Using Yeast to Study the Function of SUMO Modification in Pol III Transcription

Catherine Wu, Zheng Wang, Tony Hunter

The Salk Institute for Biological Studies, Molecular and Cellular Biology Laboratory

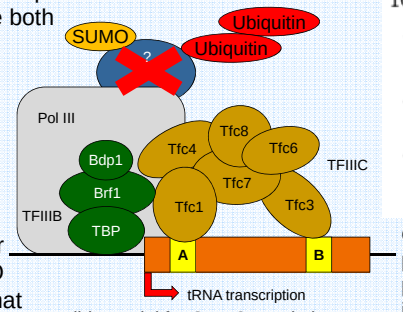
San Diego, California

Introduction

The Life Sciences Summer Institute (LSSI) connects high school students to San Diego's Life Sciences Industry since 2005. Students complete a one-week pre-internship "boot camp" training followed by 7-weeks of paid research work experience.

This summer, I not only got the chance to research in an actual laboratory, I also was able to meet new students like me, interested in science. The boot camp and subsequent lab experience were both extremely educational.

The goal of my research this summer was to identify how SUMO, small ubiquitin-like modifier, regulates the RNA Polymerase III (Pol III) transcription pathway in yeast, which is responsible primarily for the transcription of tRNA. SUMO is a post-translational modifier that can alter the structure and function of proteins through a process known as SUMOylation.

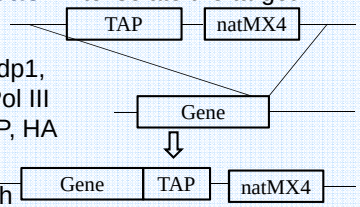


Possible model for SUMO regulation of Pol III – an unidentified subunit of Pol III complex is SUMOylated and degraded, inhibiting transcription.

Methods

Two primary approaches:

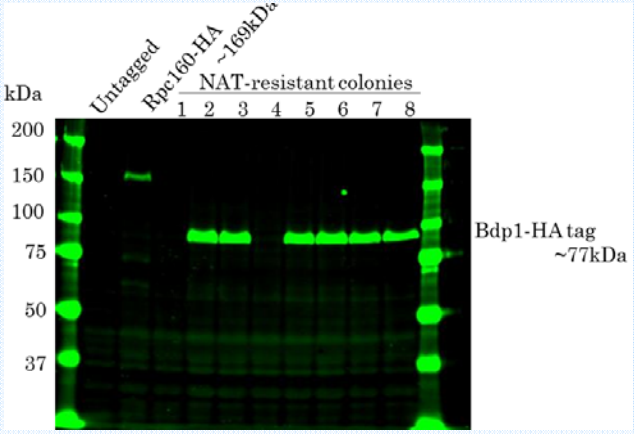
- **Biochemical approach** - to isolate the target SUMOylated protein
  - Tag Rpc160, Brf1, Bdp1, proteins involved in Pol III transcription, with TAP, HA
  - Homologous recombination through yeast transformation



- **Genomic approach** – High-copy suppressor screen
  - Rpc128 (subunit of Pol III) sick mutant strain
  - 96-well plate yeast library transformation
    - Covers 98.5% of the genome
  - Overexpression of certain genes is meant to counter negative regulators

Results

Part 1 (Biochemical)



Compared to the untagged control and colonies 1 and 4, after performing a Western blot with anti-HA antibodies, there was a prominent band around 77 kDa for the remaining 6 colonies that indicates the tagging was successful.

Part 2 (Genomic)

Cells were transformed with yeast library plasmids from nineteen 96-well plates, and pinned onto a selective media plate in pairs. The transformation on this plate were mostly successful.

Colonies were re-pinned on 5FOA, another selective plate. Cells that could grow on this plate had taken in a gene that could suppress the rpc128 mutation. For each positive hit, I recorded the genes contained on that plasmid.

- 31 hits out of 1750 total plasmids
- Positive hits include *RPC128, ULP2* (cleaves SUMO from protein)
- Identified many genes encoding ribosome proteins and rRNA processors

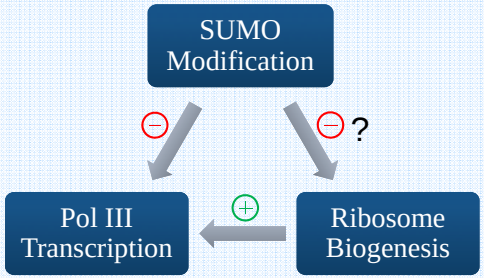
Conclusion

Part 1

- Confirmed and saved strains with each protein tagged
  - Brf1-TAP, Rpc160-TAP, Bdp1-HA
- Next steps: Immuno-precipitation with the tag to identify any SUMOylated species associated with the three proteins
- Mass spectrometry of the SUMOylated protein to determine its identity

Part 2

- Identified two expected genes *(RPC128, ULP2)*
- Screen suggests regulatory effect of ribosome biogenesis on Pol III transcription
  - Requires further confirmation of suppression for plasmid and gene
- Continue to investigate the link between SUMO, Pol III and ribosome biogenesis



SUMO was found to negatively regulate Pol III transcription, and the identification of ribosomal processors from the screen indicate that ribosome biogenesis might play an additional role in aiding Pol III transcription. This also suggests a link between SUMO and ribosome biogenesis.

Acknowledgements

I would like to thank Dr. Zheng Wang, Dr. Tony Hunter, and the Salk Institute for guiding me and allowing me to work in their laboratory. I would also like to thank LSSI and BIOCOM, as well as Dona Mapston, for giving me this opportunity. Finally, thank you to the other interns for making this summer such a wonderful experience!