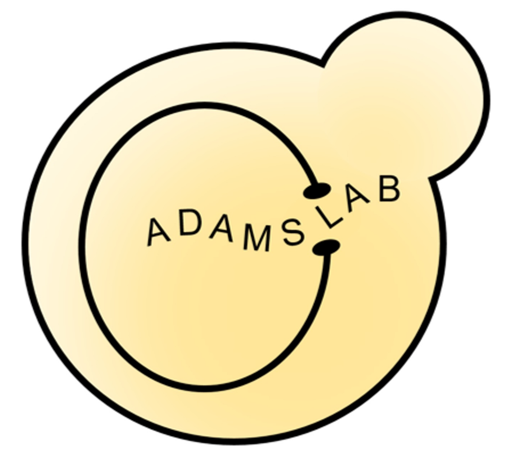
BELMONT UNIVERSITY
College *of* Sciences & Mathematics

Yeast Two-Hybrid to Observe Mex67 Interaction With Nup42 FG Domain Truncations

Authors, Rebecca L. Adams PhD

Biology Department, Belmont University, Nashville TN



Abstract

In eukaryotic cells such as the yeast Saccharomyces cerevisiae, mRNA export from the nucleus to the cytoplasm is essential for gene expression. DNA is transcribed into RNA in the nucleus and relocates to the cytoplasm to be translated into functional protein. Mature transcripts export through nuclear pore complexes (NPCs), which are doorways embedded in the nuclear envelope that permit passage of specific molecules. Selective export is mediated by NPC proteins that contain unstructured regions termed FG domains that both block non-specific transport and allow for the transit of proteins called transport proteins. One of these transport proteins is called Mex67, which binds RNA and ferries it across the NPC via interaction with FG domains. We aim to determine whether specific regions of the NPC protein Nup42 FG domain bind to Mex67 to allow mRNA export. To test this, we have cloned plasmids that contain truncations of the Nup42 FG domain to perform a yeast two-hybrid (Y2H) experiment for binding to Mex67. In this experiment, growth on selective media lacking histidine indicates interaction between Mex67 and the Nup42 truncation. These experiments will determine whether transport proteins have preferred binding sites as they cross the NPC to allow for organization of transport.

Introduction

Saccharomyces cerevisiae, commonly known as yeast, has been an effective model organism in the study of molecular biology. Yeast cells are eukaryotic and perform many of the same functions as human cells, which allows for direct comparison. Their cells center around the mechanisms of transcribing DNA into RNA and translating RNA into functional protein. In the cell, DNA is transcribed in the nucleus, and the resulting mRNA is exported into the cytoplasm to be translated into protein to give the cell function. Protein complexes along the nucleus that act as doorways for mRNA transportation are highly regulated via protein-protein interaction. Several proteins at the NPC are capable of moving macromolecules, but we focus on the Nup42 FG (phenylalanine, glycine) domain that binds Mex67 to facilitate mRNA export from the nucleus to the cytoplasm. **This project aims to identify whether specific regions of the Nup42 FG domain bind with Mex67. We hypothesize that one or multiple regions of the FG domain bind Mex67 selectively to organize transport across the NPC.** To test this, we performed a yeast two hybrid (Y2H) experiment between Nup42 and Mex67, inserting Nup42 FG domain quarters 1-4 into GAD plasmids for this analysis. These plasmids were transformed into yeast with GBD-Mex67 plasmids, and the resulting cells were placed on selective media wherein growth indicates the proteins interacted.

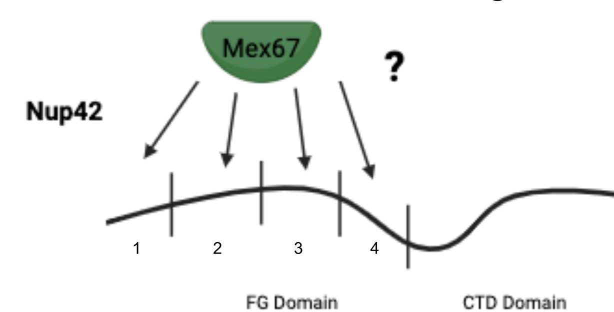


Figure 1. Mex67 binds mRNA and Nup42 to export the RNA out of the nucleus and into the cytoplasm. It travels through NPCs to ribosomes where it will serve as a message for protein. We do not know which regions of Nup42 is binding Mex67 with high affinity.

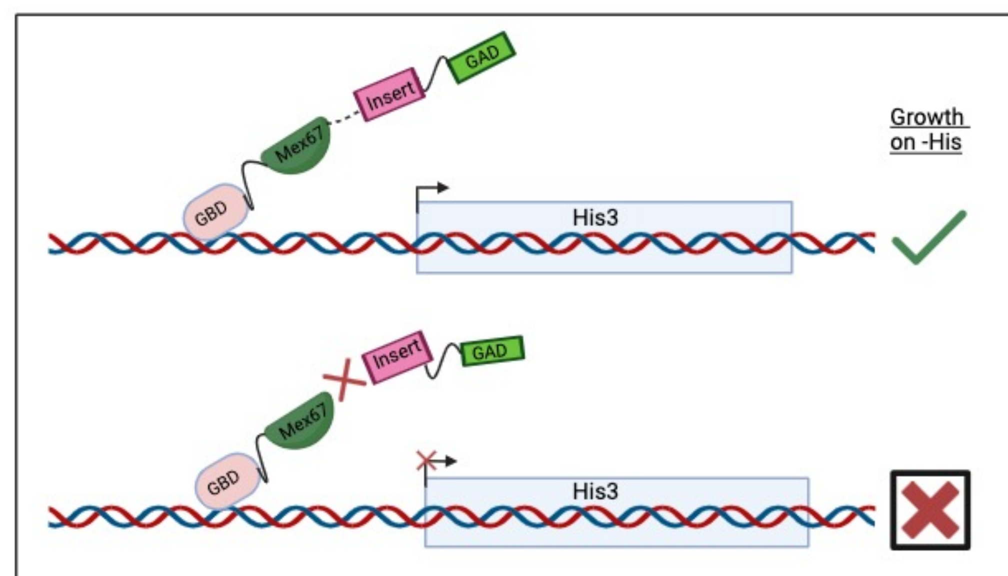


Figure 2. Yeast two-hybrid is a experiment to test if Mex67 binds with Nup42 region. If they interact then growth of yeast on -histidine plates will occur. If the two proteins do not bind, yeast will not grow on selective media.

Materials and Methods

- Miniprep to isolate plasmid
- PCR to amplify gene
- Gibson cloning to generate new plasmid
- Transformation
- Y2H

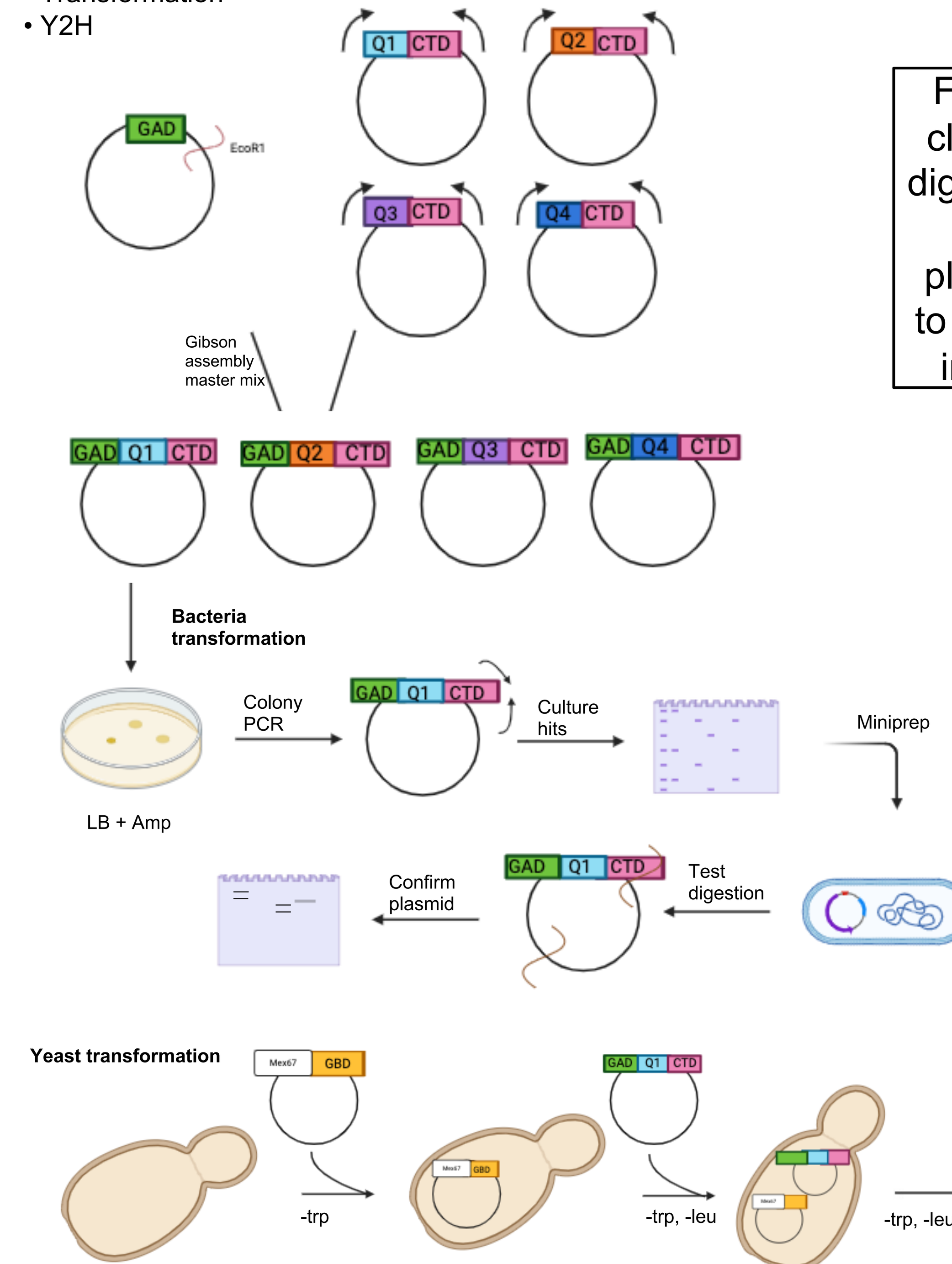
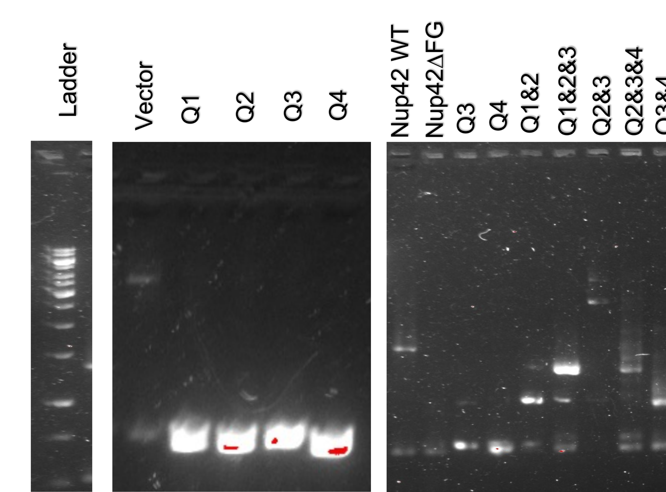


Figure 3. Plasmids were cloned via pBA 14 vector digestion and pBA 234-237 insert digestion. New plasmids were generated to for later transformations into bacteria and yeast.

Figure 4. Bacteria transformation was done to insert new plasmids with GAD and Nup42 insert into bacteria cells. Colony PCR and test digestion confirmed the new plasmids were in bacteria.

Figure 5. Yeast transformation was completed to insert GBD-Mex67 plasmid along with the generated plasmid. Y2H experiment measured if the two proteins interacted.

Results



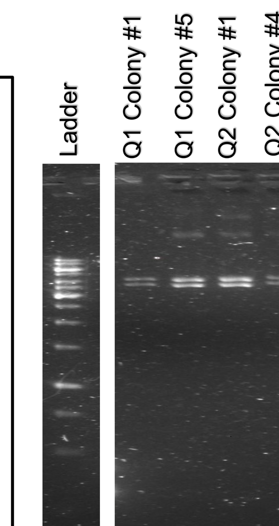


Figure 6. Successful Gibson cloning for different Nup42 truncations. Bands correlate with expected sizes in comparison to ladder.

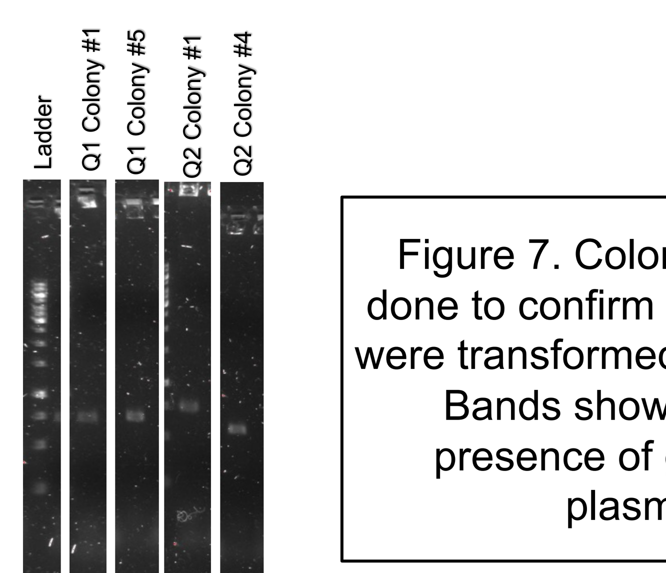
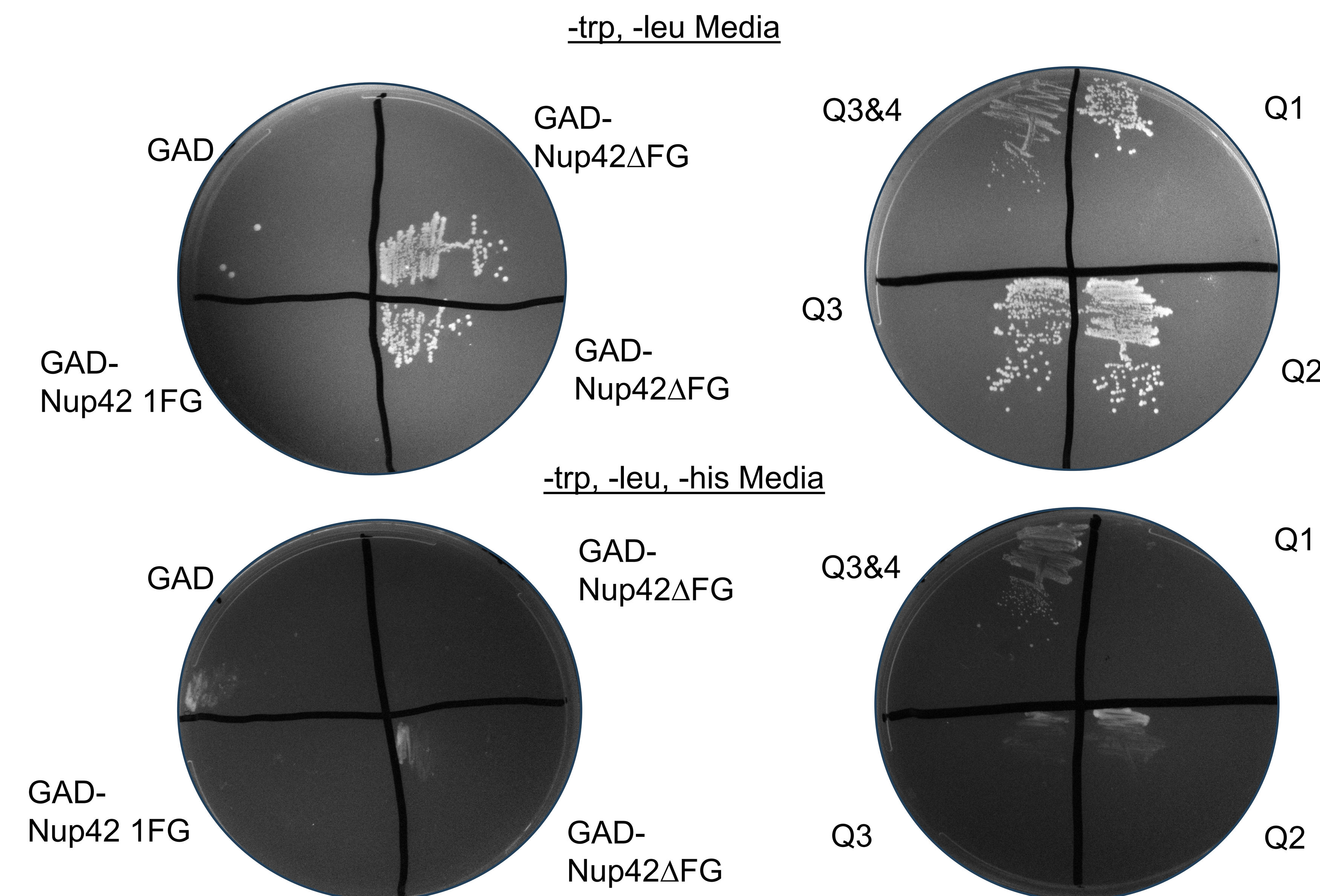


Figure 7. Colony PCR was done to confirm new plasmids were transformed into bacteria. Bands shown confirm presence of generated plasmid.

Ladder | Nup42ΔFG Colony #2 | Nup42ΔFG Colony #3 | Q1&2&3 Colony #3 | Q2&3&4 Colony #5

Figure 8 and 9. Restriction enzyme test digestion was done. Two bands shown indicate plasmids were cut properly and new plasmids were obtained.

Results, continued



Discussion and Future Directions

With the transformation of GBD-Mex67 and GAD-Nup42-CTD plasmids into yeast cells, some of the Nup42 inserts could have interactions with Mex67. It cannot be concluded that Q1 binds with Mex67 to transport mRNA; the control shows minimal growth while no growth is shown on the –his media. Quarters 2 and 3 however show some growth which indicates these sections of Nup42 could have interacted with Mex67. Quarter 3&4 on –his media show some growth, but it cannot be concluded that the 4th quarter is contributing to interaction between proteins. The negative controls with GAD-Nup42 without the FG domain show growth that is unexpected. Therefore, this experiment cannot be concluded with successful data on whether the generated plasmids had interactions with GBD-Mex67 plasmids. Future steps is to test digest other colonies from bacterial cells and transform those into yeast to further observe. Not all generated plasmids could be used for Y2H experiment because of zero colony growth after yeast transformation on -trp, -leu media. The transformations should be redone to get sufficient growth and observe protein interactions via –his media. Also, this would allow for a positive control to be present when comparing the media. Future steps should include repeating the experiment with proper controls to better analyze Nup42 FG domain interaction with Mex67.

Acknowledgements and References

I would like to thank Belmont University's Biology Department for allowing me to conduct research with the proper resources. I would also like to thank Dr. Adams for the exceptional guidance and knowledge she provided throughout the duration of this project.

De Magistris P. The Great Escape: mRNA Export through the Nuclear Pore Complex. Int J Mol Sci. 2021 Oct 29;22(

Folkmann AW, Noble KN, Cole CN, Wente SR. Dbp5, Gle1-IP6 and Nup159: a working model for mRNP export. Nucleus. 2011 Nov-Dec

Adams RL, Mason AC, Glass L, Aditi, Wente SR. Nup42 and IP_6 coordinate Gle1 stimulation of Dbp5/DDX19B for mRNA export in yeast and human cells. Traffic. 2017 Dec;18

Katahira J, Strässer K, Podtelejnikov A, Mann M, Jung JU, Hurt E. The Mex67p-mediated nuclear mRNA export pathway is conserved from yeast to human. EMBO J. 1999 May 4;18

Adams RL, Terry LJ, Wente SR. Nucleoporin FG domains facilitate mRNP remodeling at the cytoplasmic face of the nuclear pore complex. Genetics. 2014 Aug;197