The role of SUMOylation in Cdc48 mediated stress granule clearance

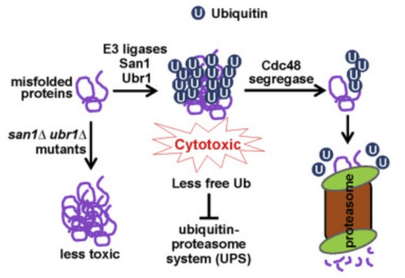
Karina Frey, Austin Folger, and Dr. Yanchang Wang
Department of Biomedical Sciences

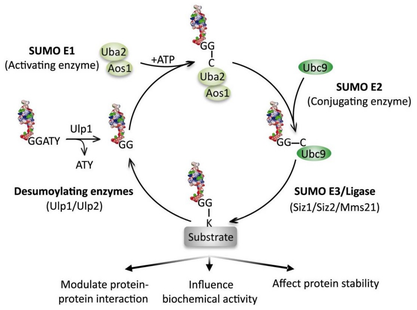
FLORIDA STATE UNIVERSITY
COLLEGE OF MEDICINE

Introduction

SUMO (small ubiquitin-like modifier) is a protein that can be added to substrate proteins in a process called SUMOylation, which is a cellular post translational modification. SUMOylation functions in protein translocation, DNA replication, stress response, and protein degradation. Stress granules (SGs) are condensates of mRNA and misfolded proteins that form during stress. This promotes cell survival by sequestering inactive mRNAs and toxic misfolded proteins from the cellular environment until the stress is resolved. Stress granules are cleared by various mechanisms, but we focus on Cdc48 complex mediated SG clearance. The Cdc48 complex is an ATPase that serves as a segregase/unfoldase that can unfold misfolded proteins before targeting them to the proteosome. The Cdc48 complex consists of three subunits: the Cdc48 core particle, Ufd1, and Npl4. While Npl4 contains a ubiquitin interacting motif, Ufd1 contains a SUMO interacting motif (SIM). This allows for the recognition of SUMOylated substrates. The protein components of SGs can be SUMOylated and ubiquitinated, which aids their clearance. However, the role of SUMOylation in Cdc48 mediated clearance is unknown.

Overview of Cdc48 complex and SUMOylation





Methods/Results

This research lab evaluates the relationship between SUMOylation, ubiquitination, and cytotoxic stress response in an *S. cerevisiae* yeast model after being exposed to stress, such as treatment with heat shock. The experiment took course over three hours total to observe stress granule formation and respective clearance.

- Inoculated cell samples in 10mL of YPD overnight
- At each time point we took 1mL of sample for imaging or western blotting
- Heat shock cultures for 15 minutes after taking time 0 by placing into 42ºC water bath
- Take T15 and place in room temperature water bath of 25ºC (30ºC for temperature sensitive mutants) and take time points every 30 minutes for two hours
- Collected data through cell imaging and western blotting to visualize the proteins

Figure 1

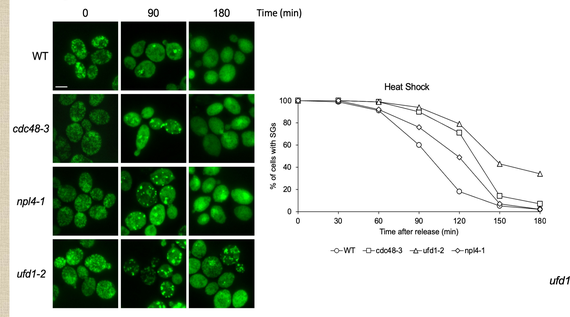


Figure 1: Stress granule formation and clearance in Cdc48 and cofactor mutants

Figure 2

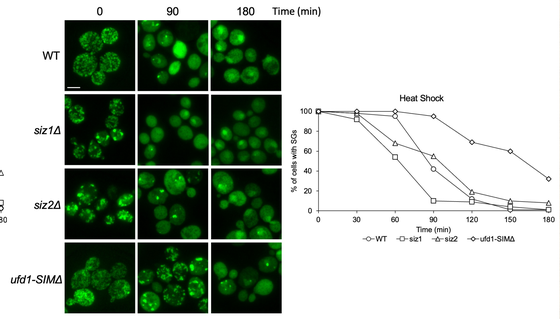


Figure 2: Stress granule formation and clearance in other SUMO proteins and Ufd1 SUMO interacting motif (SIM)mutant

Figure 3

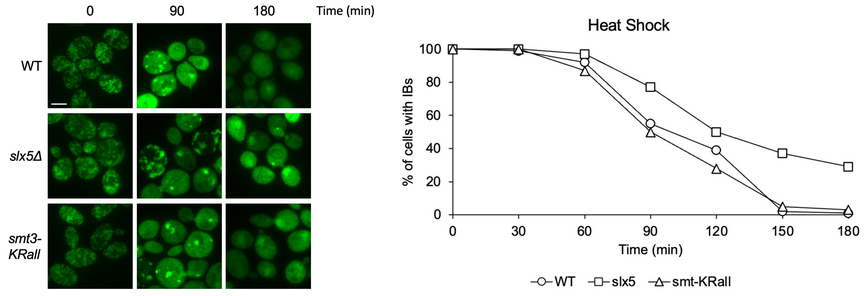


Figure 3: Stress granule formation and clearance in slx5 STUbL (SUMO Targeting Ubiquitin Ligase) deletion mutant and monoSUMOylation only mutant, smt3-KRall

Figure 4

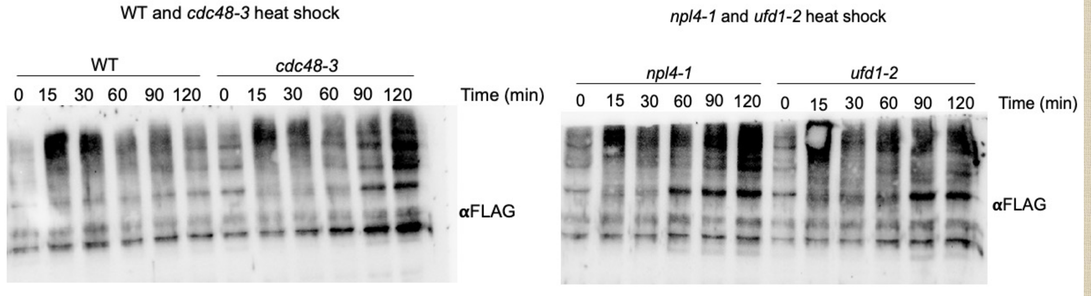


Figure 4: Global SUMOylation during stress granule formation and clearance in WT and Cdc48 complex temperature sensitive mutants

Discussion

We observed how Cdc48-mediated clearance was affected in various SUMOylation and ubiquitination mutants. We found that SG clearance was negatively affected in mutants where SUMOylation and ubiquitination were hindered. In addition, we found that SG clearance was negatively impacted in Cdc48 complex mutants.

This research furthers our comprehension of the mechanical machinery that occurs with stress granule clearance with the aid of SUMOylation. We intend to repeat heat shock experiments to confirm the results and proceed with a different type of stress treatment to the cells (arsenite) to further our insight on how SUMO proteins interact with the Cdc48 complex to perform stress granule clearance from the cell.

Acknowledgements

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References

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