Effects of NOM1 on Ribosome Biogenesis

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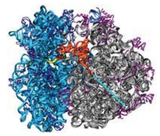
Introduction:

Ribosome Biogenesis

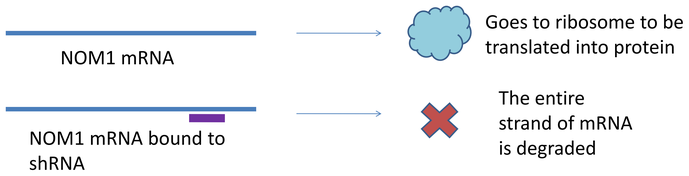


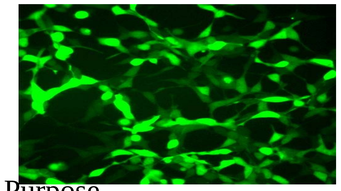
The 40S and 60S ribosomal subunits work together to translate messenger RNA into protein.

- Ribosomes translate genetic information from messenger RNA into proteins, and are therefore necessary for cell growth. Disruption of ribosome biogenesis leads to arrest in cell growth and replication, and has been identified as a precursor to some cancers.
- Eukaryotic ribosomes are composed of 40S and 60S subunits. The 40S subunit includes one segment of ribosomal RNA and approximately 30 proteins. The 60S subunit is composed of three segments of rRNA and approximately 50 proteins. Both are necessary to translate proteins from mRNA.
- NOM1 was identified by the Conklin lab because of its location at a breakpoint on chromosome 7 associated with acute myeloid leukemia. Functional studies of NOM1 have demonstrated that it:
  - Is required for cell growth and cell replication.
  - Localizes to the nucleolus.
  - Interacts with and targets several proteins to the nucleolus including Protein Phosphatase I, the oncogene MSP58 and the RNA helicase eIF4AIII.
  - Is required for production of 40S ribosomes.

Knockdown Studies

We have investigated the function of NOM1 by decreasing its expression in cultured cells using a procedure known as RNA interference (RNAi). In RNAi, a short RNA is synthesized that binds an mRNA of interest, targeting it for degradation.
In our experiments, the short RNAs were synthesized using a virus (GIPZ) that also encodes green fluorescent protein (GFP). GFP expression identifies cells infected by the virus. In the experiments below, GIPZA encodes a short RNA that binds to and targets NOM1 mRNA for degradation and GIPZN is a non-targeting control.





GIPZ viruses encode a fluorescent protein, so successfully infected cells fluoresce green.

Purpose

My project involved cloning a mutation into NOM1 that makes the NOM1 mRNA resistant to the GIPZA encoded short RNA. Wild type and mutant versions of this GIPZA resistant construct will be introduced into GIPZA expressing cells to determine which can rescue cell growth. One mutant we will test is NOM1 m307, which is altered so that it cannot bind Protein Phosphatase I. If a mutant does not rescue cell growth, we can conclude that it lacks an essential binding site.

The A Resistant Mutation

The A Resistant mutation enables expression of NOM1 in the presence of GIPZA virus. The modified mRNA does not bind the short RNA that binds wild-type NOM1.

Wild type NOM1
Short RNA targeting NOM1 binds efficiently. The mRNA is degraded.

NOM1 A Resistant Mutation
Short RNA targeting NOM1 does not bind because it does not match in the critical binding region. The mRNA is translated into protein.

The A Resistant mutation inhibits the short RNA binding without altering the NOM1 protein by utilizing the redundancy in the genetic code. Each set of three nucleotides codes for one amino acid, but one amino acid is indicated by multiple codes. The third base in a set of three is known as the wobble base, because it can often be altered and still indicate the same amino acid. This is why the A mutation changes every third nucleotide for six amino acid codes.

AAA	TTC	TGT	GAA	TAT	GAA
AAG	TTT	TGC	GAG	TAC	GAG
Lys	Phe	Cys	Glu	Tyr	Glu

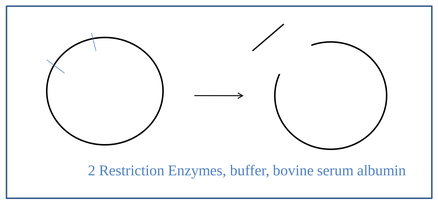
Wild Type NOM1

NOM1 A Resistant Mutation

Different sequences code for the same amino acids

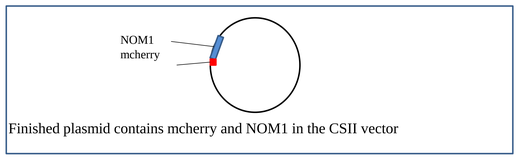
Method:

• I started with two mutations of NOM1 in TOPO vector: NOM1 1-860 A Resistant and NOM1 1-860 m307 A Resistant. Digesting with the proper restriction enzymes cut the NOM1 DNA out of the plasmid.



I used gel extraction to separate the vector from the plasmid in both cases.
These pieces could then be ligated together.
I repeated the process using a CSII vector that contains a gene for a red fluorescent tag called mCherry.

The red fluorescent tag is useful for identifying cells expressing the vector, similar to GFP in the GIPZ viruses.

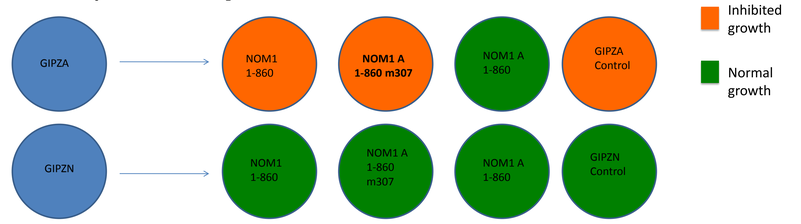


Rescue Experiment Plan

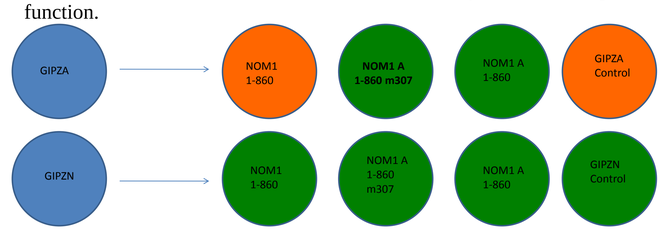
- Introduce GIPZA and GIPZN viruses
- Verify NOM1 knock-down by measuring the amount of NOM1 mRNA using quantitative PCR
- After 9-10 days, we expect the cells with reduced NOM1 to stop growing. At this point, the different variants of NOM1 will be added.
- Observe growth by comparing the concentration of cells under different treatments. This can be done quantitatively using crystal violet assay.

Theoretical Results

If the GIPZA cells rescued with NOM1 A Resistant mutation resume growth and those rescued with NOM1 A Resistant m307 mutation do not, we conclude that the interaction with PP1 is necessary for NOM1 to perform its essential function.



If the GIPZA cells rescued with NOM1 A Resistant m307 also resume growth, then we conclude that the interaction with PP1 is not necessary for NOM1 to perform its essential function.



This experiment will also reveal the effects of over-expression of NOM1.

Future Directions

We plan to conduct the rescue experiment described.
Additional work will use similar experiments to test different mutations of NOM1, revealing which segments and binding regions participate in its essential function.

Acknowledgements

I am very thankful for the guidance and support from Dr. Kathleen Conklin in conducting this project. I also appreciate the technical help and theoretical lessons from Samantha Van Hove.