Functions of NOM1 Required for Cell Growth and Proliferation

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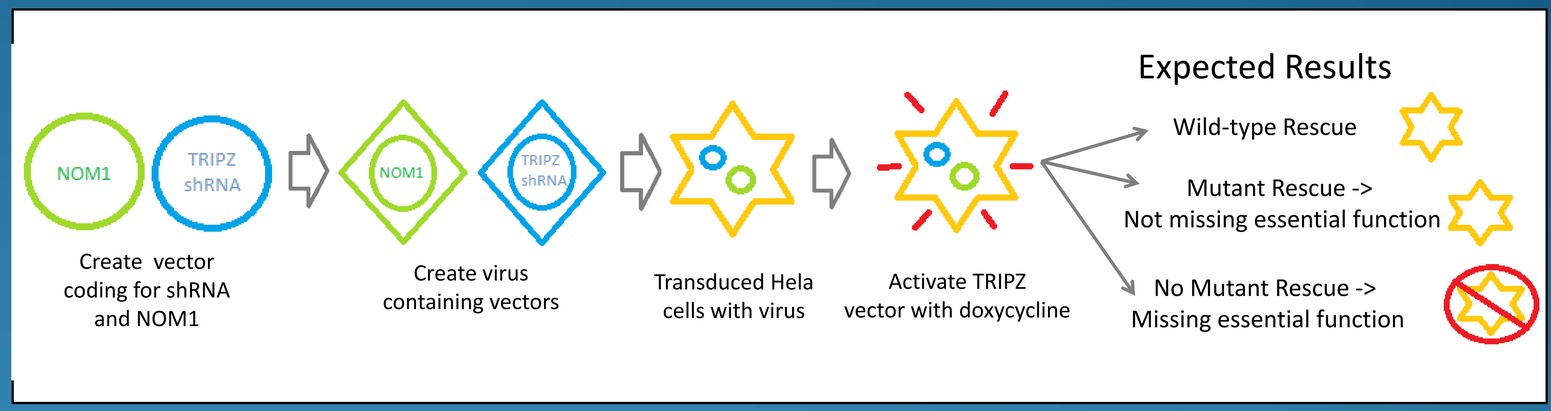
Introduction

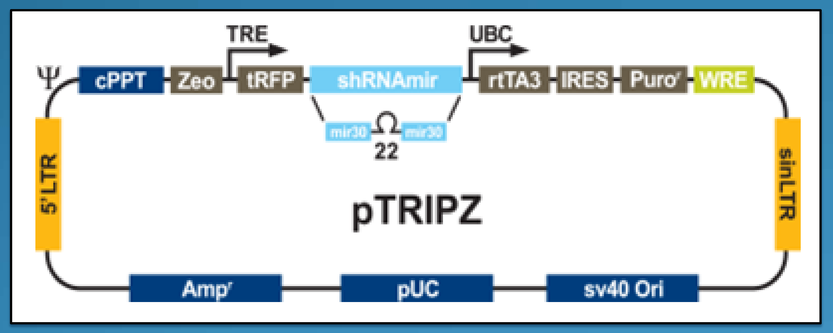
NOM1 is a highly conserved protein that is required for ribosome biogenesis. Decreased levels of NOM1 result in cells that are smaller and that fail to proliferate. I am investigating functions of NOM1 required for cell growth and proliferation. My approach is to decrease levels of NOM1 and then determine whether I can rescue these cells with either wild-type or mutant versions of NOM1. The functions I am testing include:

1. NOM1's ability to localize to and to target proteins to the nucleolus, the sight of ribosome biogenesis. Expression construct NOM1 247-860 lacks this function.

2. NOM1's ability to bind Protein Phosphatase I (PPI), a protein required for cell signaling, cell division, and metabolism. Expression construct NOM1 1-860 m307 lacks this function.

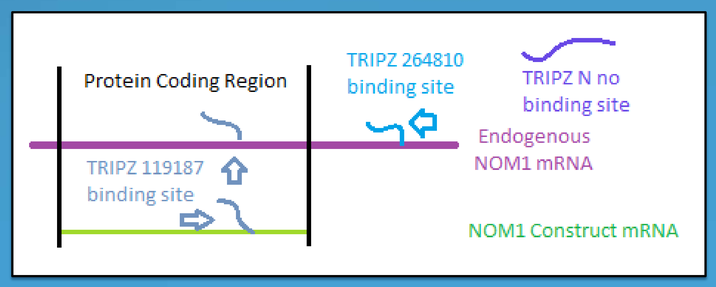
Methods





TRIPZ and shRNAs

TRIPZ (Open Biosystems) is a tet-inducible shRNA vector. By adding doxycycline to cells containing the vector, an shRNA is expressed. The shRNA binds to a specific mRNA targeting it for degradation thereby decreasing expression of the corresponding protein. The shRNAs used in this experiment are: (1) TRIPZ 119187 that targets both endogenous and vector-encoded NOM1, (2) TRIPZ 264810 that targets only endogenous NOM1 mRNA but not the mRNA encoded by our NOM1 constructs, and (3) TRIPZ N that encodes a control, non-targeting shRNA.

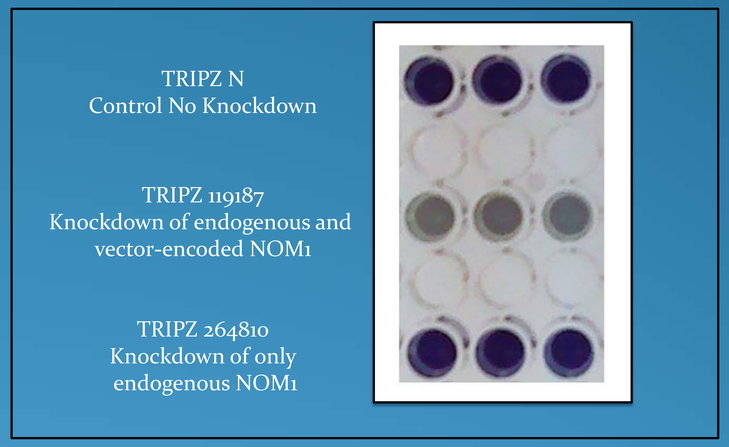


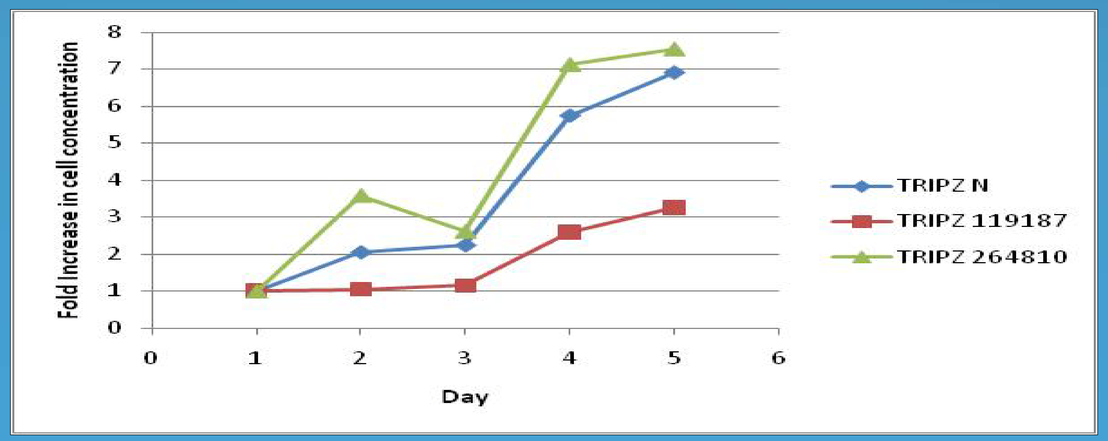
TRIPZ 119187, TRIPZ 264810 shRNA binding sites.

Results

The picture to the right shows results from an MTT assay. In this assay, MTT is converted to a blue color when incubated in the presence of living cells; the depth of color is proportional to the cell number. Data collected over 5 days is shown in the graph below.

- As expected, cells expressing NOM1 and the non-targeting control virus grew well during the course of the experiment.
- Also as expected, cells expressing NOM1 and the TRIPZ 119187 shRNA grew very poorly due to the loss of both the endogenous and vector-encoded NOM1.
- However, cells expressing wild-type NOM1 and TRIPZ 264810 grew as well as the controls, suggesting that, although this shRNA reduces levels of endogenous NOM1, cell growth can be rescued by the vector-encoded protein.





Growth of cells expressing wild-type NOM1 and TRIPZ virus.

What's Next...

There is still work to do. The experiment will be continued by repeating the MTT assay with the other NOM1 constructs (NOM1 247-860 and NOM1 1-860 m307). Progression through the cell cycle and cell size will also be assessed by flow cytometry. Further, controls will be added to ensure the shRNAs are efficiently reducing the expression of NOM1 and causing cell growth and proliferation defects. Finally, the entire experiment will be repeated in normal cells.