Dynamic changes in protein abundance and phosphorylation in yeast stress response

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***Short abstract*— Yeast cells can sense changes to their environment and rapidly change their protein expression profiles. This rapid signaling in the cell is made possible by phosphorylation of key proteins. Thus, environmental changes lead to cell signaling changes. Here we describe global protein phosphorylation over time in the yeast proteome in response to oxidative stress. We observe phosphorylation changes over two hours at each 10-minute interval under steady-state growth in miniature chemostats, where we can precisely control growth rate. The data shown in this work provides quantifiable changes over time, under controlled conditions, amenable to mathematical models.**

***Keywords* — Proteomics, Mass spectrometry, phosphorylation, cell signaling, chemostat, yeast, *Saccharomyces cerevisiae*.**

THE environmental stress response (ESR) was described by Gasch *et al*[1] by subjecting the baker's yeast to a battery of stressors, ranging from heat and hypo-osmotic shock to oxidative stress through which they discovered a common gene expression pattern by quantitating mRNA on microarrays. They also determined key gene expression patterns unique to some stressors for a particular expression pattern.

Quantitating peptides as opposed to mRNA transcripts provides a more direct measurement of the functional units of the cell, which are the proteins themselves. Here we robustly interrogate the temporal proteome response induced by oxidative stress. This has two important features for predictive modeling of cellular regulatory systems: 1) Quantitative change over time of modifications to the cell signaling network. 2) Experimental control over environmental growth conditions dictating growth rate.

We performed experiments using chemical oxidant menadione, first as a single time point —ten minutes for phosphopeptide analysis, and three hours for protein expression— and later over a time course of two hours, collecting samples every ten minutes. These experiments reflect induced, repressed and neutral protein changes across hundreds of proteins and twelve time points. In the protein experiment, we identified an average of 14,000 unique peptides and 2,500 unique proteins in a single 3-hour mass spectrometry analysis. In this experiment, proteins showing >2-fold changes upon oxidative stress were enriched for proteins involved in redox reactions and proteins relevant to free-radical mitigation activity. We observed most (2/3) of the dynamic changes in the proteome were attributed to a decrease in protein levels. The subset of the proteome with induced expression represents many of the ESR genes and redox signaling enzymes. This subset significantly overlaps with previous observations with mRNA measurements. Our phosphorylation analysis resulted in 5,700 unique peptides (1,200 unique proteins). These two sets of experiments reveal half and one-quarter of the yeast's entire genome, respectively.

Within only 10 minutes of a 1mM menadione stress, significant changes in the phosphoproteome were observed. In contrast to the protein data, the cellular signaling, measured through the phosphoproteome, shows more inductive changes than repressive ones as was shown by others[2]. Data from early time points is important, as it reveals rapid phosphorylation changes and protein degradation. The chemostats employed here are special yeast culture chambers, which allow media and nutrients to be dripped in at a constant rate, while old media and cells are simultaneously dripped out in the effluent. Meanwhile, the yeast cells are growing under control of a limiting nutrient, which in this case is glucose. These data from steady-state chemostat growth provide a reduction of noise, common to batch cultures. We will also present a comparison and discussion between cultures in batch and chemostats, and their effect in building dynamic models of the proteome. Future work will aim to incorporate methods learned at the q-bio summer school to better predict oxidative stress pathways in the yeast cell.

REFERENCES

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