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The Role of Med13 in Proteaphagy

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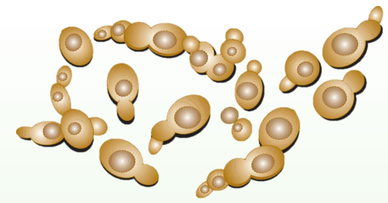


Abstract

Regulation of proteasomes is important for adaptation to cellular stress. Previous studies have shown that following starvation stress, proteasomes are targeted for destruction by autophagy. However, how cells control proteasomes in response to nitrogen starvation remains unclear. This study delves into the intricate interplay between Med13, proteaphagy, and stress response regulation, aiming to elucidate their roles in cellular survival mechanisms. It focused on the highly conserved Cdk8 kinase module (CKM) of the Mediator complex a that plays a pivotal involvement in cellular signaling and gene regulation under stress conditions. During the investigation, we asked if the degradation of specific proteasome subunits was dependent upon Med13, a member of the CKM. The results suggest a novel role of Med13 in the degradation of the Rpn8 proteasome subunit, shedding light on its specific impact on proteasome dynamics during stress. Moving forward, the research endeavors to validate and refine these initial findings through further experimentation and analysis. Replication of experiments and additional time courses, particularly focusing on the Rpn11 subunit, are planned to bolster the observed effects of Med13 on specific proteasome subunits. Furthermore, the exploration of Med13's involvement in proteaphagy under different stress conditions, such as carbon starvation, promises to broaden our understanding of stress-induced cellular responses. In conclusion, this research underscores the critical role of Med13 in cellular survival mechanisms during nitrogen starvation and its potential implication in proteasome degradation. These findings contribute significantly to the comprehension of stress response regulation and hold promise for future investigations into proteaphagy and related cellular processes.

Background & Significance

- Following adverse environmental cues, cells reprogram transcription, translation, and protein degradation pathways. This reprogramming is essential for maintaining homeostasis and cell survival. Dysregulation of these pathways is linked with many diseases, including cancer and neuropathies. It remains unclear how these pathways coordinate their responses following stress.
- The highly conserved CDK8 kinase module (CKM) of the Mediator complex comprises cyclin C, its cognate kinase Cdk8, Med13, and Med12. In budding yeast, the CKM acts predominantly as a repressor of stress response genes (SRGs), including autophagy (*ATG*) genes.
- Following nitrogen starvation, the CKM dissolves, relieving the repression of *ATG* genes. Importantly, Med13 has a second job in the cytoplasm, promoting the autophagic degradation of RNA-processing body (P-body) assembly factors following starvation stress. This role is important as Med13, but not other CKM members, is required for survival following starvation.
- In this SMRF project, we tested if Med13 is required for the autophagic degradation of proteasomes (coined proteaphagy) following starvation stress. 26S proteasomes are conserved multi-subunit complexes that degrade ubiquitinated targets during many processes. We tested different subunits of the 19S and 20S complexes.
- The reason for so doing was that the autophagic degradation of P-bodies and proteasomes use many similar proteins, but molecular details on how proteaphagy is regulated are unclear. **These studies are important as understanding the interplay between Med13, proteaphagy, and stress response regulation is vital for uncovering potential therapeutic targets to manage cellular stress and future cancer therapies.**

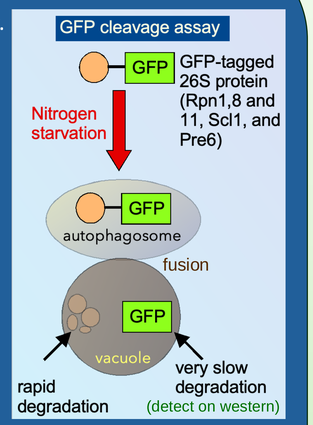
Methods

GROWTH
- Grew cells with GFP-tagged proteasomal proteins to mid-log.

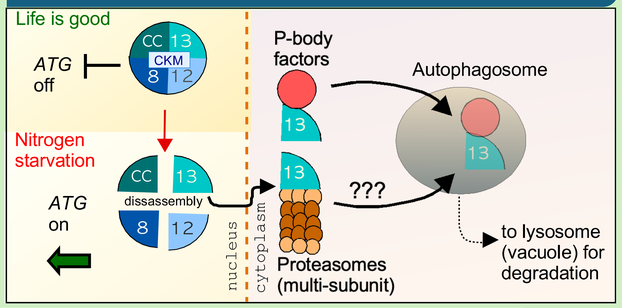
STARVATION
- Placed cells in media lacking nitrogen (induces autophagy)
- time points at 0, 6, and 24 hours

ANALYZE
- Western Blot Analysis of GFP tagged proteins. Pgk1 was used as a loading control.
- iBright Analysis Software to quantify the cleavage of the protein

REPEAT
- Ran the experiment again to confirm the data
- Reran in Carbon starvation to analyze any difference

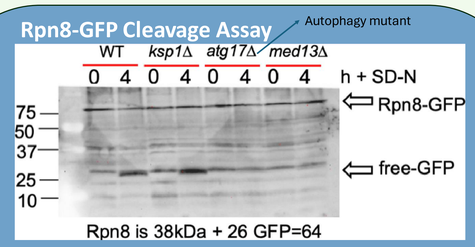


Model being tested



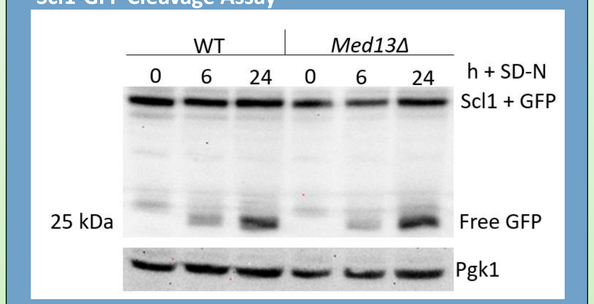
Results

Rpn8-GFP Cleavage Assay

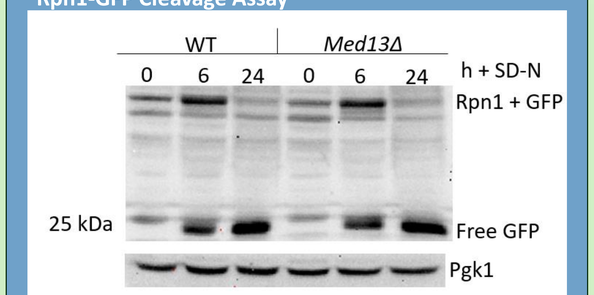


Rpn8 is 38kDa + 26 GFP=64

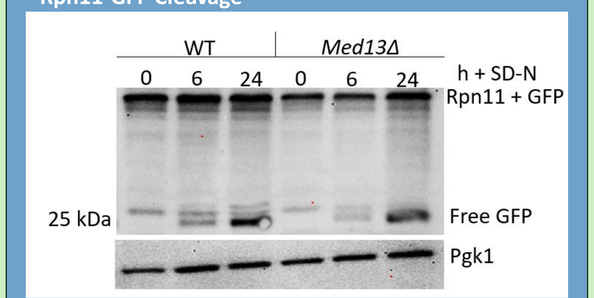
Scl1-GFP Cleavage Assay



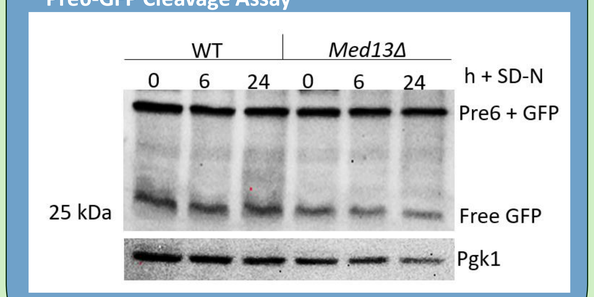
Rpn1-GFP Cleavage Assay



Rpn11-GFP Cleavage



Pre6-GFP Cleavage Assay



Western blot analysis of the GFP tagged proteins shown following nitrogen starvation.

Discussion & Conclusion

- Med13 may not significantly influence the degradation of Scl1, Rpn1, Rpn11, and Pre6 proteasome subunits during stress, indicating a potential specificity for the Rpn8 subunit.
- Initial experiments showed no difference in Med13 mutants, but repeated time courses, particularly concerning Rpn11, provided a more comprehensive assessment.
- Additional investigations under nitrogen and carbon starvation conditions did not support the hypothesis of Med13's regulation of proteasomes through specific subunits.
- Our research enhances understanding of cellular stress responses, underscores the CKM's role in stress response regulation, and suggests a potential involvement of Med13 in proteaphagy.
- These findings provide new avenues for exploring the dynamic interplay between stress response pathways and proteasome regulation.

Future Directions

- Confirm results with biological replicates.
- Confirm Rpn8 cleavage assay
- Test whether proteaphagy requires Med13 in carbon starvation

References