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A Modified MudPIT Separation Identified 4,488 Proteins in a System Wide Analysis of Quiescence in Yeast

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Abstract

A modified multidimensional protein identification technology (MudPIT) separation was coupled to an LTQ Orbitrap Velos mass spectrometer and used to rapidly identify the near complete yeast proteome from a whole cell tryptic digest. This modified on-line two dimensional liquid chromatography separation consists of 39 strong cation exchange steps followed by a short 18.5 min reversed-phase (RP) gradient. A total of 4,269 protein identifications were made from 4,189 distinguishable protein families from yeast during log phase growth. The "Micro" MudPIT separation performed as well as a standard MudPIT separation in 40% less gradient time. The majority of the yeast proteome can now be routinely covered in less than a days' time with high reproducibility and sensitivity. The newly devised separation method was used to detect changes in protein expression during cellular quiescence in yeast. An enrichment in the GO annotations 'oxidation reduction', 'catabolic processing' and 'cellular response to oxidative stress' was seen in the quiescent cellular fraction, consistent with their long lived stress resistant phenotypes. Heterogeneity was observed in the stationary phase fraction with a less dense cell population showing reductions in KEGG pathway categories of 'Ribosome' and 'Proteasome', further defining the complex nature of yeast populations present during stationary phase growth. In total 4,488 distinguishable protein families were identified in all cellular conditions tested.

Keywords

MudPIT; *Saccharomyces cerevisiae*; Quiescence

INTRODUCTION

One major objective of proteomics is to measure and quantitate the total protein content in samples over a variety of different cellular conditions. With the recent improvements in instrumentation we are progressing ever closer to this goal. Several publications have reported the near complete coverage of the *S. cerevisiae* proteome. In 2008 Godoy et al reported identifying 4,399 proteins in yeast.¹ This approach involved large quantities of cellular extract, extensive fractionation and long instrument analysis time (275 LC/MS/MS runs). Further improvements in instrumentation have reduced analysis time and in 2011 Nagaraj et al reported identifying 4,206 proteins in 6 replicate 4 hour UHPLC separations coupled to a Q-Exactive mass spectrometer.² Peng and colleagues, used multiple proteolytic enzymes and extensive fractionation to identify a total of 4,475 distinguishable protein families.³ Additionally, Single Reaction Ion Monitoring (SRM) approaches are capable of detecting proteins present at 50 copies per cell or less.⁴ This work was recently expanded

SUPPORTING INFORMATION This material is available free of charge via the Internet at <http://pubs.acs.org>.

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with the completion of a complete spectral reference library containing spectra for 97% of genome predicted peptides.⁵ However, the number of proteins detected in a single run is limited by the dwell time of each SRM transition and the precision of the chromatographic retention times for accurate scheduling of the targeted analyses. In this work we introduce a straight forward, on-line approach for detecting the yeast proteome based on a modified MudPIT separation technique capable of identifying a total of 4,488 distinguishable protein families in three cellular conditions of yeast.

With the introduction of the next generation Velos LTQ ion trap mass spectrometer major improvements in ion transfer efficiency and reduced ion trap scan time were made.^{6,7} This resulted in shorter duty cycle times and increased sensitivity. In this work our aim was to develop a fast in-line two dimensional chromatography approach which was well matched with the increased scan speeds of the new generation of Velos LTQ Orbitrap hybrid mass spectrometer. The MudPIT two dimensional separation combines SCX (strong cation exchange) and RP chromatography, and has demonstrated the ability to increase the fractionation power and dynamic range of a peptide separation.⁸⁻¹⁰ This technology has the added benefit of separating the peptide mixture online, eliminating the possibility of sample loss from repeated exposure to new, active surfaces and simplifies the analysis. A typical MudPIT separation consists of 9 SCX salt steps each followed by a 155 minute RP gradient. This separation can take approximately 24 hours of gradient time. In this work we introduce a modified MudPIT separation which reduces separation time by 40% with no loss in protein identification and test its use by analyzing the yeast proteome.

We chose to apply the newly developed Micro MudPIT to the analysis of cellular quiescence in the yeast *S. cerevisiae*. In this field there has been increased interest in this process as it may lead to a better understanding of the causal genes and cellular mechanisms behind aging, and cell cycle regulation.^{11,12} We used the recently revised Tfold program, part of the PatternLab for proteomics software suite to perform a pairwise comparison using spectral counting to determine changes in protein abundance during cellular quiescence.

METHODS

Growth and Isolation of Log Phase and Stationary Phase Yeast

S288C *S. cerevisiae* strain was obtained from ATCC. All samples were prepared in triplicate. 250 ml of log phase cells were grown at 30 °C in YPD media (1% bacto-yeast extract, 2% bacto-peptone, 2% dextrose) to an optical density of 0.6 at 600 nm. The culture was harvested by centrifugation at $3,000 \times g$ for 5 min at 4 °C and washed twice with 10 ml of sterile water. The resulting pellet was snap frozen in liquid nitrogen and placed in -80 °C until lysis. Stationary phase cells were grown at 30 °C in YPD media for 7 days. Two distinct cell fractions were isolated using Percoll density gradient (GE Healthcare, 17-0891-02) method developed by Allen and colleagues.¹³ A Percoll solution was prepared by diluting 9:1 (vol/vol) Percoll with 1.5 M NaCl producing a final concentration of 167 mM. Gradients were formed in 15 ml Corex tubes by centrifuging at 19,240 g_{av} for 15 min. 15 ml of the SP culture (200 OD₆₀₀) was pelleted and resuspended in Tris buffer (167 mM NaCl, 10 mM Tris pH 7.5), layered on top of the prepared gradient, and centrifuged at 400 g_{av} for 60 min in a swinging bucket rotor at room temperature. Fractions were removed from the gradient washed two times and snap frozen in liquid nitrogen and placed in -80 °C until lysis.

Lysis

The YeastBuster protein extraction reagent (Novagen, 71186-4) was used to lyse cell pellets. The procedure was identical to the manufacturer's protocol with the addition of 0.5 g of 0.5

MM zirconia beads (rpi Research, 9834) per 1 gram of cell pellets. During the 15 min incubation time the lysates were vortexed three times for 30 seconds with one minute rest on ice between cycles. The addition of the bead beating step improved the protein yield from the dense, lower fraction stationary phase cells. Protein concentration was determined using a non-interfering protein assay kit (Calbiochem, 488250).

In Solution Digestion

To remove any interfering surfactants protein lysates were precipitated using chloroform and methanol. A 100 µg aliquot of lysate was adjusted to 200 µl with MilliQ H₂O. 600 µl of methanol, 150 µl chloroform, and 450 µl of H₂O was added. The solution was vortexed then centrifuged at 18,000 g_{av} for one minute. The upper aqueous layer was removed and 450 µl of methanol added vortexed a second time and centrifuged at 18,000 g_{av} for two minutes. The supernatant was removed and the pellet was resuspended in 60 µl 8 M urea in 100 mM Tris pH 8.5. The samples were reduced for 20 minutes by adjusting the solution to a final concentration of 5 mM tris(2-carboxyethyl)phosphine then carboxyamidomethylated in a final concentration of 10 mM iodoacetamide for 15 minutes. Finally the sample was digested by diluting to a final concentration of 2 M urea, adding four µg of sequencing grade modified trypsin (Promega, V5111), and incubating at 37°C while shanking overnight.

Multidimensional Protein Identification Technology (MudPIT)

The digested proteins were analyzed using a modified MudPIT separation that has been described previously.^{9,10} A brief description of the method and the modifications are listed below. The biphasic trapping portion of a two stage MudPIT column was prepared by creating a Kasil frit (PQ Corporation, 1624) on one end of a section of 250 µm i.d. fused-silica capillary. This segment was filled with 2.5 cm of 5 µm Partisphere SCX (Whatman, 4621-1507) followed by 2 cm of 5 µm Aqua C18 (Phenomenex, 04A-4299) using slurry packing to create the trapping/SCX column. The analytical portion of the MudPIT column was prepared by pulling a 100 µm i.d. fused-silica capillary using a laser puller (Sutter Instrument, P-2000) and slurry packing with of 3 µm Aqua C18 (Phenomenex, 04A-4311) to produce a 12 cm column. Forty µg of the digested yeast lysate was pressure-loaded onto a biphasic trapping column and desalted with 5% acetonitrile with 0.5% formic acid for 1 hr at 2 µl a minute prior to MS analysis. After desalting the biphasic column was connected to the analytical column using an Upchurch union (Upchurch Scientific, Oak Harbor, WA) and placed in a custom column heater set at 40 °C. An Agilent 1100 quaternary HPLC pump (Palo Alto, CA) was used with a split flow configuration to produce a column flow rate of ~400 nl/min. The buffer system is as follows: water/acetonitrile/formic acid (95:5:0.1, v/v/v) as buffer A (pH ~2.6), water/acetonitrile/formic acid (20:80:0.1, v/v/v) as buffer B, and buffer A including 500 mM ammonium acetate as buffer C (pH ~6.8).

Gradient Conditions

Standard Gradient Conditions—An initial 90 min gradient of 0-100% buffer B was used as the first step and 8 additional steps consisting of a 10 min salt pulse followed by 5 min of 0% B and then a 155 min RP gradient containing a 130 min 0-40% buffer B gradient and a 25 min 40-100% B gradient were used. The second step used a 10% buffer C and 90% buffer A salt pulse. The salt concentration increased in 10% increments for the next four steps followed by two 20% increment increases and a final 100% buffer C salt step.

Modified Gradient Conditions—To better utilize the SCX dimension of the separation 39 ammonium acetate salt steps were used in combination with a short RP gradient preceding each step. A 10 min gradient of 0-100% buffer B was used as a first step, followed by 39 additional gradient steps with each individual step consisting of 1 min at

100% buffer A, 2 min at X% buffer C, 0.5 min at 100% buffer A, 18.5 min gradient from 0-45% buffer B, 0.5 min gradient from 45%-100% B. The X% used during the 2 min buffer C period consisted of increasing 2% increments from 2-30% buffer C, increasing 2.5% increments from 32.5-65% buffer C, increasing 5% increments from 70-100% C, followed by 3 90% buffer C with 10% buffer B. A slightly longer 22.5 min gradient from 0-45% buffer B was used in place of the 18.5 min gradient from 0-45% buffer B in the set of experiments run on the Velos Pro Orbitrap Elite instrument.

Mass Spectrometric Analysis

The peptide eluted from the MudPIT separation was directed into a Velos Orbitrap or a Velos Pro Orbitrap Elite mass spectrometer (Thermo Fisher Scientific). The instrument was run in data-dependent mode using the settings below. ESI voltage, 2.5 kV; inlet capillary temperature, 275 °C; Orbitrap full scan automatic gain control target, 10k ions; LTQ Velos MSn scan automatic gain control target, 10k ions; Orbitrap full scan maximum injection time, 250 ms; LTQ Velos MSn scan maximum injection time, 50 ms; normalized collision energy, 35; dynamic exclusion time, 35 sec; early expiration, enabled. A high resolution survey scan was acquired in the Orbitrap mass analyzer at a resolution of 60 000 at $m/z=400$ and 20 MS/MS scans on the 20 most intense ions was acquired on the LTQ Velos mass analyzer.

Data Analysis

MS2 mass spectra were analyzed using the following software protocol. Integrated Proteomics Pipeline (IP2, <http://www.integratedproteomics.com/>) was used for peptide and protein identification. Tandem mass spectra were extracted from the Thermo RAW files using RawExtract 1.9.9.¹⁴ MS2 spectra were searched with ProLuCID¹⁵ using a combined forward and reverse yeast database (database file curated by the *Saccharomyces* Genome 05-Jan-2010 version). The spectral search parameters considered fully- and half- tryptic peptides with a 50 ppm precursor mass window. Carbamidomethylation (+57.02146) of cysteine was set as a static peptide modification and oxidation of methionine (+15.9949) was set as a variable peptide modification. Candidate peptides were filtered to a 1% protein FDR and a 10 ppm precursor mass window with statistical consideration taken for tryptic and posttranslational status using DTASelect.¹⁶ Only proteins belonging to distinguishable protein families were reported. ANOVA analysis of the spectral count data was performed to determine differently expressed proteins present in one of the three conditions. The Integrated Proteomics Pipeline was used for this analysis. Proteins were considered significant to one growth condition using the following criteria; minimum of 5 spectral counts detected in all three replicate measurements in one growth condition, a minimum two fold increase in abundance in one condition, and an ANOVA p value of 0.02 or lower. All spectral count data was normalized using normalized spectral abundance factors for individual MudPIT runs.¹⁷ The data is available at <http://fields.scripps.edu/published/YeastJPR2013/>.

Pairwise comparisons between growth conditions were performed using spectral count data on an updated version of the TFCat program from the PattenLab for Proteomics software suite.^{18,19} The following parameters were used to select differently expressed proteins: Spectral count data was normalized using total signal and three non-zero replicate values were required in each condition. A Benjamini and Hochberg q -value of 0.02 (2% FDR) was set. A variable fold-change cutoff for each individual protein was calculated according to the t-test P-value using an F-Stringency value automatically optimized by the TFCat software and lowly abundant proteins were removed using an L-stringency value of 0.2.¹⁹

RESULTS AND DISCUSSION

Micro MudPIT separation

The development of the dual trap system for the LTQ Velos created a fast scanning mass spectrometer.^{6,7} Coupled with the high ion capacity S-lens, the system is able to scan at fast speeds with excellent ion statistics. By interfacing the LTQ Velos with the Orbitrap a fast scanning tandem mass spectrometer was married to a high resolution mass analyzer for accurate measurement of precursor ions. We reasoned that the increased scan speed of tandem mass spectrometer would allow for the introduction of a more complex mixture in a given time permitting the use of a faster RP gradient. Running a separation in this manner has the potential benefit of reducing the overall instrument operation time, allowing for higher sample throughput. A faster gradient also has the advantage of reducing analyte peak width therefore increasing the intensity of the eluting peptides.

To test the effectiveness of a modified MudPIT gradient, an initial bench mark of proteome coverage using a MudPIT separation of 9 steps was performed. The widely used *S. cerevisiae* S288C strain grown to log phase in rich media was chosen as a standard sample. Forty ug of a tryptic digest was loaded on a MudPIT column and run with 9 chromatographic steps, comprised of a 10 min salt pulse followed by a 155 min RP gradient (see methods section). The experiment was done in triplicate with the total gradient time of a single experiment lasting 24.2 hours. The resulting spectra were searched using ProLuCID with a protein false discovery rate of one percent, a precursor delta mass cutoff of 10 parts per million, a minimum of one peptide identified per protein, and only genomic loci with uniquely matching peptides were considered for protein identification. In total 4,126 distinguishable protein families were detected in the three experiments (4,206 protein groups) with an average of 3,844 distinguishable protein families detected in individual experiments (figure 1A).

Forty ug of the same standard yeast sample was separated using a modified MudPIT approach consisting of 39 two minute salt steps each followed by an 18.5 minute RP gradient. Once again the experiment was run in triplicate and the data searched using ProLuCID as described above. As with the standard MudPIT separation a similar number of proteins were identified. These included 4,189 distinguishable protein families (4,269 protein groups) from the combined triplicate run with an average of 3,814 distinguishable protein families in each individual run (figure 1A). The modified MudPIT separation is capable of performing as well as a standard MudPIT gradient but in 40% less time.

Analysis of the three MudPIT LC-MS/MS runs resulted in an average of $29,952 \pm 1,456$ unique peptides identified as compared with the three Micro MudPIT runs where $28,528 \pm 1,844$ unique peptides were identified. Together the three MudPIT runs identified a total of 41,826 unique peptide sequences and the Micro MudPIT runs identified 44,154 unique peptide sequences (figure 1B).

Due to the data dependent nature of shotgun proteomics, a common problem is the absence of a peptide or protein measurement in a replicate sample. This occurs because of the variability of the process of precursor peak selection. Interestingly, the frequency of protein identification of the standard MudPIT separation was quite good with 3,517 proteins of the total 4,126 proteins (85%) identified in all three replicates. The Micro MudPIT reproducibility was slightly less with 3,323 proteins of the total 4,189 proteins (79%) identified in all three replicates (figure 2). As expected the peptide level overlap was less with 19,173 peptides out of 41,826 identified in all three replicate standard MudPIT runs (46%), and 15,016 out of 44,154 identified in all three replicate Micro MudPIT runs (34%) (figure 3). Protein sequence coverage was also similar with the standard MudPIT combined

run median of 25% and the Micro MudPIT combined run median of 23% (figure 4A). Apparently, the increased speed of MS2 fragmentation on the Velos Orbitrap also increased protein coverage in a single run, leading to increased reproducibility seen between the technical replicates.

The coverage of the yeast protein open reading frames in this work was just under two thirds of the predicated reading frames present in the yeast genome. This is similar to the coverage observed in the Godoy, Nagaraj, and Peng studies.^{1,2,3} Over the four studies we observe a total of 3496 proteins in all four experiments and a cumulative total of 4984 proteins were detected when all four studies were combined. As the protein identifications are summed between the studies we see the total number of identifications converge, suggesting the proteome of yeast in laboratory log phase growth conditions is close to being comprehensively covered (supplemental fig 1). Currently there are 1,623 predicted protein encoding open reading frames yet to be observed in log phase growth using a shotgun proteomics approach.

Dynamic range of the Micro MudPIT accessible proteome

To better understand the extent of the yeast proteome accessible to detection with the Micro MudPIT method, an estimation of the relative protein amounts present was calculated using the Exponentially Modified Protein Abundance Index (emPAI) and the values were compared to the absolute copy number of each protein.²⁰ The emPAI calculation uses the number of observed peptides divided by the number of observable peptides (PAI) then raises 10 to the power of that value minus one, $10^{\text{PAI}-1}$. emPAI values were calculated for all detected proteins with a spectral count of 5 or above and a log-log plot was made using the corresponding cell copy number determined by Ghaemmaghami and colleagues using a TAP-fusion library (figure 4B).²¹ Using the cell copy number data as a reference the Micro MudPIT method was found to cover a substantial dynamic range, detecting proteins with 50-100 copies per cell to proteins present at $1e^6$ copies per cell. The use of the emPAI calculation and the TAP-fusion cellular copy numbers indicate that our data set covers a large dynamic range. Interestingly, as was previously reported by Peng et al. we see a weak correlation between the emPAI and TAP-fusion expression data ($R^2 = 0.43$) possibly due to a protease bias.³

Proteome changes in yeast quiescence

With the establishment of Micro MudPIT as a quick and robust approach we wanted to apply it to measure quantitative changes in a biological system. Recent interest in yeast quiescence (a reversible non-dividing cellular state occurring during stationary phase (SP) growth) and its use as a model system for cellular aging provides an interesting subject of study.¹¹ Several articles cover the latest developments in the understanding of the quiescent state of yeast.^{12,22,23} Proteomic methodologies have been used to study yeast quiescence, providing insight into the remodeling of the proteome. However, these studies were either limited in scope, using 2D gel electrophoresis^{24,25} or were non MS based²⁶. Moreover, SP cultures contain subpopulations with a mixture of quiescent and non-quiescent cells further complicating the profiling of a homogeneous population of quiescent cells. Only the GFP-fusion based proteomics approach by Davidson et al addressed the complication of this cellular heterogeneity.^{13,26} Here we apply the Micro MudPIT analysis to yeast in log phase growth, and two populations of stationary phase yeast, quiescent and non-quiescent cells. We used a spectral counting method to ascertain changes in protein abundance.

Triplicate samples of the autotrophic S288C strain were cultured in rich media to log phase (0.6 OD₆₀₀) and to SP (7 day growth). Quiescent and non-quiescent cellular fractions were isolated from the SP culture using percoll gradients and density centrifugation as described

by Allen et al.¹³ Cells were lysed, protein content determined, and 40 ug was digested with trypsin as described in the materials and methods section. Three biological replicates were run for log, quiescent, and non-quiescent samples using the Micro MudPIT technique on a Velos Pro Orbitrap Elite. ProLuCID was used for protein identification with the identical conditions listed above. The resulting protein identification, peptide identification, and sequence coverage numbers are displayed in figure 5.

To determine the differences in protein abundance between the samples label free spectral count analysis was employed. Spectral counting uses the number of identified MS2 mass spectra of a protein as an indication of the abundance of a protein.²⁷ We used spectral counting, which is a quick and efficient means of quantitating changes between samples with similar capabilities of stable isotope labeling, to quantify the differently expressed proteins between each condition.²⁸

First a pairwise comparison between each of the conditions was performed. This analysis was done using a newly revised version of Tfold, a component of the PatternLab for Proteomics software suite.^{18,19} This analysis consisted of a simple student's t-test of proteins present in all three replicate measurements followed by a Benjamini-Hochberg correction for multiple hypothesis testing with a FDR cutoff of 0.02. A variable fold-change cutoff calculation was used to select differently expressed proteins. This calculation uses the proteins t-test p-value and fits a power-law function to the data set to determine an appropriate fold change for each measurement. For a full explanation of the software see Carvalho et al.¹⁹ Next, low abundance proteins, those with low p values and a significant fold change but low spectral count values were removed using the L-stringency calculation in Tfold. This function uses the sample mean of all protein quantitation values and a user specified L-stringency value to calculate a spectral count cutoff specific for each pairwise comparison. Total proteins quantitated, number of differently expressed proteins determined, L-stringency spectral count cutoff, and other Tfold parameters used in the pairwise comparison between the three sample conditions are listed in Table 1. The differently expressed proteins in the three pairwise comparisons are listed in Table S1.

Enrichment analysis of the protein sets from quiescent fraction analysis with a 2 fold or more change in expression levels revealed changes in the Gene Ontology (GO) terms 'oxidation-reduction' (p 10^{-31}), 'catabolic process' (p 10^{-10}), and 'cellular response to oxidative stress' (p 10^{-7}) as significantly up regulated as compared to yeast during log phase growth. The enrichment in GO annotations is consistent with the increased oxygen consumption, decreased reactive oxygen stress, increased lifespan, and stress resistant phenotypes seen in the quiescent fraction of stationary phase yeast.²⁶ Not surprisingly, log phase yeast was enriched in 'ribosomal biogenesis' (p 10^{-15}), 'rRNA processing' (p 10^{-12}), and 'cellular component biogenesis' (p 10^{-11}) GO terms. The pairwise analysis between the two stationary phase fractions highlighted several interesting GO terms associated with each. The terms 'cytoplasmic translation' (p 10^{-23}), 'proteasomal ubiquitin-independent protein catabolic process' (p 10^{-11}), and 'cellular component biogenesis' (p 10^{-8}) were associated with the increases in protein abundance in the quiescent fraction. The upper non-quiescent fraction had the several GO terms enriched including, 'small molecule metabolic process' (p 10^{-29}), 'cellular nitrogen compound biosynthetic process' (p 10^{-17}), 'cellular amino acid biosynthetic process' (p 10^{-13}), and 'amine biosynthetic process' (p 10^{-13}) as compared to log phase yeast. KEGG pathway analysis revealed enrichment in enzymes involved in alanine, aspartate, and glutamate metabolism in the non-quiescent cellular fraction. This cellular fraction also lacks active respiration and has minimal reproductive capacity.²⁶ Taken together these observations suggests the possibility that the heterogeneity observed in the stationary phase may indicate cellular cooperation with the non-quiescent fraction providing nutrient support for the long lived lower quiescent fraction. KEGG

pathway analysis revealed a reduction in the Ribosome and Proteasome pathway components in the non-quiescent fraction. A past study observed reversible relocalization of the proteasome into large cytosolic granular structures. However this study did not distinguish if the reorganization occurred for the total stationary phase population.²⁹ Our results suggest this phenomenon may only occur in the non-quiescent fraction, a characteristic further distinguishing the differences between the two cellular populations. These results further support the previous GFP-fusion and microarray analysis that the lower quiescent fraction of a stationary phase culture is distinct from other cells present in the culture.^{13,26,30} When we compared our MS based results to the GFP-fusion library study we saw good agreement. Twenty-two out of 37 (81%) GFP-fusion proteins that increased in abundance in the quiescent fraction were also found to be enriched in the micro MudPIT analysis ($p < 0.05$) (Table S2).

In addition to the pairwise analysis we used a one way ANOVA analysis between the three cellular states to find proteins specific for each cellular condition. Proteins were considered significant when they were present in three triplicate measurements in one condition, had a total of five or more spectral counts in each replicate measurement, an ANOVA p value of 0.02 or less, and had a fold increase of two or greater compared to the two other conditions (Table S3). This resulted in the log phase sample having a total of 333 uniquely expressed proteins, the upper non-quiescent fraction having 75 uniquely expressed proteins, and the quiescent fraction having a total of 236 unique proteins.

CONCLUSION

We developed the modified MudPIT approach, a fast and efficient separation based on increased SCX steps and fast RP separations. The Micro MudPIT separation provides the same protein coverage of the yeast proteome as a standard MudPIT separation in 40% less gradient time. We used this separation technique combined with a newly optimized spectral counting method to measure the changes in protein abundance in during cellular quiescence. Using this approach we obtained near complete proteome coverage of yeast with the identification of a total of 4,488 proteins detected in all three growth conditions. This is the most comprehensive coverage of the haploid yeast proteome to date.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Proteome coverage of a standard MudPIT and the Micro MudPIT. A. Number of proteins identified in individual runs and the total number identified in three triplicate runs. B. Number of peptides identified in individual runs and the total number identified in three triplicate runs.

Figure 2. Comparison of protein coverage of replicate runs using of standard MudPIT and Micro MudPIT separations. Numbers are the identified proteins in each analysis.

Figure 3. Comparison of the peptide coverage of standard MudPIT separations and Micro MudPIT separations in triplicate runs. Numbers are the identified peptides in each analysis.

Figure 4.

A. The median sequence coverage for MudPIT runs is around 19% with a triplicate combined coverage of 25%. The Micro MudPIT coverage is approximately 17% with a triplicate combined coverage of 23%. B. The dynamic range of the Micro MudPIT accessible proteome was estimated using the Exponentially Modified Protein Abundance Index (emPAI) and by using the TAP-fusion library copy numbers.²¹ emPAI values and cell copy numbers for proteins detected with 5 spectral counts or above from the combined triplicate Micro MudPIT experiment are displayed in a log-log plot.

Figure 5.

A. Proteins identified in individual runs and the total number identified in three triplicate runs for log phase, stationary phase non-quiescent and quiescent cells. B. Same as A, but at the peptide level. C. Median protein sequence coverage of individual and triplicate runs.

Table 1

Summary of protein abundance changes in the yeast growth conditions.

	Log vs. Quiescent	Non-quiescent vs. Quiescent	Log vs. Non-quiescent
Differently expressed (Variable fold change cutoff)	842 (31.6%)	807 (33%)	875 (37.8%)
Differently Expressed (2 fold change cutoff)	575 (21.6%)	569 (23.3%)	694 (30.0%)
Average Fold Change of all Proteins Quantified	0.14	0.46	0.46
Total Proteins Quantified	2663	2442	2315
L-Stringency Spectral Count cutoff	7.5	8.2	8.6