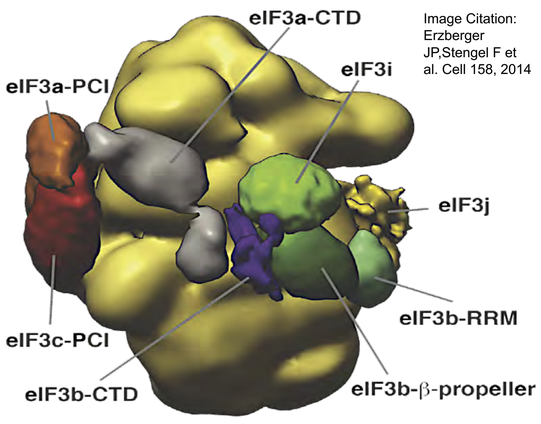


Image Citation: Erzberger JP,Stengel F et al. Cell 158, 2014

Human eIF3d participates in an alternative initiation pathway

- In stressful, nutrient-deficient environments, **human eIF3d** binds onto **specific classes of mRNAs** for the translation initiation, of which many are responsible for **cell survival** and **cellular growth .**This **noncanonical pathway** is thought to play a role in **preserving the metabolic state** of the cell (Lee et. al 2016).

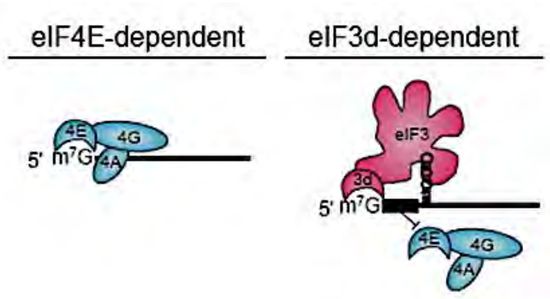


Image Citation: Lee, Amy S et al 2016

The Role of Moe1/eIF3d in Global Translation in Fission Yeast (S. pombe)

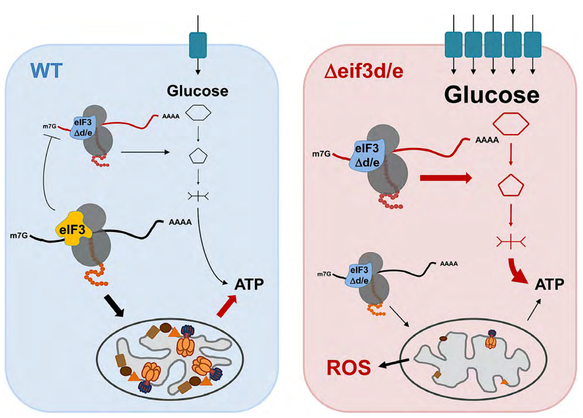


Image Citation: Shah et al., 2016

- **Fission yeast (S. pombe)** without **eIF3d and eIF3e** cannot make key components of the **mitochondrial electron transport chain**, suggesting a link between these **subunits** and **global translation activity** (Shah et al.,2016).
- The role of **Moe1/eIF3d** remains unclear and is investigated in this project.

Confirming the MOE1 Deletion (ΔMOE1)

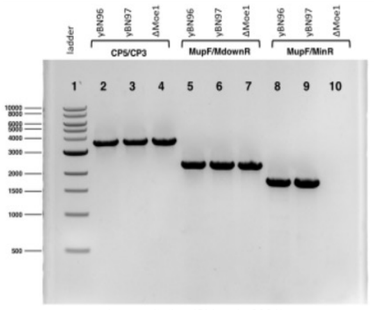
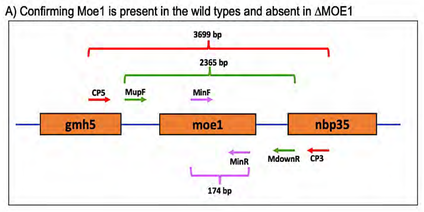


Image citation (Shimoda 2021)

2.0% agarose gel electrophoresis was utilized to verify Moe1 sequence in wild-type and KanMX4 sequence in the mutant. Well 1 was a 1kb ladder.

A) Confirming Moe1 is present in the wild types and absent in ΔMOE1



B) Confirming Moe1 is present at the right locus at chromosome 1 and absent in ΔMOE1

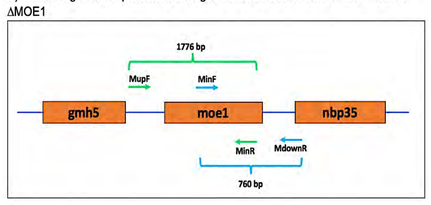


Image citation (Shimoda 2021)

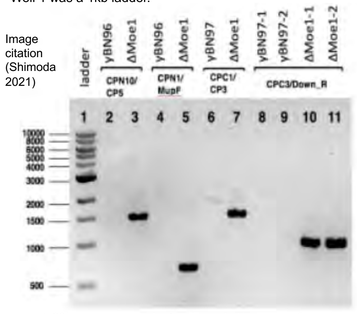


Image citation (Shimoda 2021)

C) Confirming the presence of the KanMX4 marker at the right locus in ΔMoe1

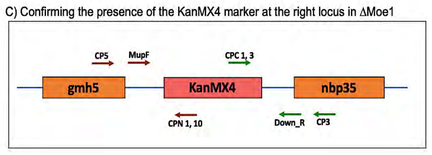


Image citation (Shimoda 2021)

A 1.0% agarose gel electrophoresis image confirmed presence of the KanMX4 selection marker in the mutant. Well 1 was a 1kb ladder. We obtained 2 samples of purified DNA for yBN96, yBN97, and ΔMOE1 each. For wells 2-7, the purer of the two samples of each strain was used. For wells 8-11, both purified samples of yBN97 and ΔMOE1 were tested.

ΔMOE1 Grows Slower than Wildtype Strains

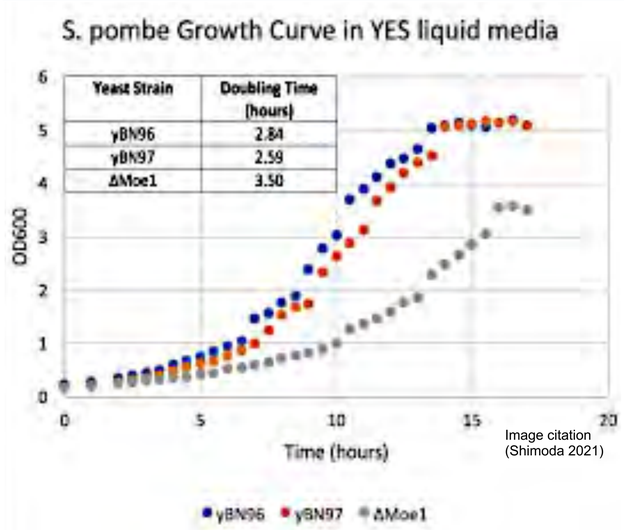


Image citation (Shimoda 2021)

- Growth curves of **mutant (ΔMoe1)** to replicate **Wild-type strains** (yBN-96 and yBN-97).
- Grew **50 mL cell cultures** at **250 RPM and 32 ºC**. We monitored their growth from a starting **optical density at 600 nm** (OD600) of 0.2 using a **UV-Vis Spectrophotometer**
- Each reading was taken every **30 minutes** until the curve levelled off.

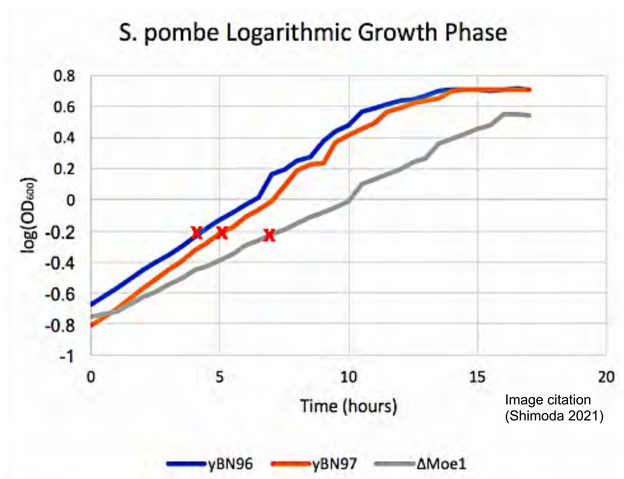
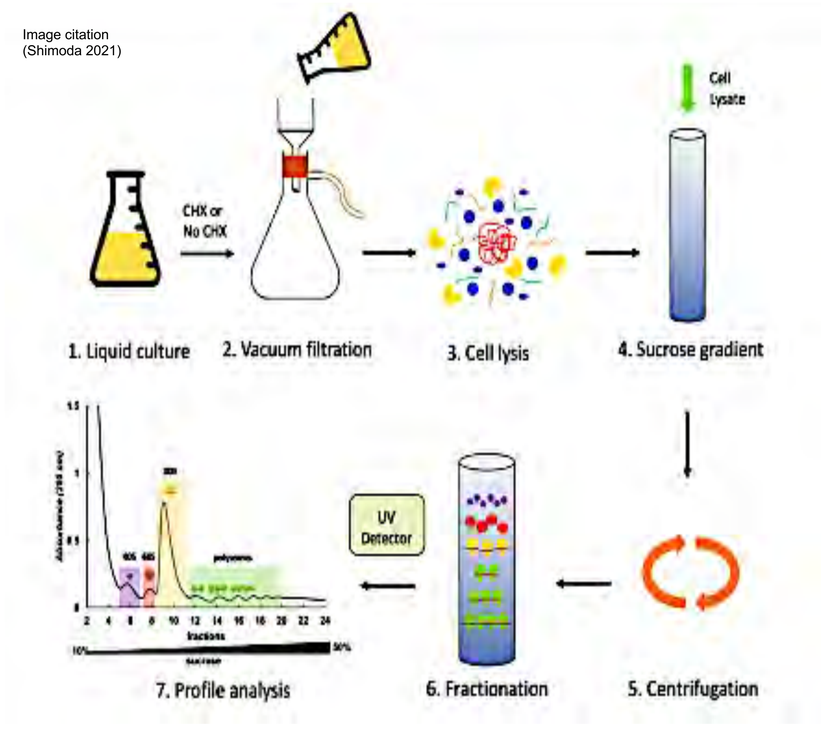


Image citation (Shimoda 2021)

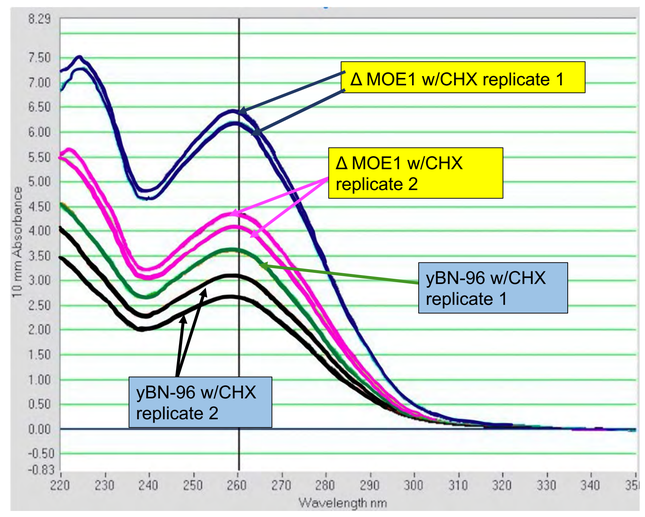
- Growth Curve for Wildtype and Mutant is treated with **logarithmic function** at OD_{600}.
- **Red X’s** indicate **mid-log phase**
- yBN-96 and yBN-97 are **comparable** strains

Polysome Profiling Monitors Global Translation throughout the Cell

Image citation (Shimoda 2021)

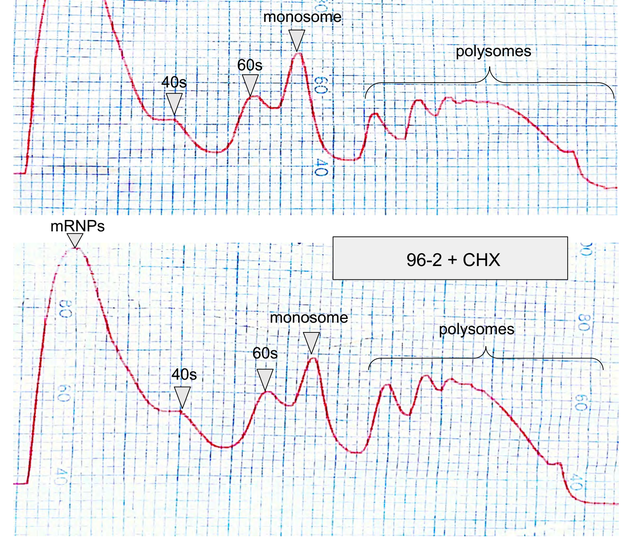


High Concentration and Purity of CHX-Treated Translational Lysates Before Polysome Analysis



- ΔMoe1 and Wildtype (yBN-96) strains with replicates were **treated with CHX**, lysed, and centrifuged.
- After diluting the lysate, **absorbance levels at a wavelength of 260 nm** (A260) were taken via a Nano-Drop.
- **Amplitude** suggests high yield. **A260/A280 ratios** suggests that RNA is at high purity.

Polysome Analysis Reports on Global Translational Status of the Cells



- **Polysome profiles** of the ΔMoe1 strain and wild type (yBN96) strain are shown.
- After harvesting and lysing the yeast cells, **30ODs sample aliquots** were layered on **10-50% w/v linear sucrose gradients.**
- The gradients were ultra-centrifuged and pumped with heavy sucrose solution through a **UV Lamp-Gradient Fractionator**

Ribosome Profiling Identifies the Position of Every Translating Ribosome in the Cell

- We hope to further our analysis with ribosome profiling. Ribosome profiling measures the translational efficiency in ΔMOE1 cells
- Analyzing the structure of mRNAs translated at various efficiencies can give insight into how eIF3d regulates translation.

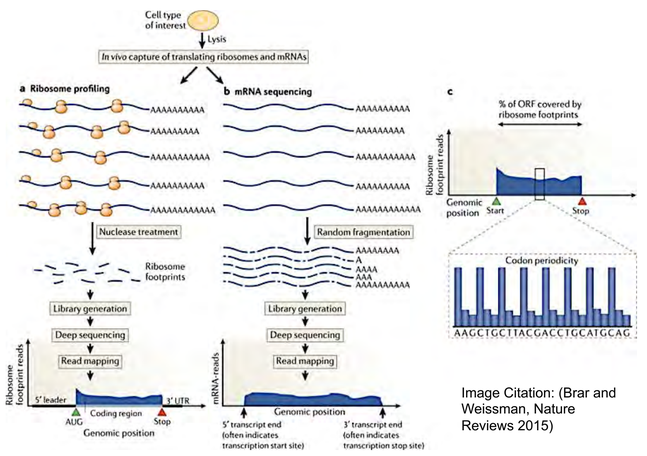


Image Citation: (Brar and Weissman, Nature Reviews 2015)

- We are currenting treating our aliquot samples with nuclease, collecting the monosomes, and performing mRNA extraction to gather the ribosome footprints.

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Acknowledgements

We would like to give a special thank you to our research mentor for the summer, Professor Colin Echeverría Aitken for guiding us through this experiment. We would also like to thank Brian Daly, the URSI director, Susan Painter, the URSI coordinator, and all the donations that continue to make research at Vassar possible.

Audio Code