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

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Summary

The Ub-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 (QTAX) is a powerful approach to identifying both stable and transient/weak 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 QTAX 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

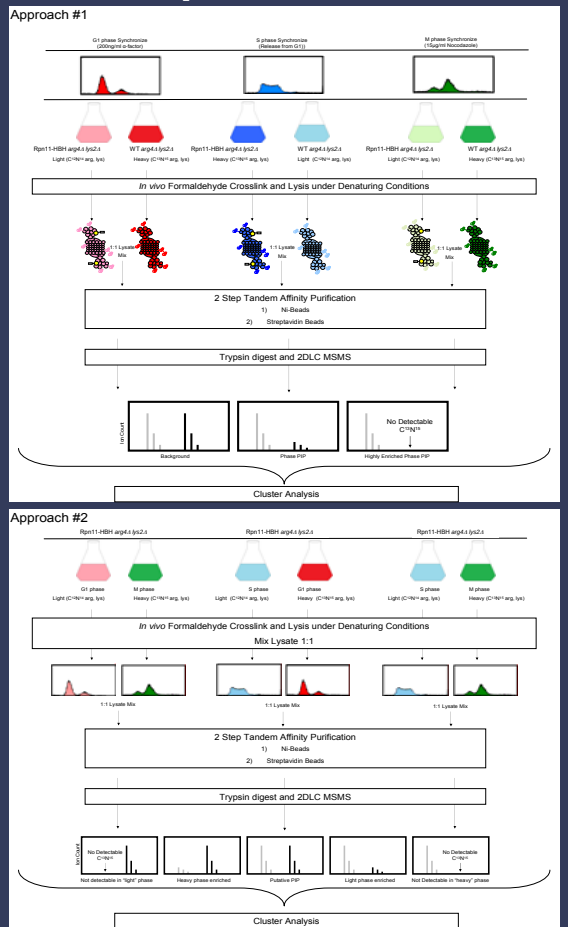

Fig.1- The QTAX strategy for quantifying cell-cycle dependent proteasome interacting proteins (PIPs). Approach #1: Identifying specific PIPs by comparing samples from Rpn11-HB and wt cells synchronized identically. Approach #2: Identifying cell-cycle dependent changes in PIPs by quantitative comparison between differentially synchronized Rpn11-HB cells.

Results

Validation

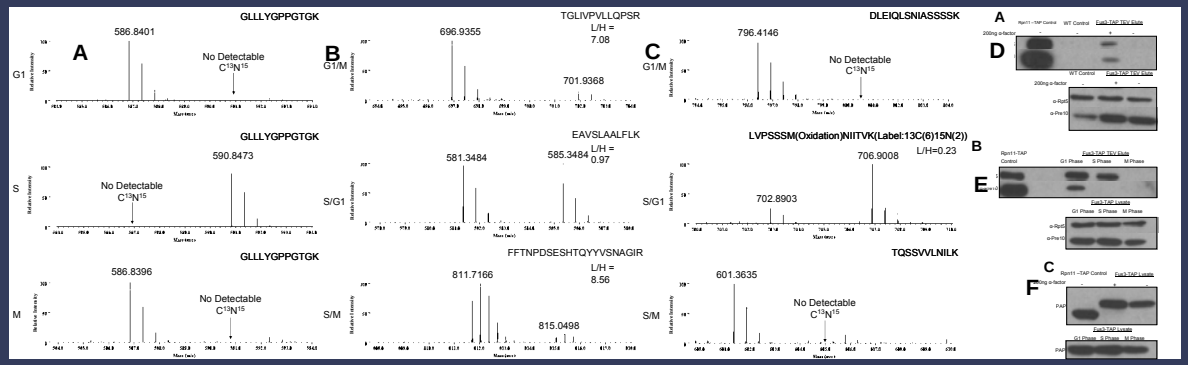

Fig.2-Identification and Validation of the selected putative cell cycle specific PIPs. A-C. Representative MS spectra of highly enriched PIP identified from approach #1 (YBR080C, Sec18) (**A**); of cell cycle dependent PIPs identified from approach #2: YLR452C, Sst2 (**B**); YBR040W (**C**); **D-E.** 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) (**E**); (**F**). Expression level of the tagged proteins. Rpn11-TAP:pos. control; WT-neg. control; Fus3-TAP: a selected PIP.

Cluster Analysis

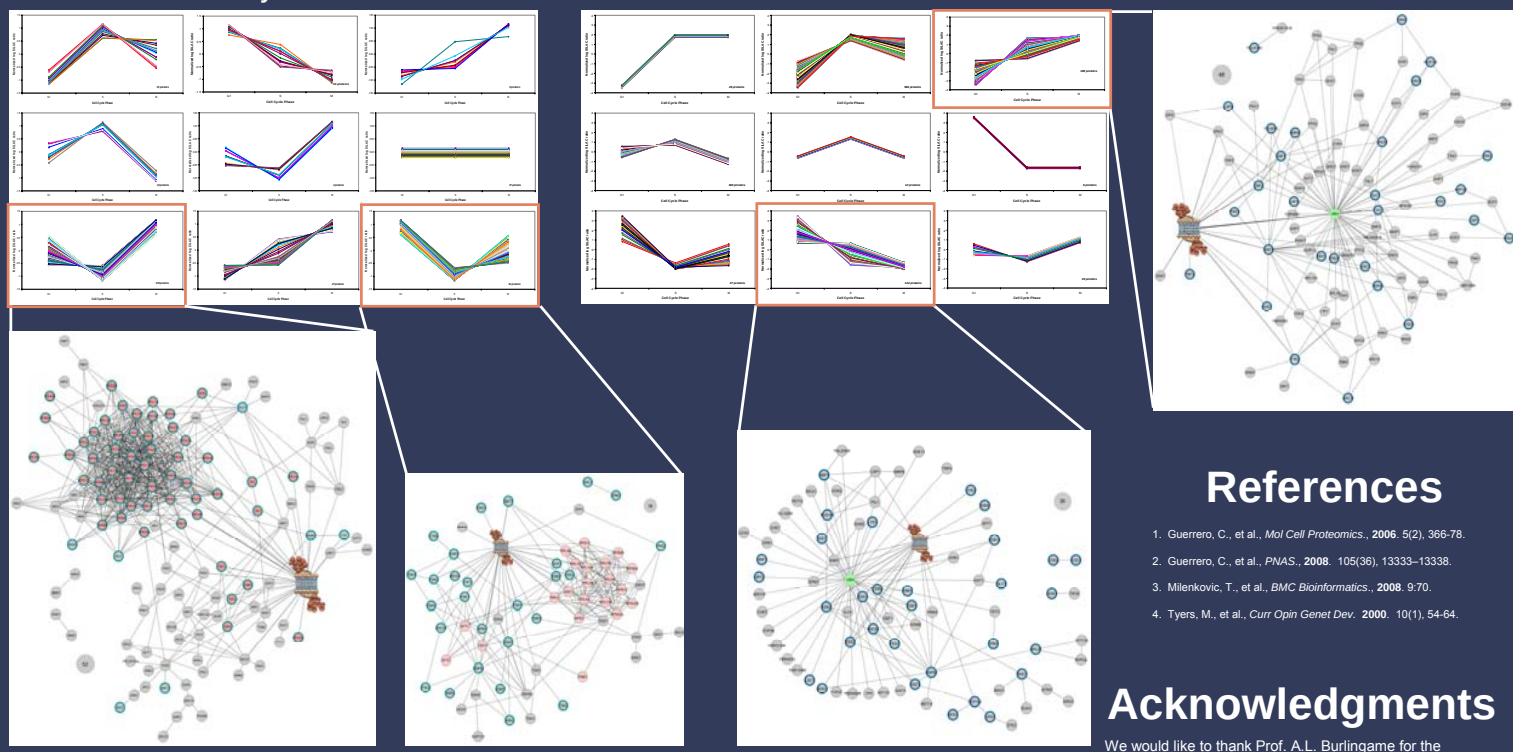

Fig.3- PIP Clusters by SILAC Ratio Profiles and Cluster-based Interaction Maps. A-B. Clusters of PIPs (grouped by similarity in SILAC ratio profiles) for Approach#1 (**A**); and Approach #2 (**B**); NOTE: for the aesthetics of the figure, any profiles which overlapped were shifted; **C-F.** Cluster-based protein interaction maps with annotated function and complex. Pink = annotated for a given complex; Blue Node Border = annotated for a given function; Ubi4 (green) = Ubiquitin; Isolated nodes with no interacting partners in a cluster are represented by a single large grey node; the node label represents the number of isolated nodes. Hit-rate represents the percentage of annotated proteins in a cluster with the given function/complex annotation; p-value represents the probability of observing the given hit-rate at random. (**C**) Protein Interaction Map for cluster from Approach #1 enriched for Protein Synthesis Function (hit-rate of 37.4% and p-value of $7.0x10^{-5}$) and Translation Complexes (hit-rate of 73.3% and p-value of $8.7x10^{-6}$); (**D**) Protein Interaction Map for cluster from Approach #1 enriched for Metabolism Function (hit-rate of 49.4% and p-value of $1.3x10^{-2}$) and Translation Complexes (hit-rate of 61.8% and p-value of $7.7x10^{-2}$); (**E**) 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; (**E**) Protein Interaction Map for cluster from Approach #2 enriched for Protein Folding Fate Function (hit-rate of 35.0% and p-value of $4.9x10^{-2}$), no enriched Complexes, no enriched complexes.

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.

References

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Acknowledgments

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