

Probing the subunit composition of the Cul3-based E3 ligase

Gabija Laskonyte
Dr. Jeffrey Singer's lab



Objectives

- Understand the function of Cul3-based E3 ligase
 - Architecture/subunits
 - Is Muf1 a subunit?

Ubiquitination

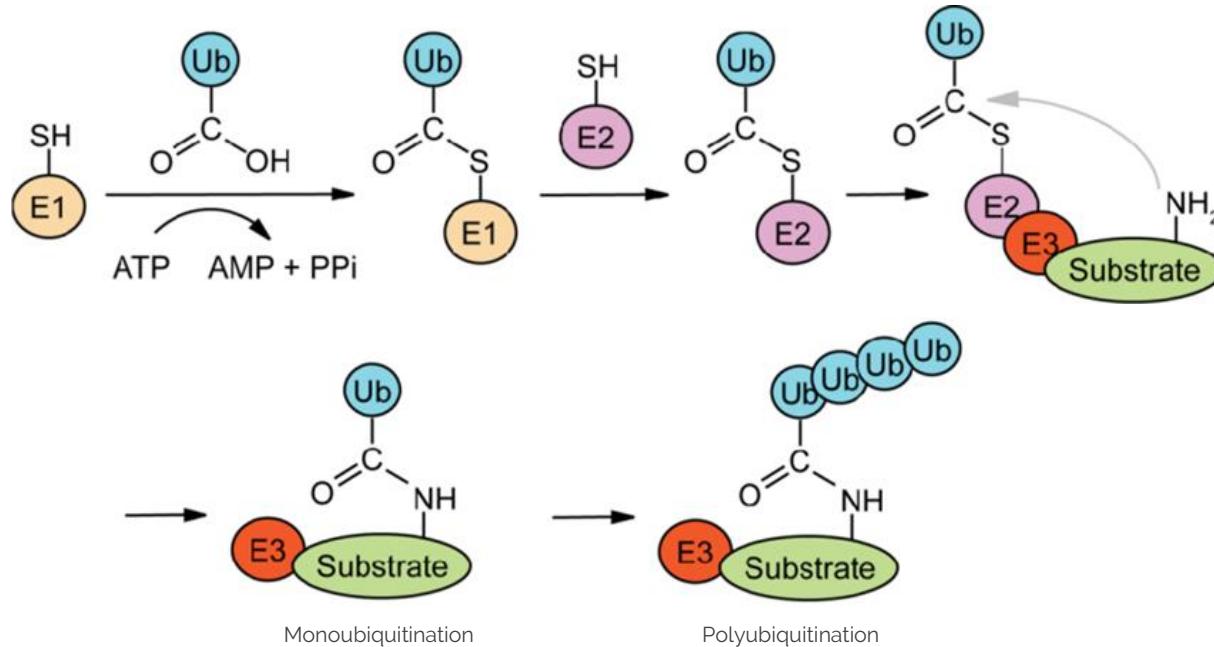


Figure 1: Ubiquitination is the process by which the protein ubiquitin is attached to a substrate via E1, E2, and E3 ligase. Ubiquitin is used as a tag to identify a protein for degradation. Degradation of proteins is essential to regulating cellular function; the presence or absence of proteins allows for the regulation of the cell cycle. Ubiquitination is regulated by the binding of molecules to the subunits of the E3 ligase. The E3 ligase mediates ubiquitination.

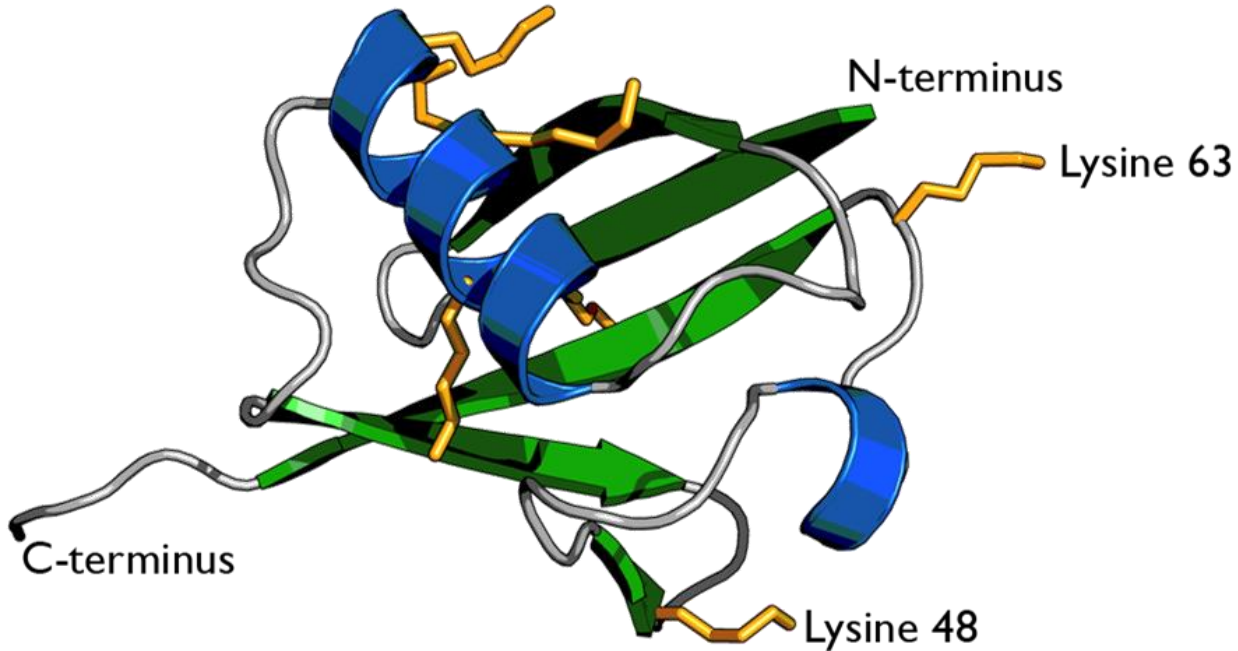


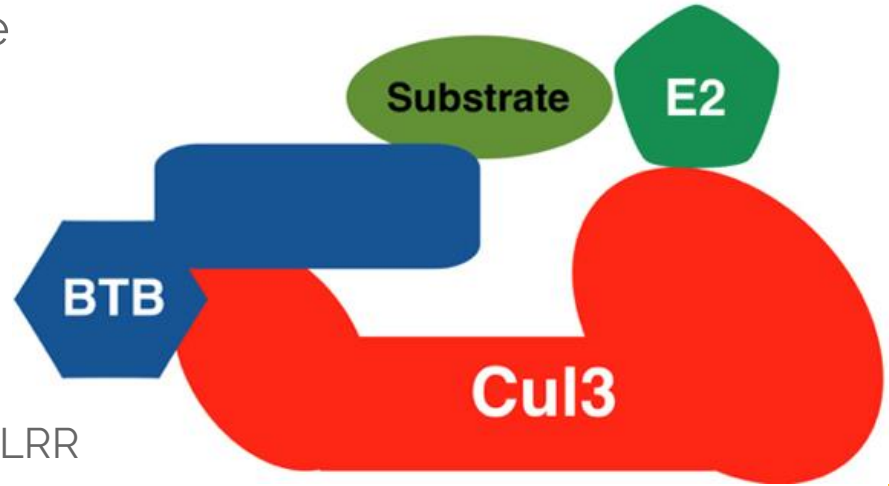
Figure 2: Shown is a diagram of the locations of the binding sites on ubiquitin, two of which are lysine amino acid binding sites. Ubiquitin is attached to target proteins using its lysine side chains. Different signals can be expressed depending on which lysine binding site is used and the amount of ubiquitin that is attached. The types of signals result in distinct changes in functionality, including a change in ability to interact with other proteins, folding, and more.

E3 ligases

- One of the functions of ubiquitination is degradation.
- Concentrations of proteins are regulated in the cell via synthesis, modification, and degradation. Enzymes mediate these processes.
- All ligases bind large molecules together.
- Swaps out its subunits to make complexes that are specific to each substrate.
 - Many types of ligases

Cullin-3 (Cul3)

- Acts as a scaffolding to create Cul3-based E3 ligase
- Dynamic function
- Subunits attach or detach depending on the substrate
 - Subunits include BTB, E2, and LRR
- Muf1 and Cul3 are known to bind to each other



Why Muf1?

- Currently, there is a limited understanding of its function.
- LRRs are under investigation as subunits of E3 ligase.
- We aim to determine whether Muf1 is a subunit of Cul3-based E3 ligase.
 - We know Muf1 can bind to Cul3
 - What else does Muf1 bind to? Understanding binding partners will help with understanding Muf1's function.

Our contribution

- **Aim:** Determine what proteins interact with Muf1.
- **Method:** Patterns in function of binding partners. May provide insight into the purpose of Muf1.
- **Expected results:** A large amount of protein-protein interactions that involve Muf1.

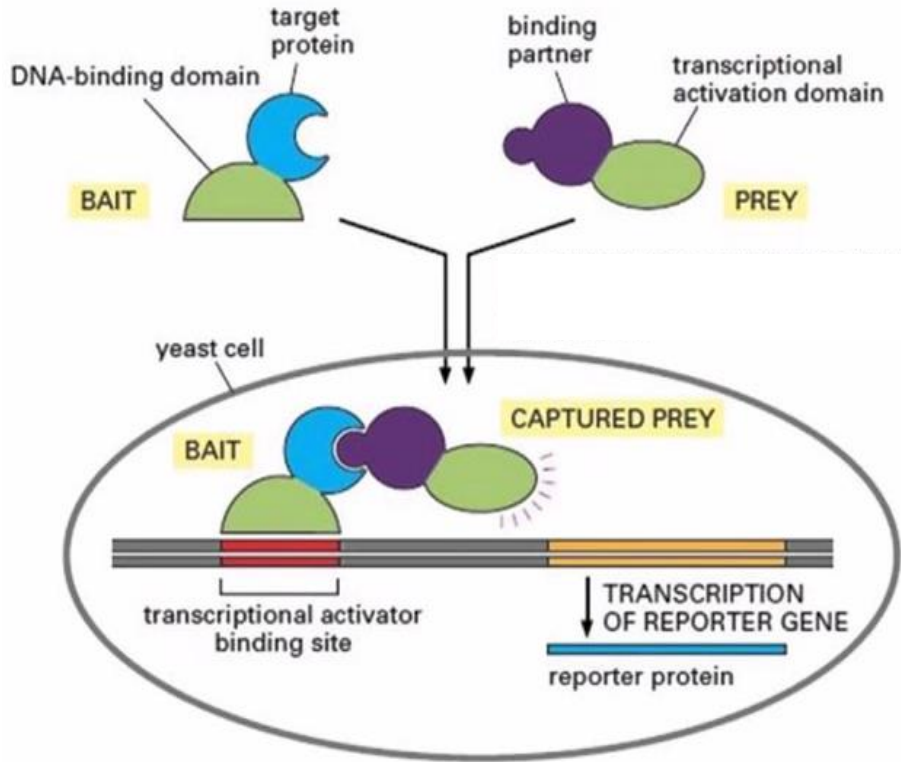


Figure 5: Simplified diagram of two hybrid screen. The two hybrid screen is used to determine the interactions between two proteins. Our target protein was Muf1.

- Bait protein attracts prey protein to form the transcription factor
- Once formed, it binds to the upstream activating sequence (UAS)
- UAS activates reporter gene
- Reporter gene codes for reporter protein
- Phenotype of cell changes

Reporter genes

- *AUR1-C* (resistant to Aureobasidin A)
- *HIS3* (grow on -Histamine media)
- *ADE2* (grow on -Adenine media)
- *MEL1* (blue colonies in X- α -Gal)

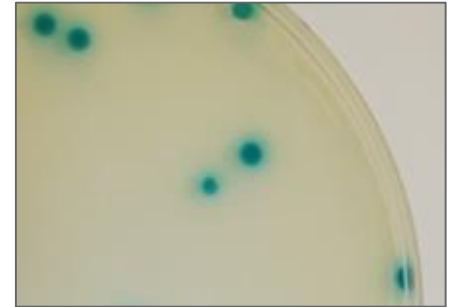
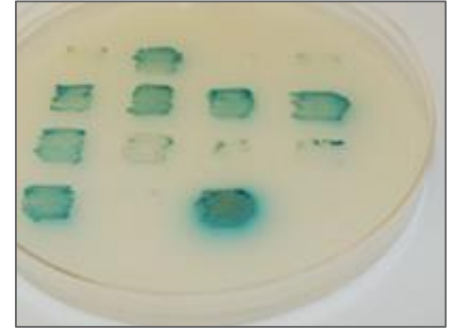


Figure 6: Yeast colonies expressing the MEL1 reporter gene, which shows the cells are positive for α -galactosidase activity.

Future research

- Relevance of Muf1 to Cul3-based E3 ligase function.
- Determine all possible subunits and their function to understand catalyzation at molecular level
 - Achieved with RNA polymerase

Acknowledgements

- **National Science Foundation** for funding, guidance, structure, and coordination through this REU program.
- **Dr. Jeffrey Singer** for his mentorship and technical proficiency.
- **Siri Vegulla, Dr. Chen, and Dr. Jiao** for mentorship and coordination.
- **Portland State University and the Collaborative Life Sciences building** for space and resources.



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