

# Systematic Interrogation of the Budding Yeast Kinome

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## Introduction

Kinases regulate many if not all cellular processes through reversible phosphorylation of myriad substrates. The systematic identification of the proteins that associate with kinases is crucial for understanding the global regulatory network that dictates cellular behaviour. Due to the difficulty in detecting the often weak or transient interactions between kinases and their substrates or regulators, the kinase interactome (kinome) is only poorly charted, even in yeast (1). We have developed a sensitive gel-free mass spectrometric method that has enabled detection of interaction partners with virtually all of the 144 kinases in budding yeast.

## Methods

The complete set of kinases were systematically interrogated using a variety of epitope tags (HA, FLAG and TAP) in small scale cultures. Kinase complexes were efficiently recovered on pre-coupled Protein A magnetic beads and rapidly washed before on-bead trypsin digestion. Samples were analyzed by nano-scale liquid chromatography coupled to an LTQ mass spectrometer. Data was analyzed using the Mascot search engine and statistically significant hits in the dataset identified with a novel in-house algorithm called SAInt (Significance Analysis of Interactome (2)).

## Results

Each of the 144 yeast kinases was successfully expressed and identified by mass spectrometry. Using SAInt, ~2,000 statistically significant ( $p=0.9-1$ ) interactions were determined. This interaction dataset expands substantially on the current published set of kinase interactions. Visualization and reduction of the overall kinase network was performed by grouping baits in close binary proximity (based on the number of shared interactors) to reveal several kinase super-hubs, such as Tor1 and Tor2. Moreover, a statistically enriched kinase-backbone links different parts of the network. Immobilized metal affinity chromatography and iTRAQ studies were also performed on selected protein-protein interactions to deduce phosphosites and determine if particular interactions are regulated by environmental signals. These experimental studies were complemented and extended by novel informatics approaches to predict kinase substrates and

phosphorylation sites (3). In summary, sensitive gel-free mass spectrometry platforms for detection of protein interactions should enable an understanding of the transient interactions that comprise the dynamic proteome.

## Innovative aspects

- Comprehensive coverage of the budding yeast kinome using gel-free nano-scale liquid chromatography and tandem mass spectrometric analysis to identify low abundance kinase interaction partners
- Novel algorithm called SAInt (Significance Analysis of Interactome) for statistically robust filtering of large datasets
- Network analysis reveals a kinase-backbone and predicted substrates identified by the NetworkKIN approach (3)

## References

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# An in Vivo Map of the Yeast Protein Interactome

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## Introduction

The elucidation of protein-protein interaction networks (PINs, or interactomes) holds the promise of answering fundamental questions about how the biochemical machinery of cells organizes matter, information, and energy transformations to perform specific functions. An essential and rarely addressed question is whether protein complexes and PINs that are reconstructed or reconstituted in vitro or removed from the normal context in which they are expressed reflect their organization in living cells. We have performed a genome-wide in vivo screen for protein-protein interactions in *Saccharomyces cerevisiae* by means of a protein-fragment complementation assay (PCA) (1).

## Methods

We performed a systematic binary screen for PPIs at a genome wide scale in *S. cerevisiae* using a PCA based on the murine dihydrofolate reductase (mDHFR), a survival-selection assay based on a methotrexate resistant mutant of DHFR (2). We inserted complementary N- and C-terminal DHFR fragment sequences directly into the genome of haploids 3' to ORFs achieving final combined coverage of 5367 (93%) of all consensus ORFs. Screening was performed on solid-phase where MATa strains (N-fragment fusions) served as baits mated individually with all MATa (C-terminal fragment) strains on high-density arrays. The resulting diploids were selected for methotrexate resistance (growth indicating an interaction). A total of 15 million screens were performed.

## Results

We identified 2770 high quality interactions among 1124 endogenously expressed proteins and many more likely meaningful interactions. Comparison with previous studies confirmed known interactions, but most were not known, revealing a previously unexplored subspace of the yeast protein interactome, notably membrane proteins. The PCA detected structural and topological relationships between proteins, providing an 8-nanometer-resolution map of dynamically interacting complexes in vivo. These include topological maps of known macroassemblies such

as the proteasome, RNA polymerase and the nuclear pore complexes for which our results suggest that in vitro determined macromolecular complexes reflect their structure in living cells. We also identified extended protein complex networks that provide insights into fundamental cellular processes, including cell polarization and autophagy, pathways that are evolutionarily conserved and central to both development and human health. The tools developed for this study will enable analysis of protein-interaction dynamics on any scale to uncover mechanisms of biochemical network regulation, spatial dynamics of protein complexes and integrated with studies of gene regulation and protein modifications will lead to a fuller understanding of how complex cellular processes are orchestrated in the living cell (3).

## Innovative aspects

- Comprehensive coverage of the budding yeast protein interactome in intact, living cells using a Protein-fragment Complementation Assay (PCA) (1, 2).
- Analysis of protein complexes and networks with spatial resolution of complex organization in living cells.
- Network analysis reveals both known and novel complexes on a genome-wide scale and opens the door to dynamic interpretation (3)

## References

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## The Yeast Nuclear Proteome: composition and complexes

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### Introduction:

To date, genome-wide characterization of protein complexes in yeast using TAP-tag technology and mass spectrometry has provided detailed insight in the wide variety of protein interactions present in *Saccharomyces cerevisiae*. In this study we utilize phosphocellulose chromatography as a protein fractionation tool preserving protein interactions as a general tool to isolate protein complexes, and as an enrichment technique for DNA binding proteins from yeast nuclei. Furthermore, we have used this technique combined with SDS-PAGE and mass spectrometry for the first comprehensive characterization of the yeast nuclear proteome.

### Methods:

A yeast nuclear extract was prepared by spheroplasting at 30 degrees for one hour and lysing in 1.1M sorbitol, 0.025% triton and inhibitors (pepstatin PMSF) using sheering force. Cells were then fractionated on a sucrose/PVP gradient and the nuclei collected in the 2.1/2.3 M sucrose interface. 2mg of the nuclear extract was applied on a phosphocellulose column and proteins were eluted in a stepwise manner using 0.1M, 0.3M, 0.5M and 0.85M NaCl. These fractions were subsequently individually separated on an SDS gel, in-gel digested and analysed using an LTQ-FTICR and LTQ-Orbitrap mass spectrometer. All spectra were processed with Bioworks 3.3.1 and the subsequent data was searched against Yeast SGD database using Mascot.

### Results:

A yeast nuclear extract was fractionated by phosphocellulose chromatography, eluting proteins in a stepwise manner using 0.1M, 0.3M,

0.5M and 0.85M NaCl. This led to the identification of over 1800 unique proteins with a minimum of 2 peptides per proteins. Among these were 773 proteins from a total of 1385 annotated as nuclear proteins in the yeast SGD database. In addition, 175 out of 202 nucleolar proteins were identified. Together this provides the largest dataset of the yeast nuclear proteome known to date. Many protein complexes were identified with full coverage in distinct high salt fractions. In the flowthrough, proteins with a low affinity for the phosphocellulose column were identified, and a vast number of proteins known to be part of protein complexes were identified eluting in various fractions, indicating that they were part of more than one complex or were present in free form. Collectively, this study provides a comprehensive overview of the yeast nuclear proteome and valuable information about protein-protein interactions without the need for tagging approaches.

### Innovative Aspects:

- Phosphocellulose chromatography as a fractionation tool preserving protein interactions, applied to the comprehensive characterization of the yeast nuclear proteome.

# Analysis of the *S. cerevisiae* metabolic network by targeted proteomics

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## Abstract

Among all cellular networks, metabolism is unique because its topology is almost completely known. Nevertheless, what is still poorly understood is how this system operates and adapts to changing conditions. To understand and model these properties, it is necessary to quantitatively measure all the elements that constitute and regulate it. However, comprehensive measurements are technically difficult, especially at the proteome level.

Here a targeted proteomics strategy based on selected reaction monitoring (SRM) was applied to generate comprehensive quantitative data sets of all key metabolic proteins and regulatory elements in *S. cerevisiae*, under a defined set of conditions.

## Method

The method consists of the following steps: (i) a list containing all key metabolic proteins in *S. cerevisiae* was generated (ii) For each enzyme proteotypic peptides were derived, either by screening proteomics data repositories (PeptideAtlas<sup>1</sup>) or by bioinformatic prediction<sup>2</sup> (iii) For each peptide a SRM assay was validated and optimized on a triple-quadrupole mass spectrometer<sup>3</sup> and (iv) the assays were used to quantitatively monitor the set of metabolic proteins in yeast samples grown under a range of metabolic conditions inducing radically different modes of metabolic operation. In all measurements a <sup>15</sup>N metabolically labeled yeast lysate was used as an internal standard. In parallel, phosphopeptide enrichment was performed on the same set of yeast digests by TiO<sub>2</sub> chromatography.

## Results

Firstly we tested the depth and sensitivity of the targeted proteomics approach. We demonstrated that proteins spanning the whole range of abundance in yeast, between 1.3E6 copies/cell and <50 copies/ cell could be detected by LC-SRM in minimally fractionated *S.cerevisiae* proteome digests. These results showed that a solid basis was established for a comprehensive targeted analysis of the metabolic proteome. Targeted proteomic assays were then developed for key metabolic proteins and applied to measure them in yeasts grown under different conditions (glucose, ethanol, galactose, complex medium, anaerobic conditions) and in a time-course along the diauxic

shift. Protein abundance data were also combined to a preliminary analysis of the protein phosphorylation changes across the same set of conditions. Results from all metabolic pathways associated to central carbon metabolism will be presented and discussed.

The data generated set us on a path towards a quantitative modelling of the dynamics of the yeast metabolism. Furthermore, the validated protein assays are made publicly available, via the first repository of SRM transitions (SRMAtlas, ISB, Seattle, WA). It contains assays for > 1200 yeast proteins and will allow other researchers to monitor the responses of sets of yeast proteins of interest to any stimulus in a high-throughput fashion.

## Innovative aspects

- Whole-range yeast protein analysis
- Targeted proteomics based on SRM applied to the metabolic network
- First repository of proteomics assays

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