Condition-Dependent Regulation of Protein Turnover Rate in Yeast

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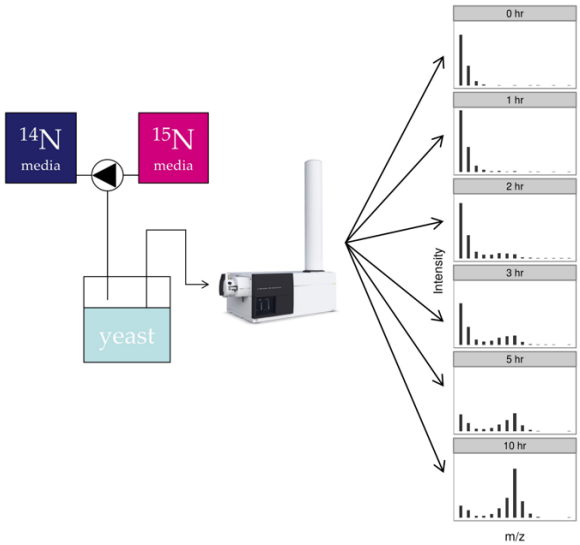
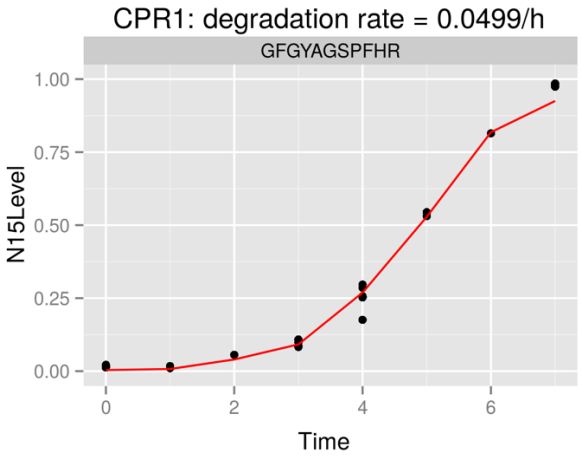
Project Goals: Despite the long history of targeted efforts to quantify protein turnover, measuring protein degradation rates in an unperturbed system at the proteome scale remains challenging. Here we aim to measure the degradation rates of a large number of proteins in nutrient-limited yeast continuous cultures (chemostats), with a focus on how degradation rates change across nutrient conditions. To this end, we switch yeast chemostat cultures into ^{15}N-labeled media and measure the dynamics of resulting protein labeling by LC-MS/MS. Through this approach, we aim to understand the extent to which protein degradation explains mismatches between protein and transcript abundance across nutrient conditions. Furthermore, we aim to uncover mechanisms controlling protein stability, which will be relevant to obtaining quantitative control of enzyme levels for optimized metabolic engineering.

Chemostat cultures provide a highly reproducible system for exploring natural variation in yeast physiology. We have previously measured transcripts[1], proteins, and metabolites[2] across 25 chemostats conditions, consisting of five different limiting nutrients each studied across five growth rates. An advantage of the chemostat setup is that biological variability across the 25 conditions is large, minimizing the impact of experimental measurement error. We were therefore surprised to observe that a median of only 19% of variation in protein abundance was explained by transcript abundance. Moreover, given that we are using relative measurements across different steady-state conditions, the discrepancy between protein and transcript levels cannot be explained by technical or biological issues impacting absolute protein and transcript abundances (e.g. MS response factors, ribosome binding strength of different mRNAs), nor differences in transcript and protein dynamics.



More careful examination of the transcript and protein data revealed stronger correlation between protein and transcript levels across growth rates for a given limiting nutrient (medians 37% – 53%), but these correlations

did not hold across limiting nutrient at specific growth rates (medians 19% - 31%). In some cases, most frequently in nitrogen limitation, we observed overt and statistically significant anti-correlation between protein and transcript abundances. This motivated us to measure protein stabilities, to see whether variability in protein degradation underlies the poor overall correlation between protein and transcript levels, and the particularly surprising cases of overt anti-correlation.



To this end, we developed an integrated experimental-computational method that uses dynamic ^{15}N-labeling to measure protein turnover in otherwise unperturbed, steady-state chemostats. We inoculate chemostats with S. cerevisiae and allow each chemostat population to stabilize. We then switch the incoming media from the natural isotope media to media that has been prepared with ^{15}N-ammonium sulfate, but is otherwise identical to the unenriched media. Switching chemostat media in this way results in an exponential decay in the amount of unlabeled nitrogen available for amino acid synthesis, so newly synthesized protein is increasingly enriched for the heavy nitrogen isotope. We sample the chemostat repeatedly over several generations after the media switch and measure the relative proportion of ^{15}N in each protein using LC-MS/MS. Using simultaneous measurements of ^{15}N enrichment in amino acids and a dynamical model of amino acid incorporation into protein, we will find the protein-specific degradation rate for each protein in each combination of nutrient condition and growth rate. We are currently in the process of completing the LC-MS/MS analysis for 9 chemostat conditions (carbon, nitrogen, and phosphorus limitation, each at 3 growth rates). We will present data on the transcript-protein correlations across the 25 chemostats conditions, as well as preliminary protein degradation results from these 9 conditions.

References

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