Quantifying Cell Cycle-Dependent Changes In Protein Interacting Network of the Yeast 26S Proteasome

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Summary

The Ut-proteasome system (UPS) mediates cell cycle progression by targeting and degrading cyclins and inhibitory proteins, and functions integrally at several cell cycle checkpoints. Quantitative analysis of tandem-affinity purified in vivo cross-linked proteins (QTAGX) is a powerful approach to identifying both stable and transient interactions of large protein complexes. Past studies have utilized this strategy to map the 26S proteasome interaction network in asynchronous cells1,2 however comprehensive analysis has yet to be applied to identify interaction network changes in response to cell cycle arrest. Using dual QTAGX approaches (Fig 1), we have been able to characterize clusters of proteasome interacting proteins (PIPs) which interact with the proteasome based on SILAC ratio profiles. Furthermore, we have identified amongst the proteins in each cluster functions and complexes which are statistically significantly enriched and we have subsequently mapped the cluster-specific proteasome interaction network. This will help us understand the underlying mechanisms of cell-cycle regulated ubiquitin/proteasome-dependent degradation pathway.

Experimental

Validation

DLEIQLSNIASSSSKDLEIQLSNIASSSSK

GLLLYGPPGTGKGLLYGPPGTGK

Results

Fig 2-Identification and Validation of the selected putative cell cycle specific PIPs. A-C: Representative MS/MS spectra of highly enriched PIP identified from approach #1 (YBR402C, Sec16) (A) of cell cycle-dependent PIPs identified from approach #2: YLR435C, Sec12 (B) and YBL040W (C). D-E: Western blot of native affinity purified samples using reciprocal Co-IP from cells treated or untreated with α-factor (D); and from synchronized cells (G1, S and M phase) (E). 

Cluster Analysis

Not detectable in “light” phase Heavy phase enriched Putative PIP Light phase enriched Not detectable in “heavy” phase

Fig 3-PPI Clusters by SILAC Ratio Profiles and Cluster-based Interaction Maps. A-B: Clusters of PIPs (grouped by similarity in SILAC ratio profiles) for Approach #1 (A) and Approach #2 (B). NOTE: for the assignment of the Cofactor requirement, values were assigned Cof. Cluster-based Protein interaction maps with annotated common and complex. Blue = annotated as a given complex. Blue Node Border = annotated for a given function. Pink = annotated as Protein Interaction Map for cluster from Approach #1 enriched for Protein Binding Function (hit-rate of 37.4% and p-value of 7.9x10^-10) and Transcription Factors (hit-rate of 37.4% and p-value of 7.9x10^-10). Cluster-based Protein Interaction Map for cluster from Approach #2 enriched for Protein Folding Fate Function (hit-rate of 54.4% and p-value of 2.6x10^-13) and Translation Components (hit-rate of 54.4% and p-value of 2.6x10^-13). Cluster-based Protein Interaction Map for cluster from Approach #2 enriched for Protein Binding Function or Cofactor Requirement (hit-rate of 45.0% and p-value of 6.8x10^-6), no enriched Complexes; 7.7x10^-2); (D) Protein Interaction Map for cluster from Approach #2 enriched for Protein Folding Fate Function (hit-rate of 30.0% and p-value of 4.6x10^-11) no enriched Complexes, no enriched complexes.

References


Acknowledgments

We would like to thank Prof. A.L. Burlingame for the developmental version of Protein Prospector. This work is supported by NIH (GM-74830), and NIH shared instrument grant S10RR023552 to L.H.

Conclusion

To our knowledge, our study is the first to examine the 26S proteasome using a proteomic approach for characterizing in vivo changes in protein interaction networks throughout the cell cycle. Our approach for obtaining cell cycle snapshots of protein complex interaction networks is a useful tool for examining how complexes are involved in and regulated by the cell cycle and will be useful for other types of biological processes including response to stress and DNA damage repair.