Comparative proteomic analysis of transition of *Saccharomyces cerevisiae* from glucose-deficient medium to glucose-rich medium

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Abstract

**Background:** When glucose is added to *Saccharomyces cerevisiae* grown in non-fermentable carbon sources, genes encoding ribosomal, cell-cycle, and glycolytic proteins are induced. By contrast, genes involved in mitochondrial functions, gluconeogenesis, and the utilization of other carbon sources are repressed. Glucose also causes the activation of the plasma membrane ATPase and the inactivation of gluconeogenic enzymes and mitochondrial enzymes. The goals of this study were to use the iTRAQ-labeling mass spectrometry technique to identify proteins whose relative levels change in response to glucose re-feeding and to correlate changes in protein abundance with changes in transcription and enzymatic activities. We used an experimental condition that causes the degradation of gluconeogenic enzymes when glucose starved cells are replenished with glucose. Identification of these enzymes as being down-regulated by glucose served as an internal control. Furthermore, we sought to identify new proteins that were either up-regulated or down-regulated by glucose.

**Results:** We have identified new and known proteins that change their relative levels in cells that were transferred from medium containing low glucose to medium containing high glucose. Up-regulated proteins included ribosomal subunits, proteins involved in protein translation, and the plasma membrane ATPase. Down-regulated proteins included small heat shock proteins, mitochondrial proteins, glycolytic enzymes, and gluconeogenic enzymes. ACh1p is involved in acetate metabolism and is also down-regulated by glucose.

**Conclusions:** We have identified known proteins that have previously been reported to be regulated by glucose as well as new glucose-regulated proteins. Up-regulation of ribosomal proteins and proteins involved in translation may lead to an increase in protein synthesis and in nutrient uptake. Down-regulation of glycolytic enzymes, gluconeogenic enzymes, and mitochondrial proteins may result in changes in glycolysis, gluconeogenesis, and mitochondrial functions. These changes may be beneficial for glucose-starved cells to adapt to the addition of glucose.

**Keywords:** Catabolite inactivation, Catabolite repression, Glycolysis, Gluconeogenesis, FBPase, *Saccharomyces cerevisiae*, iTRAQ, MALDI

**Background**

*Saccharomyces cerevisiae* is an excellent model system to study cellular responses to a variety of environmental changes such as oxidative stress, temperature, aerobic versus anaerobic conditions, and the availability of carbon or nitrogen sources [1-15]. Yeast can obtain energy through fermentation of various sugars including glucose, fructose, sucrose, galactose, melibiose, and maltose [1-6]. Yeast can also obtain energy through the utilization of non-fermentable carbon sources such as glycerol, pyruvate, acetate, and lactate [1-6,16].

Growth of yeast in non-fermentable carbon sources induces metabolic pathways required for the utilization of these carbon sources [6,16], and the addition of glucose to cells previously grown in non-fermentable carbon sources results in a rapid change in the transcriptional state of the genome [6,17]. An estimated 40% of genes in yeast alter their expression by more than two-fold within minutes.
following the addition of glucose to cells grown in a non-fermentable carbon source [17]. In general, glucose increases the expression of genes involved in glycolysis, ribosomal functions, and cell division (Figure 1) [1-6, 16-27]. Glucose also represses genes required for mitochondrial functions, genes encoding the gluconeogenic enzymes such as FBP1 (fructose-1,6-bisphosphatase), ICL1 (isocitrate lyase), PCK1 (phosphoenolpyruvate carboxykinase), and MLS1 (malate synthase) [4,6,17,26,28-30], and genes required for metabolism of sugars other than glucose and fructose (Figure 1). The repression of genes by glucose is referred to as “catabolite repression” [4,16,17,28,31-33].

Complex regulatory networks that interconnect and overlap at different levels mediate glucose-induced changes in transcriptional activation or repression. For example, the Ras/PKA pathway, the Gpr/Gpa circuit, the Sch9 pathway, the Rgt network, and the Snf1 network have important roles in transcriptional regulation of glucose-repressible or glucose-inducible genes [4,6,16,25,29,34-37].

The effect of glucose is not restricted to transcriptional activation or repression. Glucose also causes a change in the concentration of mRNA. In the presence of a non-fermentable carbon source such as glycerol, the steady-state levels of mRNA for SDH2 and CYC1 are elevated by a combination of induced transcription in the nucleus and stabilization of the mRNAs in the cytoplasm [38]. The half-life of CYC1 mRNA is reduced in de-repressed cells from 12 min to 2 min in the presence of glucose [34,38]. Likewise, the turnover rates of mRNAs for PCK1 and FBP1 are also accelerated by glucose [4,5,16,34,39,40]. In contrast, the turnover rates of mRNAs for the 40S and 60S ribosomal subunits are reduced in response to high glucose [17,38].

Glucose affects enzymatic activities not only through a change in the rate of transcription, but also through an increase in the rate of degradation of proteins. This is known as “catabolite inactivation” [41-44]. Increased degradation of fructose-1,6-bisphosphatase (Fbp1p), isocitrate lyase (Icl1p), the cytosolic malate dehydrogenase (Mdh2p), and phosphoenolpyruvate carboxykinase (Pck1p) are responsible for catabolite inactivation of these enzymes [40,45-52]. Inactivation and degradation of gluconeogenic enzymes during glucose re-feeding prevents energy futile cycles that could be detrimental to cells. In addition to the inactivation of these gluconeogenic enzymes, glucose also reduces the activities of mitochondrial enzymes including...
aconitate, cytochrome c oxidase, NADH dehydrogenase, and the mitochondrial ATPase [34,53-55].

In contrast to the inactivation of gluconeogenic enzymes and mitochondrial enzymes, glucose causes the activation of the plasma membrane H+ -ATPase (Pma1p) [4,56-58]. Pma1p pumps protons out of cells to create a proton gradient needed for the uptake of nutrients into cells. The activation of Pma1p is rapid and is a reversible process. In the presence of glucose, the Km for ATP is reduced leading to an activation of this enzyme [56]. Phosphorylation of Pma1p is critical for the process of activation [4,56-58].

Although analysis of mRNA is a powerful way to understand functional mechanisms of the entire genome, it is not sufficient for full characterization of biological systems. Much evidence has accumulated indicating that mRNA abundance is not always correlated with levels of protein expression, and changes in mRNA abundance coding for a particular protein clearly has no direct bearing on whether or not that protein is activated or inactivated by post-translational modifications, including changes in the rate of degradation. Poor correlation between transcriptome and proteome levels has been observed for proteins involved in glycolysis, gluconeogenesis, purine metabolism, and amino acid metabolism [11,59]. For instance, microarray experiments have identified several mitochondrial genes that are regulated in response to glucose. However, proteome studies indicate that mitochondrial proteins are remarkably constant whether cells are grown in glucose or in non-fermentable carbon sources [59]. Since mRNA levels are not perfect indicators of protein levels, we sought to use comparative proteomics to identify proteins that are up-regulated or down-regulated by glucose and to correlate changes in protein abundance with changes in transcription and enzymatic activities.

Glucose effects on protein expression levels have been described in previous proteomic studies that examined steady state levels of protein expression in cells grown in different carbon sources for a prolonged period of time (summarized in Table 1). Because steady state levels of proteins are determined by protein synthesis, protein degradation, or both, the observed low expression levels of gluconeogenic enzymes in cells grown in glucose are likely to result from low rates of protein synthesis. In contrast, during catabolite inactivation, existing gluconeogenic enzymes are rapidly degraded in response to glucose. In cells that are starved of glucose, gluconeogenic enzymes have half-lives of longer than 100 hours. When glucose is added to glucose-starved cells, half-lives of these enzymes are reduced to 20–40 min [48,52,60,61]. For the degradation of FBPase, cAMP is transiently increased which activates the RAS2/PKA signaling pathway [62-68]. This leads to the phosphorylation of FBPase [62-68] and subsequent degradation of this protein. Therefore, the molecular mechanisms for catabolite inactivation of gluconeogenic enzymes are fundamentally different from low rates of protein synthesis in cells grown in glucose. Given that glucose represses a large number of genes involved in different metabolic pathways, we hypothesize that glucose effects on catabolite inactivation are not restrictive to gluconeogenic enzymes. To identify new proteins that are regulated by glucose, we sought to use an experimental condition to reproduce glucose effects on catabolite inactivation of gluconeogenic enzymes.

The iTRAQ (Isobaric Tags for Relative and Absolute Quantification) technology has been used to quantify relative changes in protein ratios in cells that are grown under various growth conditions [15,69-72]. iTRAQ is a gel-free technique that uses specific reporter molecules to label primary amines of the N-termini of peptides and the side chains of lysine residues, which are then identified and quantitated using mass spectrometry [72]. We used an experimental condition that causes the degradation of gluconeogenic enzymes Fbp1p, Pck1p, Icl1p, and Mdh2p [45-52]. The identification of these enzymes as being down-regulated by glucose served as an internal control. In analysis of additional results from these experiments, we have identified proteins that were up-regulated by glucose. These included Pma1p, subunits of ribosomes, and other proteins involved in protein translation. Up-regulation of these proteins may lead to increased protein synthesis and nutrient uptake.

We have also identified proteins that were down-regulated by glucose. These included glycolytic enzymes Hxk1p, Pgi1p, and Pgm2p, gluconeogenic enzymes Fbp1p and Icl1p, mitochondrial protein Atp2p, the major mitochondrial outer membrane protein Om45p, and small heat shock proteins Hsp12p, Hsp26p, and Hsp30p.

The down regulation of Pgm2p, Fbp1p, Icl1p, Atp2p, and heat shock proteins in response to glucose is consistent with previous reports. Down-regulation of glycolytic enzymes, gluconeogenic enzymes, and mitochondrial proteins may lead to changes in glycolysis, gluconeogenesis, and mitochondrial functions when cells are transferred from glucose-deficient medium to glucose-rich medium.

Results

Proteins identified by iTRAQ

To identify new proteins that are regulated by glucose, we used an experimental condition that causes the degradation of gluconeogenic enzymes during glucose re-feeding. The experimental designs for these experiments are shown in Figure 2. Briefly, wild-type yeast cells were grown in glucose-deficient media for three days to induce gluconeogenic enzymes. Aliquots of cells were harvested at t = 0 min. The remaining cells were harvested, washed, and re-suspended in media containing fresh
Table 1 Comparison of previous proteomic studies of protein expression in yeast cells grown in various conditions

| Study                     | Francesca et al. [10] | de Groot et al. [11] | Usaite et al. [12] | Costenoble et al. [13] | Kolkman et al. [14] | Pham et al. [15] |
|---------------------------|------------------------|----------------------|--------------------|------------------------|---------------------|------------------|
| **Primary Objectives**    | Comparison of steady   | Proteomic differences in anaerobic versus aerobic growth | Comparison of steady state levels of proteins in strains deficient in SNF1/ SNF4 involved in glucose repression | Comparison of steady state levels of proteins in cells grown in glucose, galactose or ethanol. | Comparison of steady state levels of proteins in cells grown in 120 g/L (normal) to 210 g/L and 300 g/L (high) glucose for 68 hours. |
| **Analytical Platform**   | 2D-GE; Relative spot volume quantification; MALDI-TOF | Stable-isotope labeling with $^{14}$N and $^{15}$N in cultures grown in anaerobic versus aerobic conditions; 1D-PAGE; RFLP; nanoflow-LC-ESI-MS/MS | Stable isotopic labeling with $^{14}$N and $^{15}$N in wild-type, $\Delta$SNF1, $\Delta$SNF4, and $\Delta$SNF1/$\Delta$SNF4 strains; MudPIT; ESI; LTQ-Orbitrap | Targeted proteomics approach based on selected reaction monitoring (SRM) and proteotypic peptides (PTPs); ion trap MS with nanoelectrospray ion source | 2D-GE; Relative spot quantification; MALDI-MS and Nano-ESI-LC-MS/MS iTRAQ; nano-LC-ESI-MS/MS |
| **Total number of peptides/proteins identifications/quantification** | 156 protein spots changing significantly; 21 differentially expressed proteins identified by MS analysis | 1499 identified; 474 quantified proteins; 249 proteins showed differential expression levels | 2388 proteins were relatively quantified; 350 showed differential expression levels | The 228 proteins of the central carbon and amino-acid metabolic network in S. cerevisiae | 400 protein spots were detected on each 2D gel; 44 spots were relatively quantified and identified | 413 proteins were identified from 3 replicates; 237 showed differential expression between conditions |
| **Relevance to our study** | Gluconeogenic enzymes were not identified. | 1. Steady state levels of glycolytic enzymes were higher in ethanol than in cells grown in glucose. | Steady state levels of glycolytic enzymes were higher in cells grown in ethanol than in cells grown in glucose. | 1. Steady state levels of glycolytic enzymes were higher in cultures grown in glucose than cells grown in ethanol. | 1. Levels of most glycolytic enzymes were higher in 300 g/L glucose than in normal glucose. | 1. Levels of Hsp12p, Hsp26p, and other heat shock proteins were lower in cells grown in high glucose than in cells grown in normal glucose. |
glucose for 2 hours; a time period sufficient for degradation of the majority of Fbp1p, Pck1p, Mdh2p, and Icl1p proteins [45,46,48,52]. After cells were harvested, total lysates were obtained and the proteins were digested with trypsin. Resulting tryptic peptide fragments were labeled with the iTRAQ tags, with duplicate t0 cells labeled with 113 and 114 tags, whereas peptides from t2 cells were labeled with 115 and 116 tags. We have identified 591 proteins with an estimated local false discovery rate of less than 0.05. We determined the number of peptides with a confidence interval higher than 95% contributing to the ID of each of these proteins. A total of 153 proteins of these 591 were identified based on a single peptide fragment.

It has been reported previously that more than 50% of the protein IDs based on single peptides could be confirmed as correct [73]. We therefore compared the distribution of biological functions of the 438 proteins that were identified with two or more peptides (Table 2B) to the 153 proteins that were identified with a single peptide (Table 2C). We also compared the distribution of these proteins with the entire genome that contains 6310 proteins (Table 2A). Using the Gene Ontology Slim Mapper program available at the S. cerevisiae Genome Database, these proteins were classified into 44 biological processes. Based on the distribution of proteins in biological processes, it appears that we have identified more proteins involved in protein translation, ribosome biogenesis, carbohydrate metabolism, amino acid metabolism, and the generation of metabolites and energy compared to the distribution of the 6310 proteins from the entire genome (Table 2).

We next sorted these proteins based on their subcellular distributions (Table 3). Some proteins are distributed in multiple locations and have been counted more than once using this program. For the entire genome of 6310 proteins, the largest numbers of proteins (60.4%) are distributed in the cytoplasm. A significant percentage of proteins are also found in the nucleus (33.0%), mitochondria (17.8%), and in unknown locations (12.3%). For the 438 proteins that were identified using more than two peptides, the majority of proteins were found in the cytoplasm (93.2%). It appears that we have identified higher percentages of proteins located in the cytoplasm, mitochondria, ribosomes, and membrane fractions compared to the 6310 proteins from the yeast genome. For the 153 proteins that were identified with one peptide, we did not find proteins located in peroxisomes or in the extracellular region. It is possible that proteins in these compartments are difficult to be digested with trypsin and would therefore be more likely to contain fewer peptides for identification.

To be accepted as a significant quantitative difference between treatment combinations, we imposed the following criteria: the affected proteins with three of the four iTRAQ ratios significantly higher than 1.0 (p-value less than 0.05) were considered to be up-regulated (Table 4A). Conversely, proteins with three of the four iTRAQ ratios that were significantly lower than 1.0 (p-value less than 0.05) were considered to be down-regulated (Table 5A). Furthermore, the ratios of 115/113, 115/114, 116/113, and 116/114 were used as re-fed/starved ratios.

Proteins Up-Regulated by Glucose

Previous work has shown that the presence of high glucose induces the transcription of more than 90% of the ribosomal protein genes 2–4 fold within 30 minutes [17]. In one proteomic study, 22 ribosomal subunits were identified and 9 subunits increased their relative levels in response to 300 g/L glucose [15]. Because we used a different experimental condition to study glucose effects, we examined whether or not ribosomal subunits increase their relative levels when glucose starved cells were transferred to medium containing fresh glucose for 2 hours.
Table 2 Cellular functions of proteins identified and gene ontology (GO) annotations from the *Saccharomyces* genome database

| GO term FUNCTION                                      | A                | B                | C                |
|-------------------------------------------------------|------------------|------------------|------------------|
|                                                       | Genome Frequency | Peptides > 1 Frequency | Peptides = 1 Frequency |
|                                                       | n = 6310         | n = 438          | n = 153          |
|                                                       | Percent          | Percent          | Percent          |
| biological process unknown                            | 1217 19.3%       | 33 7.5%          | 14 9.2%          |
| RNA metabolic process                                 | 1210 19.2%       | 64 14.6%         | 20 13.1%         |
| transport                                             | 1047 16.6%       | 88 20.1%         | 33 21.6%         |
| translation                                           | 706 11.2%        | 129 29.5%        | 17 11.1%         |
| transcription, DNA-dependent                          | 603 9.6%         | 15 3.4%          | 7 4.6%           |
| response to stress                                    | 594 9.4%         | 61 13.9%         | 16 10.5%         |
| protein modification process                          | 583 9.2%         | 22 5.0%          | 11 7.2%          |
| cell cycle                                            | 527 8.4%         | 18 4.1%          | 6 3.9%           |
| ribosome biogenesis                                   | 411 6.5%         | 50 11.4%         | 11 7.2%          |
| DNA metabolic process                                 | 404 6.4%         | 15 3.4%          | 5 3.3%           |
| chromosome organization                               | 398 6.3%         | 18 4.1%          | 6 3.9%           |
| vesicle-mediated transport                            | 366 5.8%         | 20 4.6%          | 13 8.5%          |
| response to chemical stimulus                         | 331 5.2%         | 30 6.8%          | 11 7.2%          |
| mitochondrion organization                            | 318 5.0%         | 22 5.0%          | 8 5.2%           |
| cellular membrane organization                        | 286 4.5%         | 24 5.5%          | 10 6.5%          |
| heterocycle metabolic process                         | 269 4.3%         | 44 10.0%         | 15 9.8%          |
| cellular carbohydrate metabolic process               | 265 4.2%         | 53 12.1%         | 12 7.8%          |
| cellular amino acid metabolic process                 | 239 3.8%         | 63 14.4%         | 17 11.1%         |
| signalling                                            | 235 3.7%         | 8 1.8%           | 6 3.9%           |
| cytoskeleton organization                             | 228 3.6%         | 16 3.7%          | 11 7.2%          |
| cellular lipid metabolic process                      | 223 3.5%         | 10 2.3%          | 4 2.6%           |
| protein complex biogenesis                            | 212 3.4%         | 17 3.9%          | 10 6.5%          |
| cellular protein catabolic process                    | 198 3.1%         | 15 3.4%          | 5 3.3%           |
| cofactor metabolic process                            | 163 2.6%         | 33 7.5%          | 4 2.6%           |
| generation of precursor metabolites and energy        | 160 2.5%         | 53 12.1%         | 4 2.6%           |
| meiosis                                               | 157 2.5%         | 7 1.6%           | 2 1.3%           |
| cellular homeostasis                                  | 150 2.4%         | 20 4.6%          | 7 4.6%           |
| chromosome segregation                                | 142 2.3%         | 3 0.7%           | 1 0.7%           |
| fungal-type cell wall organization                    | 133 2.1%         | 5 1.1%           | 3 2.0%           |
| vacuole organization                                  | 130 2.1%         | 12 2.7%          | 3 2.0%           |
| sporulation resulting in formation of a cellular spore| 128 2.0%         | 10 2.3%          | 3 2.0%           |
| conjugation                                           | 117 1.9%         | 5 1.1%           | 2 1.3%           |
| cytokinesis                                           | 110 1.7%         | 4 0.9%           | 3 2.0%           |
| transposition                                         | 108 1.7%         | 1 0.2%           | 0 0.0%           |
| cellular component morphogenesis                      | 96 1.5%          | 3 0.7%           | 3 2.0%           |
| protein folding                                       | 88 1.4%          | 27 6.2%          | 3 2.0%           |
| cellular respiration                                  | 88 1.4%          | 21 4.8%          | 2 1.3%           |
| cell budding                                          | 85 1.3%          | 6 1.4%           | 3 2.0%           |
| cellular aromatic compound metabolic process          | 77 1.2%          | 13 3.0%          | 7 4.6%           |
| vesicle organization                                  | 77 1.2%          | 2 0.5%           | 3 2.0%           |
| peroxisome organization                               | 67 1.1%          | 2 0.5%           | 0 0.0%           |
We have identified subunits of ribosomes that were up-regulated by glucose (Table 4). Rps0bp and Rps8ap are components of the small (40S) ribosomal subunit, whereas Rpl3p and Rpl7bp are subunits of the 60S ribosomes [19,74,75].

We have also identified three proteins involved in different aspects of protein translation that were up-regulated. Tef2p is the translational elongation factor EF-1 alpha and functions in the binding reaction of aminoacyl-tRNA to ribosomes [76]. Tef4p is the gamma subunit of the translational elongation factor eEF1B. Tef4p stimulates the binding of aminoacyl-tRNA to ribosomes by releasing eEF1A from the ribosomal complex [77]. Lia1p is deoxyhypusine hydroxylase that catalyzes the formation of hypusine required for the modification of eIF5A [78]. Tef2p, Tef4p, and Lia1p have not been previously reported to be up-regulated by glucose.

The plasma membrane ATPase was identified as being up-regulated and was included in this group. Pma1p pumps protons out of the cell and is the major regulator of cytoplasmic pH and plasma membrane potential [4,79,80]. This protein is highly regulated by glucose both transcriptionally and post-translationally, as glucose not only induces PMA1 gene expression but also activates ATPase activity [4,79,80]. In the current study, we found that relative levels of Pma1p were higher following the addition of glucose. Pma1p is an abundant protein and is negatively regulated by Hsp30p [81]. Interestingly, we also observed a down-regulation of Hsp30p in this study (see Table 5).

### Proteins Down-Regulated by Glucose

The presence of high glucose reduces the transcription of genes involved in gluconeogenesis, glyoxylate cycle, and the TCA cycle [16,17]. Furthermore, glucose also causes the degradation of gluconeogenic enzymes. Although a recent mitochondrial proteomic study has indicated that mitochondrial proteins are remarkably constant [59], earlier studies have shown that the

| GO term                | LOCATION | A Genome Frequency | n = 6310 Percent | B Peptide >1 Frequency | n =438 Percent | C Peptide =1 Frequency | n =153 Percent |
|------------------------|----------|-------------------|-----------------|------------------------|----------------|------------------------|----------------|
| cytoplasm              |          | 3812              | 60.4            | 408                    | 93.2           | 126                    | 82.4           |
| nucleus                |          | 2080              | 33.0            | 88                     | 20.1           | 53                     | 34.6           |
| mitochondrion          |          | 1126              | 17.8            | 137                    | 31.3           | 28                     | 18.3           |
| unknown                |          | 779               | 12.3            | 4                      | 0.9            | 6                      | 3.9            |
| ER                     |          | 375               | 5.9             | 18                     | 4.1            | 17                     | 11.1           |
| ribosome               |          | 355               | 5.6             | 102                    | 23.3           | 17                     | 11.1           |
| plasma membrane        |          | 288               | 4.6             | 12                     | 2.7            | 4                      | 2.6            |
| vacuole                |          | 221               | 3.5             | 20                     | 4.6            | 2                      | 1.3            |
| cytoskeleton           |          | 208               | 3.3             | 13                     | 3.0            | 9                      | 5.9            |
| membrane fraction      |          | 208               | 3.3             | 52                     | 11.9           | 7                      | 4.6            |
| Golgi                  |          | 186               | 2.9             | 5                      | 1.1            | 7                      | 4.6            |
| cytoplasmic membrane-bounded vesicle | | 107               | 1.7             | 5                      | 1.1            | 6                      | 3.9            |
| cell wall              |          | 98                | 1.6             | 6                      | 1.4            | 1                      | 0.7            |
| peroxisome             |          | 64                | 1.0             | 7                      | 1.6            | 0                      | 0.0            |
| extracellular region   |          | 27                | 0.4             | 3                      | 0.7            | 0                      | 0.0            |

Peptide used for identification include only those having at least 95% confidence. (A) The distribution of 6310 proteins, (B) 438 proteins identified with more than one peptide and (C) 153 proteins identified with one peptide.
Table 4 Proteins up-regulated by glucose

| Name                                      | 115:113 | P     | 115:114 | P     | 116:113 | P     | 116:114 | P     | Ave  | SD     | t-test |
|-------------------------------------------|---------|-------|---------|-------|---------|-------|---------|-------|------|--------|--------|
| Rps0bp, 40S Ribosomal Subunit             | 2.1677  | 0.0324| 2.2909  | 0.0133| 1.9588  | 0.0369| 2.0324  | 0.0152| 2.1125  | 0.1274 | 0.0006 |
| Rps8bp, 40S Ribosomal Subunit             | 2.3768  | 0.0015| 3.8726  | 0.0006| 1.7865  | 0.0080| 2.8576  | 0.0088| 2.7234  | 0.7643 | 0.0298 |
| Rpl3p, 60S Ribosomal Subunit              | 1.3428  | 0.0266| 1.5136  | 0.0005| 1.1482  | 0.2264| 1.2942  | 0.0056| 1.3247  | 0.1305 | 0.0230 |
| Rpl7bp, 60S Ribosomal Subunit             | 1.9950  | 0.0129| 1.7380  | 0.0241| 1.8540  | 0.0121| 1.6290  | 0.0320| 1.8040  | 0.1360 | 0.0020 |
| Tef2p, Translational Elongation Factor EF-1 alpha | 2.4434  | 0.0134| 2.3550  | 0.0422| 2.2491  | 0.0146| 2.1677  | 0.0254| 2.3038  | 0.1044 | 0.0002 |
| Tef4p, Translational Elongation Factor eEF1B | 2.5586  | 0.0441| 1.9588  | 0.0281| 2.5119  | 0.0528| 1.9231  | 0.0275| 2.2381  | 0.2979 | 0.0055 |
| Lia1p, Deoxyhypusine Hydroxylase           | 2.9107  | 0.0115| 3.3113  | 0.0081| 2.6546  | 0.0169| 3.0479  | 0.0116| 2.9811  | 0.2372 | 0.0007 |
| Pma1p, Plasma Membrane H+-ATPase           | 1.4859  | 0.0112| 2.0701  | 0.0020| 1.5276  | 0.0224| 2.1281  | 0.0032| 1.8029  | 0.2972 | 0.0184 |

| Accession # | Name                                      | Peptides (95%) | 113:114 | P     | 115:116 | P     | t-test |
|-------------|-------------------------------------------|----------------|---------|-------|---------|-------|--------|
| gi|6323077| Rps0bp, 40S Ribosomal Subunit              | 12              | 1.0471  | 0.5788| 1.0864| 0.9352| 0.1823 |
| gi|6320949| Rps8bp, 40S Ribosomal Subunit              | 14              | 1.6596  | 0.2979| 1.3428| 0.4164| 0.1949 |
| gi|6324637| Rpl3p, 60S Ribosomal Subunit              | 23              | 1.1272  | 0.0651| 1.1695| 0.2455| 0.0902 |
| gi|6325058| Rpl7bp, 60S Ribosomal Subunit              | 14              | 0.8318  | 0.6646| 1.0186| 0.8621| 0.5701 |
| gi|90031872| Tef2p, Translational Elongation Factor EF-1 alpha | 63              | 0.9727  | 0.8135| 1.0666| 0.9691| 0.7477 |
| gi|6322769| Tef4p, Translational Elongation Factor eEF1B | 10              | 0.7244  | 0.7899| 1.0000| 0.9030| 0.5000 |
| gi|6322531| Lia1p, Deoxyhypusine Hydroxylase           | 4               | 1.1588  | 0.7297| 1.0765| 0.7288| 0.2142 |
| gi|6321430| Pma1p, Plasma Membrane H+-ATPase           | 12              | 1.4454  | 0.5425| 0.9462| 0.8061| 0.5765 |

(A) Proteins that increased their relative levels in response to glucose re-feeding. Average ratios (Avg) and standard deviations (SD) are given, along with the p-values (P) calculated for each protein's ratio by ProteinPilot™ 4.0 based on the ratios of each identified peptide. A one-sample Student's t-test was used to confirm that each protein's set of ratios for all four comparisons was significantly different from 1 and all p-values are <0.05.

(B) Ratios of 113:114; starved:starved and 115:116; re-fed:re-fed with the number of distinct peptides having at least 95% confidence and a p-value (P) greater than 0.05 calculated for each protein's ratio by ProteinPilot™ 4.0 based on the ratios of each identified peptide. A one-sample Student's t-test was used to confirm that each protein's set of ratios for both comparisons was not significantly different from 1 and all p-values are >0.05.
### Table 5 Proteins down-regulated by glucose

#### A

| Name                                | 115:113 P | 115:114 P | 116:113 P | 116:114 P | Ave     | SD      | t-test  |
|-------------------------------------|-----------|-----------|-----------|-----------|---------|---------|---------|
| Hxk1p, Hexokinase A                 | 0.3908    | 0.0002    | 0.4529    | 0.0025    | 0.5200  | 0.0015  | 0.5970  |
| Pg1p, Phosphoglucone Isomerase       | 0.6792    | 0.0267    | 0.4529    | 0.0042    | 0.7178  | 0.2011  | 0.4613  |
| Pgm2p, Phosphoglucomutase           | 0.4875    | 0.0137    | 0.4246    | 0.0068    | 0.6918  | 0.0936  | 0.6026  |
| Fbp1p, Fructose-1,6-Bisphosphatase  | 0.1528    | 0.0001    | 0.1259    | 0.0001    | 0.0731  | 0.0001  | 0.0625  |
| Icl1p, Isocitrate Lyase              | 0.3281    | 0.0001    | 0.2630    | 0.0001    | 0.2630  | 0.0001  | 0.2128  |
| Mls1p, Malate Synthase              | 0.5395    | 0.0062    | 0.4285    | 0.0019    | 0.7656  | 0.05704 | 0.5916  |
| Ach1p, Acetyl CoA Hydrolase          | 0.4831    | 0.0021    | 0.5152    | 0.0036    | 0.6546  | 0.0213  | 0.6855  |
| Atp2p, Mitochondrial ATP Synthase Subunit Beta | 0.4966    | 0.0059    | 0.2938    | 0.0053    | 0.6252  | 0.0219  | 0.3597  |
| Om45p, Mitochondrial Outer Membrane Protein | 0.4613    | 0.0045    | 0.5702    | 0.0144    | 0.4656  | 0.0020  | 0.5649  |
| Hsp12p, Heat Shock Protein          | 0.3565    | 0.0091    | 0.3251    | 0.0945    | 0.2630  | 0.0020  | 0.2466  |
| Hsp26p, Heat Shock Protein          | 0.5445    | 0.0130    | 0.5861    | 0.0162    | 0.3698  | 0.0013  | 0.4018  |
| Hsp30p, Heat Shock Protein          | 0.3981    | 0.0922    | 0.3221    | 0.0446    | 0.2312  | 0.0393  | 0.1905  |

(A) Proteins that decreased their relative levels in response to glucose re-feeding. Average ratios (Avg) and standard deviations (SD) are given, along with the p-values (P) calculated for each protein's ratio by ProteinPilot™ 4.0 based on the ratios of each identified peptide. A one-sample Student's t-test was used to confirm that each protein's set of ratios for all four comparisons was significantly different from 1 and all p-values are <0.01.

#### B

| Accession # | Name                                | Peptides (95%) | 113:114 P | 115:116 P | t-test  |
|-------------|-------------------------------------|----------------|-----------|-----------|---------|
| gi|6321184 | Hxk1p, Hexokinase A | 20 | 1.3804 | 0.7068 | 0.4962 | 0.7992 |
| gi|6319673 | Pg1p, Phosphoglucone Isomerase | 26 | 0.6368 | 0.3942 | 0.9204 | 0.2883 |
| gi|817863 | Pgm2p, Phosphoglucomutase | 18 | 0.8241 | 0.7017 | 0.6730 | 0.2974 |
| gi|6323409 | Fbp1p, Fructose-1,6-Bisphosphatase | 7 | 0.8166 | 0.1819 | 1.9953 | 0.1888 |
| gi|6320908 | Icl1p, Isocitrate Lyase | 14 | 0.7870 | 0.2416 | 1.2589 | 0.1990 |
| gi|6324212 | Mls1p, Malate Synthase | 13 | 0.7586 | 0.5169 | 0.7112 | 0.2358 |
| gi|6319456 | Ach1p, Acetyl CoA Hydrolase | 16 | 1.0375 | 0.6989 | 0.7311 | 0.1846 |
| gi|84028178 | Atp2p, Mitochondrial ATP Synthase Subunit Beta | 20 | 0.6081 | 0.9613 | 0.8472 | 0.4900 |
| gi|730224 | Om45p, Mitochondrial Outer Membrane Protein | 17 | 1.2023 | 0.5644 | 0.9008 | 0.6866 |
| gi|836740 | Hsp12p, Heat Shock Protein | 35 | 0.9462 | 0.2305 | 1.3428 | 0.4280 |
| gi|6319546 | Hsp26p, Heat Shock Protein | 41 | 1.0965 | 0.8963 | 1.4859 | 0.1689 |
| gi|6319869 | Hsp30p, Heat Shock Protein | 6 | 0.7943 | 0.4318 | 1.7701 | 0.5325 |

(B) Ratios of 113:114; starved:starved and 115:116; re-fed:re-fed with the number of distinct peptides having at least 95% confidence and a p-value (P) greater than 0.05 calculated for each protein's ratio by ProteinPilot™ 4.0 based on the ratios of each identified peptide. A one-sample Student's t-test was used to confirm that each protein's set of ratios for both comparisons was not significantly different from 1 and all p-values are >0.05.
mitochondrial F1 subunits decrease their levels in glucose-repressed cells [82]. In our current study, we have identified 12 proteins that were down-regulated by glucose (Table 5A). These proteins are involved in gluconeogenesis and mitochondrial functions. Small heat shock proteins were also identified in this group. Down-regulation of proteins in these functional groups correlates with the observation that genes in these same categories are repressed by glucose.

For glycolytic enzymes that were down-regulated by glucose, Hxk1p is involved in the phosphorylation of glucose at the C6 position in the first irreversible step in glucose metabolism [83,84]. Pgi1p (phosphoglucoisomerase) catalyzes the interconversion of glucose-6-phosphate and fructose-6-phosphate [85]. Our iTRAQ data indicate that relative levels of Hxk1p and Pgi1p were reduced following the addition of glucose. Pgm2p (Gal5p) is the major isofrom of phosphoglucomutase that catalyzes the interconversion of glucose-1-phosphate to glucose-6-phosphate. As such, Pgm2p is involved in glycolysis, the pentose phosphate pathway, and the metabolism of glycogen, galactose, and trehalose [86]. Glucose not only represses transcription of PGM2 but also reduces activity of Pgm2p [17,86,87]. Consistent with the down-regulation of the PGM2 gene and its activity, the addition of glucose to glucose-starved cells caused a decrease in the abundance of Pgm2p (Table 5).

As mentioned, we used the known glucose-induced degradation of four gluconeogenic enzymes as our internal control. Indeed, we detected a very dramatic decline in protein levels for Fbp1p and Icl1p. Fbp1p is a key enzyme in the irreversible steps of gluconeogenesis. Fbp1p converts fructose-1,6-bisphosphate to fructose-6-bisphosphate, and the gene coding for Fbp1p is repressed by glucose [4,34]. Furthermore, this protein is also degraded in response to glucose [45-51]. Consistent with these observations, levels of Fbp1p were reduced following the addition of glucose. Another gluconeogenic enzyme, Mdh2p, was identified in this study and showed a decrease in abundance following the addition of glucose. However, the p-value was higher than 0.05. As such, this protein was not included in Table 5. Pck1p is another key enzyme in the irreversible step of gluconeogenesis. It catalyzes the formation of phosphoenolpyruvate from oxaloacetate. Pck1p was also identified in this study and showed a dramatic decrease in levels in response to glucose [88]. Icl1p catalyzes the formation of succinate and glyoxylate from isocitrate [88], whereas Mls1p catalyzes the formation of malate from glyoxylate in the glyoxylate cycle [89]. Levels of these proteins were reduced following the addition of glucose.

ACH1 encodes CoA transferase that hydrolyses acetyl-CoA and transfers CoASH from succinyl-CoA to acetate. Glucose causes the repression of the ACH1 gene and a reduction in Ach1p activity [30]. We showed that levels of Ach1p were also reduced when glucose was added to glucose-starved cells (Table 5).

It has been documented that glucose suppresses genes encoding mitochondrial proteins and reduces the activity of several mitochondrial enzymes such as NADH dehydrogenase, aconitase, cytochrome c oxidase, and the mitochondrial ATPase [34,82]. The presence of high glucose not only reduces ATPase activity but also decreases levels of the F1 subunits [82]. F1 consists of Atp1p and Atp2p and is the catalytic subunits of the ATPase [90-92]. Our data showed that levels of Atp2p were also reduced when glucose was added to glucose-starved cells. Another mitochondrial protein Om45p was also down-regulated by glucose. Om45p is a major constituent of the mitochondrial outer membrane, however, the function of Om45p is currently unknown.

We have also identified three heat shock proteins that were down-regulated. Hsp12p is a small heat shock protein localized to the plasma membrane [93,94]. Hsp26p is a heat shock protein with chaperone activity [95]. Hsp30p is a stress-responsive protein localized to the plasma membrane. Hsp30p is reported to negatively regulate Pma1p [81]. Down-regulation of Hsp30p may relieve the inhibitory effects of Hsp30p on Pma1p. Interestingly, many of these proteins such as Hxk1p, Mls1p, Om45p, Hsp26p, and Ach1p were up-regulated when cells were grown in glucose-limited conditions [9]. Here, we show that when glucose-starved cells were transferred to medium containing fresh glucose, levels of these proteins were reduced. Therefore, we suggest that these proteins are highly regulated by the availability of glucose.

Expression of Subunits in Protein Complexes

Because many proteins do not function on their own but as part of larger protein complexes, we next examined how subunits of some well-described protein complexes are regulated following the regime of glucose depletion and re-addition (Table 6). Phosphofructokinase (PFK) is a key enzyme in glycolysis and catalyzes the formation of fructose-1,6-bisphosphate from fructose-6-phosphate and ATP. PFK is a hetero-oligomeric enzyme composed of four alpha (Pfk1p) and four beta subunits (Pfk2p) [96]. Glucose not only induces the transcription of the PFK1 and PFK2 genes but also stimulates the activity of the PFK enzyme [17,27]. Therefore, we examined
whether or not up-regulation of PFK activity correlates with an increase in protein expression. We have identified both Pfk1p and Pfk2p in our study. However, levels of these proteins did not change significantly before or after the addition of glucose for 2 hours (Table 6).

The plasma membrane ATPase is a large protein required for acidification of the cell and is up-regulated by glucose (see Table 4). The vacuole ATPase is responsible for the acidification of the vacuole and is a multi-subunit complex consisting of the V0 and V1 subunits [97,98]. Glucose regulates the activity of the vacuolar ATPase in a different way. In the presence of low glucose, V0 and V1 are disassembled and the V1 subunit is distributed in the cytoplasm [97,98]. The addition of glucose to glucose-depleted cells does not change levels of the V-ATPase subunits but causes the V0 and V1 subunits to assemble into a functional ATPase [97,98]. We have identified 6 out of the 15 subunits of the V-ATPase subunits, and levels of these proteins did not change significantly following the addition of glucose to glucose-starved cells. Our results are consistent with the notion that glucose regulates the function of the V-ATPase primarily through the assembly of the V0 and V1 subunits.

Next, we sought to examine how subunits of the mitochondrial ATPase were regulated by glucose. The mitochondrial F0/F1 ATP synthase is a large, evolutionarily conserved complex of enzymes required for ATP synthesis [90,91]. This complex of enzymes consists of a membrane-bound F0, a soluble F1 component, a central and a peripheral stator [90,91]. Interestingly, the assembly of the F1 subunit is independent of the F0 subunit [90,91]. We have identified 11 of 17 subunits of F0/F1 ATP synthase. Levels of Atp1p, Atp2p, and Atp3p were reduced following the addition of glucose. However, most of the ATP synthase subunits did not change their expression levels. Therefore, not all the ATP synthase subunits decreased their levels to the same extent when glucose was added to glucose-starved cells.

The presence of glucose increases mRNAs 2–4 fold for more than 90% of the genes encoding ribosomal proteins [17]. Therefore, we examined whether or not glucose causes similar changes in the abundance of ribosomal subunits. Ribosomes are highly conserved large ribonucleoprotein (RNP) particles consisting of a small 40S subunit and a large 60S subunit required for protein synthesis [17,19,74,75]. The 40S subunit has 32 proteins and the 60S subunit contains 42 proteins [19,74,75]. We have identified 25 subunits of the 40S ribosome and 38 subunits of the 60S ribosome (Table 6). The subunits that showed significant changes in abundance are highlighted. For the 40S ribosomal subunit, fewer than 6 subunits increased their levels, while 19 did not show significant changes. For the 60S ribosomal subunits, fewer than 10 subunits increased their abundance but 28 subunits did not change their levels. Therefore, less than 30% of the ribosomal subunits showed an increase in their abundance in response to glucose under our conditions.

To validate our proteomic data, we examined changes in levels of proteins in response to glucose. Cells expressing Lia1p-GFP, Fbp1p-GFP, Icl1p-GFP, Mls1p-GFP, and Hsp30p-GFP were starved of glucose and then transferred to medium containing high glucose for 0, 2, and 3 hours. Levels of these proteins were then examined by Western blotting with anti-GFP antibodies (Figure 3). In response to glucose, levels of Lia1p-GFP increased. In contrast, levels of Fbp1p-GFP, Icl1p-GFP, Mls1p-GFP, and Hsp30p-GFP were reduced when glucose was added to glucose-starved cells. Therefore, these results confirmed our proteomic data that Lia1p is up-regulated by glucose, whereas Fbp1p, Icl1p, Mls1p, and Hsp30p are down-regulated by glucose.

**Discussion**

In this paper, we report the identification of 591 proteins from yeast cells grown in glucose-deficient medium and transferred to glucose-rich medium for 2 hours using the iTRAQ and MALDI techniques. A previous study by Kolkman et al has identified 928 proteins that were expressed in carbon and nitrogen limitations and 759 proteins were quantified using the SILAC (stable isotope labeling with amino acids in cell culture) and ESI (electrospray ionization) techniques [9]. In another study by Pham et al using the iTRAQ and ESI techniques, 451 proteins were identified and 246 were quantified [15]. When we compared the overlap of proteins identified in these three studies, 150 proteins were present in all three studies (Figure 4). 365 proteins overlapped between our study and the study by Kolkman et al and 188 proteins overlapped between our study and the study by Pham et al. Furthermore, 188 proteins were unique to our study, whereas 370 proteins were unique to the study by Kolkman et al and 36 proteins were unique to the study by Pham et al. Given that each study identified unique sets of proteins, these methods appear to complement each other. Again, each of these studies used different experimental conditions. Hxk1p, Mls1p, Acl1p, Om45p, and Hsp26 were up-regulated under carbon limitation in the study by Kolkman et al (Table 7). Hxk1p, Pgi1p, and Pgm2p were up-regulated, whereas Tef2p, Hsp12p, and Hsp26p were down-regulated in high glucose (300 g/L) (Table 7) in the study by Pham et al.

It has been reported that glucose induces miRNA levels 2–4 fold for more than 90% of the genes encoding ribosomal proteins [17]. When relative levels of ribosomal proteins were examined in our study, not all ribosomal subunits increase their abundance in response to glucose. For the key glycolytic enzyme phosphofructokinase, levels of Pfk1p and Pfk2p were similar whether cells were glucose starved or glucose replenished for 2 hours. We suggest that
| Accession # | Name | Peptides (95%) | 113:114 | 115:116 | 115:113 | 116:113 | 115:114 | 116:114 |
|-------------|------|---------------|--------|--------|--------|--------|--------|--------|
| **Phosphofructokinase (2/2)** | | | | | | | | |
| gi|6321679 | Pfk1p | 18 | 0.9098 | 0.9817 | 0.9817 | 0.9908 | 0.9817 | 0.9908 |
| gi|172140 | Pfk2p | 16 | 0.9727 | 0.9727 | 0.9908 | 1.0093 | 0.9727 | 0.9908 |
| **Vacualar H + Atpase (6/15)** | | | | | | | | |
| gi|6320016 | Tfp1p | 21 | 0.9376 | 0.9550 | 0.9550 | 0.9908 | 0.9036 | 0.9376 |
| gi|6324844 | Vph1p | 3 | 0.9462 | 1.0000 | 1.0093 | 1.0093 | 0.9638 | 0.9550 |
| gi|6319603 | Vma2p | 10 | 0.9550 | 0.9908 | 0.9727 | 0.9727 | 1.0864 | 0.8790 |
| gi|6324907 | Vma4p | 3 | 0.8954 | 0.8872 | 0.9727 | 1.0864 | 0.8790 | 0.9817 |
| gi|6322770 | Vma5p | 1 | 0.9376 | 1.0568 | 1.2359 | 1.1482 | 1.1588 | 1.0864 |
| gi|6325293 | Vma13p | 2 | 0.8630 | 1.5136 | 1.5560 | 1.0186 | 1.3552 | 0.8872 |
| **F0/F1 ATP synthase (11/17)** | | | | | | | | |
| gi|56404985 | Atp1p | 26 | 0.8790 | 1.1066 | 0.8017 | 0.7244 | 0.7178 | 0.6546 |
| gi|84028178 | Atp2p | 20 | 0.6081 | 0.8472 | 0.4966 | 0.6252 | 0.2938 | 0.3597 |
| gi|6319513 | Atp3p | 4 | 1.0471 | 0.6368 | 0.4742 | 0.7379 | 0.5012 | 0.7727 |
| gi|6325179 | Atp4p | 3 | 1.0186 | 1.0186 | 0.9638 | 0.9376 | 0.9908 | 0.9638 |
| gi|849218 | Atp5p | 5 | 1.0000 | 1.0471 | 0.8017 | 0.7516 | 0.8017 | 0.7586 |
| gi|6322836 | Atp7p | 2 | 0.9376 | 0.9462 | 0.9120 | 0.9462 | 0.8630 | 0.8954 |
| gi|6323326 | Atp14p | 2 | 1.0765 | 1.0471 | 0.962 | 0.8872 | 1.0280 | 0.9638 |
| gi|6324084 | Atp15p | 1 | 0.7379 | 1.0375 | 1.0568 | 1.0093 | 0.7943 | 0.7586 |
| gi|849198 | Atp17p | 2 | 0.9817 | 0.8241 | 0.7727 | 0.9290 | 0.7656 | 0.9204 |
| gi|6324495 | Atp15p | 1 | 0.8091 | 0.9908 | 1.0965 | 1.0965 | 0.9036 | 0.8954 |
| gi|6320529 | Tim11p | 4 | 0.8872 | 0.9376 | 0.8630 | 0.9120 | 0.7727 | 0.8166 |
| **Small 40S Ribosomal Subunit (25/32)** | | | | | | | | |
| gi|6323077 | Rps0bp | 12 | 1.0471 | 1.0864 | 2.1677 | 1.9588 | 2.2909 | 2.0324 |
| gi|665976 | Rps1ap | 18 | 1.0362 | 1.2246 | 0.8551 | 0.6918 | 1.1272 | 0.9120 |
| gi|6321315 | Rps2p | 9 | 1.0864 | 1.1695 | 1.0965 | 0.9290 | 1.2023 | 1.0280 |
| gi|6322605 | Rps4ap | 20 | 1.1272 | 1.1482 | 1.6144 | 1.3804 | 1.8365 | 1.5704 |
| gi|895891 | Rps5p | 11 | 0.8166 | 1.2706 | 1.7378 | 1.3428 | 1.4191 | 1.1695 |
| gi|6327517 | Rps6ap | 8 | 0.8630 | 1.3305 | 1.9231 | 1.4723 | 1.7701 | 1.3183 |
| gi|758292 | Rps7bp | 6 | 1.1066 | 1.0765 | 1.1169 | 1.0280 | 1.2359 | 1.1376 |
| gi|6320949 | Rps8bp | 14 | 1.6596 | 1.3428 | 2.3768 | 1.7865 | 3.8726 | 2.8576 |
| gi|6319666 | Rps9bp | 14 | 1.0186 | 1.1912 | 1.8707 | 1.5417 | 1.9055 | 1.5704 |
| gi|889940 | Rps12p | 4 | 0.8954 | 0.9550 | 1.0186 | 1.0568 | 0.9204 | 0.9550 |
| gi|798915 | Rps13p | 4 | 1.0186 | 1.0186 | 1.0568 | 1.0280 | 1.0864 | 1.0568 |
| gi|730453 | Rps14bp | 6 | 1.0280 | 1.1588 | 1.1482 | 0.9817 | 1.1912 | 1.0186 |
| gi|6324533 | Rps15p | 5 | 1.0765 | 1.0568 | 1.0093 | 0.9550 | 1.0965 | 1.0280 |
| gi|9755341 | Rps16ap | 8 | 1.0375 | 1.1376 | 1.2823 | 1.1272 | 1.3552 | 1.1803 |
| gi|642297 | Rps18ap | 13 | 0.8790 | 1.0375 | 1.5849 | 1.4454 | 1.4322 | 1.3305 |
| gi|6324451 | Rps19ap | 8 | 1.0093 | 1.0965 | 1.0568 | 0.9550 | 1.0765 | 0.9817 |
| gi|730687 | Rps20p | 5 | 1.0765 | 1.0666 | 1.1376 | 1.0568 | 1.2359 | 1.1482 |
| gi|84028229 | Rps21ap | 6 | 0.9727 | 1.2589 | 2.2491 | 1.7539 | 2.2080 | 1.7539 |
| gi|6325389 | Rps23bp | 6 | 0.9550 | 1.0280 | 1.3183 | 1.2706 | 1.2706 | 1.2134 |
| gi|730648 | Rps24ap | 13 | 0.8954 | 1.2942 | 1.7378 | 1.3305 | 1.5704 | 1.2023 |
| gi|83288131 | Rps25bp | 2 | 1.0765 | 1.1376 | 1.1376 | 0.9908 | 1.2359 | 1.0765 |
| Protein Complex | Subunit | Control | Control |
|-----------------|---------|---------|---------|
| gi|730459 | Rps26bp | 4 | 1.0375 | 0.9908 | 1.1066 | 1.1066 | 1.1588 | 1.1482 |
| gi|730460 | Rps27bp | 5 | 0.8954 | 1.0765 | 1.2134 | 1.1169 | 1.0965 | 1.0093 |
| gi|85695430 | Rps28bp | 3 | 1.0375 | 0.8872 | 1.0186 | 1.1376 | 1.0765 | 1.2023 |
| gi|730461 | Rps29ap | 8 | 0.9204 | 0.9036 | 1.0568 | 1.1588 | 0.9817 | 1.0765 |
| gi|6323196 | Rps31p | 12 | 1.3428 | 0.8017 | 0.8872 | 1.1272 | 1.2474 | 1.4859 |

**Ratios that are significantly different from 1.0 (p < 0.05) in bold.**

113:114; starved:starved and 115:116; re-fed/re-fed (control ratios). 115:113, 116:113, 115:114, 116:114; re-fed:starved.
other mechanisms such as allosteric stimulation, protein modifications, or subunit assembly are likely to play more important roles in the regulation of the PFK activity.

One of the well-known effects of glucose regulation is the activation of the plasma membrane ATPase [4,56-58]. In this study, we have observed an increase in relative levels of Pma1p following the addition of glucose for 2 hours. For the same period of time, we did not find significant changes in the abundance of 6 out of the 15 subunits of the vacuole ATPase. The mitochondrial ATP synthase consists of 17 subunits. Of the 11 subunits that we have identified, 3 subunits reduced their abundance, while 8 other subunits did not change their levels. Atp1p and Atp2p are components of the catalytic F1 subunit which is known to be repressed by glucose [82]. Down-regulation of these subunits by glucose may be sufficient to cause a reduction in the ATPase activity. This is consistent with the finding that glucose-repressed cells contain fewer F1 particles in mitochondria as observed by electron microscopy of negatively stained mitochondria membranes [82]. It has been reported that many of the mitochondrial proteins such as Atp1p, Atp2p, Atp4p, Atp5p, Atp15p, Atp16p, and Atp20p are phosphorylated [99]. We suggest that protein degradation, protein modifications, allosteric inhibition, and subunit assembly may all contribute to the known decreased activities of mitochondrial enzymes that consist of multiple subunits.

Proteins that were observed to be down-regulated in the current experiments in response to glucose include several previously reported to be down-regulated such as Pgm2p, Fbp1p, Icl1p, Mls1p, Ach1p, Atp1p, Hsp12, Hsp26p, Hsp30p, and several proteins that were not previously reported to be down regulated, such as Hxk1p, Pgi1p, and Om45p. It is known that the transcription of ACH1, HXK1, HSP12, and HSP26 genes are repressed by glucose [24,28]. The levels of the corresponding proteins were also observed in the current work to be reduced in response to glucose addition.

A dramatic decline in protein levels in response to glucose was observed for the gluconeogenic enzymes Fbp1p and Icl1p. Malate synthase is involved in the gluconeogenesis/glyoxylate pathway. Transcription of MLS11 is repressed by glucose [16]. Furthermore, Mls1p activity is reduced following the addition of glucose [43]. Down-regulation of Fbp1, Icl1p, Mls1p, and Hsp30p by glucose was confirmed by Western blotting using cells that expressed GFP tagged proteins (Figure 3).

In summary, glucose up-regulates proteins involved in protein synthesis and nutrient uptake (Table 7). It also down-regulates small heat shock proteins, mitochondrial proteins, and proteins involved in gluconeogenesis (Table 7). For up-regulated proteins, glucose increases the abundance of several of the 40S and 60S ribosomal subunits, Tef2p, Tef4p, and Lia1p. Increased expression...
Table 7 Proteins that were up-regulated and down-regulated by glucose in our study and the comparison with the studies by Kolkman et al. [9] and Pham et al. [15]

| Function          | Up-regulated in our study             | Kolkman et al. [9] | Pham et al. [15] |
|-------------------|---------------------------------------|--------------------|------------------|
| Ribosome          | Rps0bp, 40S Ribosomal Subunit          | identified         | not identified   |
| Ribosome          | Rps8bp, 40S Ribosomal Subunit          | identified         | not identified   |
| Ribosome          | Rpl3p, 60S Ribosomal Subunit           | identified         | not identified   |
| Ribosome          | Rpl7bp, 60S Ribosomal Subunit          | not identified     | not identified   |
| Translation       | Tef2p, Translational Elongation Factor EF-1 alpha | not identified | Down-regulated in high glucose |
| Translation       | Tef4p, Translational Elongation Factor eEF1B | identified | not identified   |
| Translation       | Lia1p, Deoxyhypusine Hydroxylase       | identified         | not identified   |
| Nutrient Uptake   | Pma1p, Plasma Membrane H+ --ATPase     | Down-regulated under carbon limitation | not identified   |

**Glycolysis**
- Hxk1p, Hexokinase A
- Pgi1p, Phosphoglucone Isomerase
- Pgm2p, Phosphoglucomutase
- Fbp1p, Fructose-1,6-Bisphosphatase
- Icl1p, Isocitrate Lyase
- Mis1p, Malate Synthase

**Gluconeogenesis**
- Fbp1p, Fructose-1,6-Bisphosphatase
- Icl1p, Isocitrate Lyase
- Atp2p, Mitochondrial ATP Synthase Subunit Beta
- Om45p, Mitochondrial Outer Membrane Protein
- Hsp12p, Heat Shock Protein
- Hsp26p, Heat Shock Protein
- Hsp30p, Heat Shock Protein

Kolkman et al examined steady state expression of proteins in cells grown in two different conditions [9]. Carbon-limited medium contained 19 mM (NH4)2SO4 and 42 mM glucose. Nitrogen limited medium contained 7.5 mM (NH4)2SO4 and 330 mM glucose. Hxk1p, Ach1p, Mls1p, Om45p, and Hsp26p were up-regulated under carbon limitation, whereas Pma1p was down-regulated under carbon limitation. Pgi1p, Icl1p, and Atp2p were identified but showed no significant change in carbon limitation versus nitrogen limitation.

Pham et al examined steady state levels of proteins in cells grown in 120 g/L (normal) to 210 g/L and 300 g/L (high) concentrations of glucose for 68 hours [15]. Hxk1p, Pgi1p, and Pgm2p were up-regulated in high glucose, whereas Tef2p, Hsp12p, and Hsp26p were down-regulated by high glucose.
of these proteins may lead to an increase in protein synthesis. Glucose also up-regulates the plasma membrane ATPase, which is needed for the uptake of nutrients. In addition, glucose causes down-regulation of a number of proteins involved in glycolysis/gluconeogenesis, the TCA cycle, and the glyoxylate cycle (Figure 5). Although the significance of down-regulation of Hxk1p and Pgi1p is not clear at present, down-regulation of Pgm2p may lead to an increase in glycolysis. Pgm2p is the major enzyme that catalyzes the interconversion of glucose-6-phosphate and glucose-1-phosphate. Down-regulation of Pgm2p may reduce levels of glucose-1-phosphate required for the pentose pathway and the synthesis of glycogen, galactose, and trehalose. Consequently, more glucose-6-phosphate is available for the glycolytic pathway. Hence, down-regulation of Pgm2p may result in an increase in glycolysis. Glucose also causes the down-regulation of gluconeogenic enzymes, which leads to a decrease in gluconeogenesis. Down-regulation of Ach1p may cause a decline in the utilization of acetate when glucose is present. As Atp1p and Atp2p are components of the F1 catalytic subunits of the mitochondrial ATPase, decreased expression of these proteins may be sufficient to reduce the activity of the ATPase. The significance of down-regulation of these heat shock proteins in response to glucose is not presently known. One possibility is that...
some of these proteins need to be removed in order for cells to adapt to the new environments. For instance, Pma1p is negatively regulated by Hsp30p. Down-regulation of Hsp30p may remove the inhibitory effects of Hsp30p leading to the activation of Pma1p. Activated Pma1p may then stimulate the uptake of nutrients into the cells.

Conclusions
Using the iTRAQ technique, several of the enzymes that are known to be regulated by glucose were identified in our study. Furthermore, we have also identified new glucose-regulated proteins that have not been previously reported to be regulated by glucose. Up-regulation of ribosomal proteins and proteins involved in protein translation may increase protein synthesis. Up-regulation of the plasma membrane ATPase may result in enhanced nutrient uptake. Down-regulation of glycolytic enzymes, gluconeogenic enzymes, and mitochondrial proteins may lead to changes in glycolysis, gluconeogenesis, and mitochondrial functions. These changes may be beneficial for cells to adapt to the new environments.

Methods
Cell Culture and Media
Yeast cells (BY4742, MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) were grown in glucose-starved conditions in YPKG containing 1% yeast extracts, 2% peptone, 1% potassium acetate, and 0.5% glucose for 3 days and then shifted to YPD media containing 1% yeast extracts, 2% peptone, and 2% glucose for 2 hrs.

iTRAQ Sample Preparation
Cells were lysed in 500 mM Hepes (pH 7.2), 50 mM MgSO4, 1 mM EDTA, and 1% SDS by vortexing with glass beads. A Bio Rad Protein Assay was used to measure the protein concentration in each sample. Protein from each sample (140 μg) was processed according to the Applied Biosystems iTRAQ kit, with the exceptions that iodoacetamide was used as the alkylation agent rather than MMTS and trypsin digestion was performed with Promega Sequencing Grade Trypsin (#: V511) overnight at 48°C. Protein from each sample (7 μg) was removed before and after addition of trypsin and digestion was confirmed by electrophoresis and Coomassie Blue staining. iTRAQ labeling was performed as follows: wild type S. cerevisiae cultures were starved of glucose for three days in YPKG medium and extracted peptides from duplicated samples were tagged with reagents 113 and 114. Similarly, duplicated samples from 3 day starved wild type S. cerevisiae cultures, which had been re-fed with glucose for 2 hours, were trypsin digested and tagged with reagents 115 and 116. These peptides were combined and subjected to 2D-LC separation and one MS/MS run.

2D-LC Separation and Mass Spectrometry
After iTRAQ labeling, the peptides from all separate labeling reactions were mixed together, dried down, and re-suspended in 10 ml of 10 mM ammonium formate, pH 3.6, in 20% acetonitrile/80% water. The combined peptides were separated by strong cation exchange separation (SCX) followed by reverse phase C18 nanoflow-LC separation, and spotted onto a stainless steel MALDI target plate, for a total of 370 spots per original SCX fraction.

SCX Separations were performed on a Passivated Waters 600E HPLC system, using a 4.6 × 250 mm Poly-SULFOETHYL Aspartamide column (PolyLC, Columbia, MD) at a flow rate of 1 ml/min. Buffer A contained 10 mM ammonium formate, pH 3.6, in 20% acetonitrile/80% water. Buffer B contained 666 mM ammonium formate, pH 3.6, in 20% acetonitrile/80% water.

The gradient was Buffer A at 100% (0–22 minutes following sample injection), 0% → 40% Buffer B (22–48 min), 40% → 100% Buffer B (48–49 min), 100% Buffer B isocratic (49–56 min), then at 56 min switched back to 100% Buffer A to re-equilibrate for the next injection. The first 28 ml of eluant (containing all flow-through fractions) were combined and separated by strong cation exchange separation (SCX) followed by reverse phase C18 nanoflow-LC separation.

For the 2nd dimension separation by C18 reverse phase nanoflow LC, each SCX fraction was auto injected onto a Chromolith CapRod column (150 × 0.1 mm, Merck) using a 5 μl injector loop on a Tempo LC MALDI Spotting system (ABI-MDS/Sciex). Buffer C was 2% acetonitrile, 0.1% trifluoroacetic acid and filtered prior to reverse phase C18 nanoflow-LC separation.

The C18 elution gradient was 95% Buffer C/5% Buffer D (2 μl per minute flow rate from 0–3 min, then 2.5 μl per minute from 3–8.1 min), 5% Buffer D → 38% Buffer D (8.1–40 min), 38% Buffer D → 80% Buffer D (41–44 min), 80% Buffer D → 5% Buffer D (44–49 min) (initial conditions). Flow rate was 2.5 μl /min during the gradient, and an equal flow of MALDI matrix solution was added post-column (7 mg/ml recrystallized CHCA (a-cyano-hydroxycinnamic acid), 2 mg/ml ammonium phosphate, 0.1% trifluoroacetic acid, 80% acetonitrile).

The combined eluant was automatically spotted onto a stainless steel MALDI target plate every 6 seconds (0.6 μl per spot), for a total of 370 spots per original SCX fraction. Each MALDI target plate was analyzed in a data-dependent manner on either an ABI 5800
MALDI TOF-TOF or an ABI 4800 MALDI TOF-TOF. The MS spectra were taken from 5500 total MALDI Spots, averaging 500 laser shots per spot at laser power 2800. In a data-dependent manner, 12767 MS/MS spectra were taken from those same MALDI Spots, using up to 2600 laser shots per spectrum at laser power 3200, with CID gas at 1.2 to 1.3 × 10⁻⁶ Torr.

Data analysis
All the data sets from the different plates were analyzed simultaneously; with protein identification and quantitation performed using the Paragon Algorithm [100] as implemented in ProteinPilot™ 4.0 software (ABSciex). ProteinPilot™ search parameters were set as follows: cysteine alkylation: iodoacetamide, ID focus: biological modifications, and search effort: thorough. We searched the combined spectra against the species-specific (S. cerevisiae) NCBI-nr database concatenated with a reversed "decoy" version of the same database plus 156 common lab contaminants identified by Keller et al [101]. The species-specific (S. cerevisiae) NCBI-nr database used was from Jan. 4, 2010 and contained 36621 protein sequences. The identification results from the Paragon Algorithm search were further filtered through the use of a very stringent Local False Discovery Rate (FDR) estimation calculated from the Proteomics System Performance Evaluation Pipeline (PSPEP) program [102-104]. This was used to reduce the potential number of false positive protein identifications. The FDR estimation is based on the number of hits obtained while simultaneously searching the "decoy" database, which is the exact reverse of each protein sequence [103,104].

For our list of identified proteins, we required protein IDs with a local FDR estimate of <5%. By these stringent criteria, 591 proteins were identified. Detailed protein and peptide information and a full list of protein IDs are available as supplemental data, see Additional file 1: Table S1 and Additional file 2: Table S2, and from Proteomecommons.org Tranche using the following hash:vnaxedOvABNSXenjTjTPwRoK7W7hoEZ9aeFVfIkx0Vv + mqhFYNWeulsZt3ezLkoybyFyXZ64wBcmMUIK6MjwwA AAAAAABxQ==. For the quantitative analyses, it is assumed that most proteins will not differ between two experimental conditions, and therefore the distribution of observed ratios should center near a median of 1.0 (a ratio of 1.0 would represent no relative change in that protein's level between the two conditions being compared). With this assumption, a small data-dependent auto-bias correction was applied to each set of iTRAQ ratios such that the adjusted distribution of iTRAQ ratios observed had a median value of 1.0 (or 0 in log space), thus normalizing against any small discrepancies in total protein amount labeled or efficiency of individual iTRAQ labeling in the different samples. The relative change in protein ratios between three day low glucose conditions and glucose re-feeding for 2 hrs was accepted as significant for proteins with a p-value less than 0.05 as calculated by ProteinPilot™ 4.0 based on the ratios of each identified peptide in at least three out of four comparisons and proteins with more than 1 peptide identified with at least 95% of confidence. A one-sample Student's t-test was used to confirm that each protein's set of ratios for all four comparisons was significantly different than 1. From the identifiers (gi numbers) obtained from searching against the NCBI-nr database, we used the Gene Ontology Slim Mapper program available in the Saccharomyces Genome Database (Stanford University) to search for the function and subcellular location for each protein. The overlap in identified proteins between other MS-based proteomics studies was compared using the online tool BioVenn [105]. The alignment of primary amino acid sequences of 12 proteins that are down-regulated by glucose is also available, see Additional file 3.

Western blotting
Cells that expressed Lia1p-GFP, Fbp1p-GFP, Icl1p-GFP, Mls1p-GFP, and Hsp30p-GFP were purchased from Invitrogen. Cells that expressed Lia1p-GFP, Fbp1p-GFP, Mls1p-GFP, and Hsp30p-GFP were purchased from Invitrogen. These cells were starved of glucose for three days and re-fed with glucose for 0, 2, and 3 hours. Total lysates were prepared and proteins were separated by SDS-PAGE. Proteins were transferred to nitrocellulose membrane and blotted with anti-GFP antibodies (Abcam) followed by HRP conjugated goat anti-rabbit antibodies (GE Healthcare). Proteins were detected with the ECL kit from PerkinElmer, Inc.

Additional files

| Additional file 1: Table S1. | Proteins identified with a local FDR <5%. |
| Additional file 2: Table S2. | Peptide summary information for iTRAQ experiment. |
| Additional file 3: Alignment of primary amino acid sequences of 12 proteins that are down-regulated by glucose. |

Abbreviations
iTRAQ: Isobaric Tags for Relative and Absolute Quantification; MALDI: Matrix-assisted laser desorption/ionization; FDR: False Discovery Rate; MS: Mass Spectroscopy; SCX: Strong Cation Exchange.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
BJG participated in sample preparations, data collection, data analysis, and drafting of the manuscript. BAS was involved in data collection, data interpretation, and the drafting of the manuscript. HLC participated in the design of the experiments and the drafting of the manuscript. All three authors approved the order of the authorship. All authors read and approved the final manuscript.

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References

1. Carlson M: Regulation of glucose utilization in yeast. Curr Opin Genet Dev 1998, 8:550–556.

2. Ludin K, Jiang R, Carlson M: Glucose-regulated interaction of a regulatory subunit of protein phosphatase 1 with the Snf1 protein kinase in Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 1998, 95:6245–6250.

3. Fraenkel DG: The top genes: on the distance from transcript to function in yeast glycolysis. Curr Opin Microbiol 2003, 6:198–201.

4. Gancedo JM: The early steps of glucose signalling in yeast. FEMS Microbiol Rev 2006, 32:673–704.

5. Gancedo JM: Yeast carbon catabolite repression. Microbiol Mol Biol Rev 1998, 62:334–361.

6. Zaman S, Lippman SI, Zhao X, Broach JR: How Saccharomyces responds to glucose control in Saccharomyces cerevisiae: a genome-wide screen identifies eight novel GID genes and indicates the existence of two degradation pathways leading to transcriptional regulation of genes involved in the activation of glycolysis in yeast. Mol Microbiol 1997, 25:483–493.

7. de Groot E, Bebelman JP, Mager WH, Nielsen J: Very low amounts of glucose cause repression of the stress-responsive gene HSP12 in Saccharomyces cerevisiae. Microbiology 2000, 146(Pt 2):367–375.

8. Griifffen G, Mager WH, Plant J: Nutritional upshift response of ribosomal protein gene transcription in Saccharomyces cerevisiae. FEMS Microbiol Lett 1994, 123:137–144.

9. Siemens N, Sijlle HT, Verbeek JM, Verrips CT: The glucose-6-phosphate-1-epimerase reaction is essential for normal glucose repression in Saccharomyces cerevisiae. Eur J Biochem 1993, 214:121–127.

10. Moore PA, Sagiocco F, Wood RM, Brown AJ: Yeast glycolytic mRNAs are differentially regulated. Mol Cell Biol 1991, 11:5330–5337.

11. Siestra LN, Nouwen NP, Verbeek JM, Verrips CT: Regulation of glycolytic enzymes and the Crabtree effect in glucose-limited continuous cultures of Saccharomyces cerevisiae. Yeast 1993, 9:767–785.

12. Beulens M, Mboony K, Geerts L, Gladines D, Dettmerman K, Jans AW, Thevelein JM: Studies on the mechanism of the glucose-induced cAMP signal in glycolysis and glucose repression mutants of the yeast Saccharomyces cerevisiae. Eur J Biochem 1988, 172:227–231.

13. Lee FJ, Lin LW, Smith JA: A glucose-repressible gene encodes acetyl-CoA hydrolase from Saccharomyces cerevisiae. J Biol Chem 1990, 265:7413–7418.

14. Efing K, Stahlberg A, Hohmann S, Gustafsson L: Transcriptional responses to glucose at different glycolytic rates in Saccharomyces cerevisiae. Eur J Biochem 2004, 271:4855–4864.

15. Cohen R, Holland JP, Yoko T, Holland MJ: Identification of a regulatory region that mediates glucose-dependent induction of the Saccharomyces cerevisiae enolase gene ENO2. Mol Cell Biol 1986, 6:2287–2297.

16. Lascaris R, Piwowarski J, van der Spek H, Teixeira de Mattos J, Grivell L, Blom J: Overexpression of HAP4 in glucose-derepressed yeast cells reveals post-transcriptional regulation of key cellular processes. Microbiology 2007, 153:3894–3878.

17. Usate R, Wohlischlegel J, Venable JD, Park SK, Nielsen J, Olson L, Yates III JR: Characterization of global yeast quantitative proteome data generated from the wild-type and glucose repression Saccharomyces cerevisiae strains: the comparison of two quantitative methods. J Proteome Res 2008, 7:266–275.

18. Costenoble R, Picotti P, Reiter L, Stallmach R, Heinemann M, Sauer U, Aebersold R: Comprehensive quantitative analysis of central carbon and amino-acid metabolism in Saccharomyces cerevisiae under multiple conditions by targeted proteomics. Mol Syst Biol 2011, 7:564.

19. Kolkmann A, Olthoorn MM, Heeremans CE, Heck AJ, Slipier M: Comparative proteome analysis of Saccharomyces cerevisiae grown in chemostat cultures limited for glucose or ethanol. Mol Cell Proteomics 2005, 4:1–11.

20. Pham TK, Chong PK, Gao CNS, Wright PC: Proteomic analysis of Saccharomyces cerevisiae under high gravity fermentation conditions. J Proteome Res 2006, 5:3411–3419.

21. Klein CJ, Olthoorn L, Nielsen J: Glucose control in Saccharomyces cerevisiae: the role of Mgl1 in metabolic functions. Microbiology 1998, 144(Pt 1):13–24.

22. Yin Z, Wilson S, Hauser NC, Tournu H, Hoheisel JD, Brown AJ: Glucose triggers different global responses in yeast, depending on the strength of the signal, and transiently stabilizes ribosomal protein mRNAs. Mol Microbiol 2003, 48:713–724.

23. Werner JR: The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 1999, 24:437–440.

24. Venema J, Tollever D: Ribosome synthesis in Saccharomyces cerevisiae. Annu Rev Genet 1999, 33:261–311.
Changes in the enzyme activities of Saccharomyces cerevisiae.

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http://www.proteomesci.com/content/10/1/40

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89. Hartig A, Simon MM, Schuster T, Daugherty JR, Yoo HS, Cooper TG: Differentially regulated malate synthase genes participate in carbon and nitrogen metabolism of S. cerevisiae. Nucleic Acids Res 1992, 20:5677–5686.

90. Saltzgaber-Muller J, Kunapuli SP, Douglas MG: Nuclear genes coding the yeast mitochondrial adenosine triphosphatase complex. Isolation of ATP2 coding the F1-ATPase beta subunit. J Biol Chem 1983, 258:11465–11470.

91. Boyer PD: The ATP synthase – a splendid molecular machine. Annu Rev Biochem 1997, 66:717–749.

92. Rak M, Zeng X, Briere JJ, Tzagoloff A: Assembly of F0 in Saccharomyces cerevisiae. Biochim Biophys Acta 2009, 1793:108–116.

93. Stone RL, Mataese V, Magee BB, Magee PT, Bernlohr DA: Cloning, sequencing and chromosomal assignment of a gene from Saccharomyces cerevisiae which is negatively regulated by glucose and positively by lipids. Gene 1990, 96:171–176.

94. Praekelt UM, Meacock PA: HSP12, a new small heat shock gene of Saccharomyces cerevisiae: analysis of structure, regulation and function. Mol Gen Genet 1990, 223:97–106.

95. Bentley NJ, Fitch JT, Tuite MF: The small heat-shock protein Hsp26 of Saccharomyces cerevisiae assembles into a high molecular weight aggregate. Yeast 1992, 8:95–106.

96. Heinisch J: Isolation and characterization of the two structural genes coding for phosphofructokinase in yeast. Mol Gen Genet 1986, 202:75–82.

97. Parra KJ, Kane PM: Reversible association between the V1 and V0 domains of yeast vacuolar H+–ATPase is an unconventional glucose-induced effect. Mol Cell Biol 1998, 18:7064–7074.

98. Bond S, Forgac M: The Ras/cAMP/protein kinase A pathway regulates glucose-dependent assembly of the vacuolar (H+)-ATPase in yeast. J Biol Chem 2008, 283:36513–36521.

99. Ohlmeier S, Hiltunen JK, Bergmann U: Protein phosphorylation in mitochondria – a study on fermentative and respiratory growth of Saccharomyces cerevisiae. Electrophoresis 2010, 31:2869–2881.

100. Shilov IV, Seymour SL, Patel AA, Loboda A, Tang WH, Keating SP, Hunter CL, Nuwaysir LM, Schaeffer DA: The Paragon Algorithm, a next-generation search engine that uses sequence temperature values and feature probabilities to identify peptides from tandem mass spectra. Mol Cell Proteomics 2007, 6:1638–1655.

101. Keller BO, Sui J, Young AB, Whittal RM: Interferences and contaminants encountered in modern mass spectrometry. Anal Chim Acta 2008, 627:71–81.

102. Tang J, Gao M, Deng C, Zhang X: Recent development of multi-dimensional chromatography strategies in proteome research. J Chromatogr B Analyt Technol Biomed Life Sci 2008, 866:123–132.

103. Elias JE, Haas W, Faherty BK, Gygi SP: Comparative evaluation of mass spectrometry platforms used in large-scale proteomics investigations. Nat Methods 2005, 2:667–675.

104. Tang WH, Shilov W, Seymour SL: Nonlinear fitting method for determining local false discovery rates from decoy database searches. J Proteome Res 2008, 7:3561–3567.

105. Hulsen T, de Vlieg J, Akema W: BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. BMC Genomics 2008, 9:488.

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