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Feasibility of Protein Turnover Studies in Prototroph *Saccharomyces cerevisiae* Strains

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Abstract

Quantitative proteomics studies of yeast that use metabolic labeling with amino acids rely on auxotrophic mutations of one or more genes on the amino acid biosynthesis pathways. These mutations affect yeast metabolism and preclude the study of some biological processes. Overcoming this limitation, it has recently been described that proteins in a yeast prototrophic strain can also be metabolically labeled with heavy amino acids. However, the temporal profiles of label incorporation under the different phases of the prototroph's growth have not been examined. Labeling trajectories are important in the study of protein turnover and dynamics, in which label incorporation into proteins is monitored across many time points. Here we monitored protein labeling trajectories for 48 h after a pulse with heavy lysine in a yeast prototrophic strain and compared them with those of a lysine auxotrophic yeast. Labeling was successful in prototroph yeast during exponential growth phase but not in stationary phase. Furthermore, we were able to determine the half-lives of more than 1700 proteins during exponential phase of growth with high accuracy and reproducibility. We found a median half-life of 2 h in both strains, which corresponds with the cellular doubling time. Nucleolar and ribosomal proteins showed short half-lives, whereas mitochondrial proteins and other energy production enzymes presented longer half-lives. Except for some proteins involved in lysine biosynthesis, we observed a high correlation in protein half-lives between prototroph and auxotroph strains. Overall, our results demonstrate the feasibility of using prototrophs for proteomic turnover studies and provide a reliable data set of protein half-lives in exponentially growing yeast.

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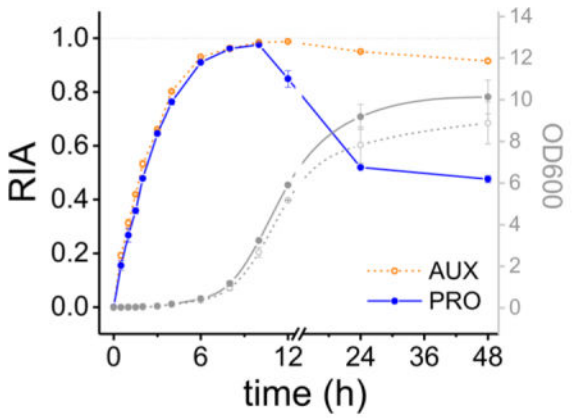
The authors declare no competing financial interest.

Supporting Information

Raw and search result files that can be downloaded from the PRIDE database (ref 13) with the accession PXD001823 and additional information as mentioned in the text, including supporting figures and the complete data set of normalized protein intensities and protein half-lives in the yeast prototroph. This material is available free of charge via the Internet at http://pubs.acs.org.



Measurements of protein abundances and kinetics are essential to decipher cellular adaptation mechanisms. Quantitative proteomic experiments measure protein abundances and provide snapshot pictures of cellular states. Because the abundance of an individual protein is regulated by a dynamic balance between its synthesis and degradation, the analysis of protein turnover can shed light on the cellular regulatory mechanisms. Protein turnover information can also reconcile the discrepancies observed between mRNA and protein levels.[1,2] However, despite its importance, the study of protein turnover is still a rare practice in proteomics. Several efforts have been made in recent years with the development of different metabolic pulse labeling strategies[3–6] based on stable isotope labeling by amino acids in cell culture (SILAC).[7] These pulsed SILAC (pSILAC) methodologies, as well as standard SILAC experiments, rely on the use of cells that can incorporate, but cannot synthesize, a specific amino acid, being therefore dependent on its availability in the medium. Microorganisms, such as the yeast *Saccharomyces cerevisiae*, are able to biosynthesize all amino acids being prototrophic for them. Thus, SILAC and pSILAC experiments require using auxotrophic mutant strains, where one or several genes encoding enzymes of amino acid biosynthetic pathways are genetically deleted.

Auxotrophic mutants may complicate the biological interpretation of some experiments; for example, biasing the results toward amino acid metabolism or other related cellular processes. Furthermore, the dependence on auxotrophic strains may limit the experimental design, restricting the use of existing yeast lab strains, industrial strains, strains from the wild, and mutant library collections that are not auxotrophic for the correct amino acid on SILAC-based quantitative proteomics studies. Despite these limitations, yeast auxotrophic mutants have been widely used by the yeast community, and exclusively used in quantitative proteomic studies with SILAC labeling in yeast.

Interestingly, it has been recently proved that proteins in prototrophic yeast strains can be efficiently labeled with isotope-labeled amino acids,[8,9] because amino acid incorporation from the culture medium is less energetically demanding than their internal biosynthesis. Dilworth et al. measured the aggregate amino acid incorporation at different doublings in exponential log-phase *S. cerevisiae* and showed that labeling reached its maximum after seven doublings, paralleling the predicted incorporation based on population doubling.[8]

Fröhlich et al. termed the approach native SILAC (nSILAC) and showed similar labeling efficiency in auxotroph and prototroph *S. cerevisiae* after 10 doublings. Further, they provided a proof-of-principle application into an osmotic stress SILAC experiment, overcoming the limitations of using auxotrophic mutant strains.[9]

Nevertheless, the application of the native SILAC approach to the study of protein dynamics in *S. cerevisiae* remains elusive. In order to test the feasibility of using prototrophic *S. cerevisiae* for protein dynamic studies (native pulsed SILAC, npSILAC), we monitored the incorporation of isotopically labeled lysine in a yeast prototrophic strain and compared the resulting half-lives with those of a lysine auxotrophic counterpart. The high reproducibility and correlation between both strains validates the npSILAC approach and provides a reliable reference tool for the scientific community.

EXPERIMENTAL SECTION

Cell Culture

All experiments were performed in duplicate with diploid *S. cerevisiae* strains from the FY background, a wild-type prototrophic strain (parental strains FY4H and FY3G), and a lysine auxotrophic counterpart (parental strains FY4 and FY1856) generated by the insertion of a Ty retrotransposon into *LYS2* gene in both alleles (*lys2–128Δ*/*lys2–128Δ*), resulting in a lysine auxotrophy due to transcriptional block. Both strains were grown in synthetic complete medium containing 6.7 g/L yeast nitrogen base, 2 g/L dropout mix (Bufferad, Lake Bluff, IL) containing all amino acids except lysine, and 2% glucose. Light lysine, containing natural abundance isotopes (Sigma-Aldrich, St. Louis, MO) or heavy-labeled ($^{13}C_6/^{15}N_2$) lysine (Cambridge Isotope Laboratories, Andover, MA), was added to a final concentration of 0.436 mM. Cells were precultured in 5 mL of medium containing light lysine overnight at 30 °C. Then 400 mL of heavy medium was inoculated from the precultures to OD_{600} = 0.02, and cells were grown for 48 h, collecting samples at 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, and 48 h into the labeling (Figure 1). At each time point, OD_{600} was measured and cells were collected directly into ice-cold tubes. Cells were harvested and centrifuged at 10 000*g* for 5 min at 4 °C. The pellet was washed twice with ice-cold ultrapure water, the supernatant was discarded, and the yeast pellet was frozen in liquid nitrogen and stored at −80 °C.

Sample Preparation

Cell pellets were thawed on ice and resuspended in lysis buffer composed of 8 M urea, 300 mM NaCl, 50 mM Tris, pH 8.2, 5 mM dithiothreitol (DTT), phosphatase inhibitors (50 mM NaF, 50 mM sodium β-glycerophosphate, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate), and protease inhibitors (EDTA-free, Roche, Nutley, NJ). Sample processing from cell lysis through elution of purified peptides was performed following the in-StageTip (ST) method described previously[10] with some modifications. Briefly, 100 μL of cell suspension containing approximately 20 μg protein (assuming 3 pg of protein per cell) was loaded directly onto bottom-sealed STs containing 14-gauge four-layered Empore C_{18} material (3M, St. Paul, MN) plugs already conditioned with methanol and 80% acetonitrile, 0.1% acetic acid, and equilibrated with 0.1% acetic acid. The enclosed ST was filled with ~100 μL of 0.5 mm diameter zirconia/silica beads (BioSpec, Bartlesville, OK) and placed

inside the bead-milling adaptor. Cells were lysed by four repetitions of bead beating (1 min beating, 1.5 min rest). Lysate protein concentration was measured by Bradford assay (Biorad, Hercules, CA). Already reduced protein was alkylated of free thiols by addition of 15 mM iodoacetamide in the dark for 30 min. The alkylation reaction was quenched with 5 mM DTT. Urea concentration was diluted 2-fold with 50 mM Tris pH 8.9 and 2 mM calcium chloride. Proteolytic digestion was performed by addition of lysyl endopeptidase (LysC, Wako Chemicals, Richmond, VA), at 1:50 enzyme to protein ratio, and incubation at room temperature overnight. The digestion was quenched by addition of 10% TFA to pH < 2. Bottom seals from ST were removed, and samples were centrifuged at 2000*g*. Peptides were desalted by three continuous washes with 0.1% acetic acid and directly eluted into a 96-well plate with 60 μL of 80% acetonitrile, 0.1% acetic acid. Samples were dried down by vacuum centrifugation and stored at −20 °C until liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis.

LC–MS/MS Analysis

Peptides were resuspended in 4% formic acid, 3% acetonitrile and loaded onto a 100 μm i.d. × 3 cm precolumn packed with Maccel C_{18} 3 μm diameter, 200 Å pore size reversed-phase material (The Nest Group, South-borough, MA). Peptides were separated on a 75 μm i.d. × 40 cm analytical column packed with the same material and heated to 50 °C. The gradient was 13–32% acetonitrile in 0.125% formic acid. Eluted peptides were online analyzed in a hybrid quadrupole–Orbitrap (QExactive) mass spectrometer (Thermo Fisher, Bremen, Germany) using data-dependent acquisition in which the 20 most abundant ions on an MS scan where selected for fragmentation by beam-type collision-activated dissociation (HCD), and fragmented ions were excluded from further selection during 40 s. Full MS scans were acquired in centroid mode from 300 to 1500 *m*/*z* at 70 000 fwhm (full width at half-maximum) resolution with a maximum injection time of 100 ms and fill target of 3×10^6 ions. MS/MS fragmentation spectra were collected at 17 500 fwhm with maximum injection time of 50 ms, using a 2.0 *m*/*z* precursor isolation window and fill target of 5×10^4 ions. Acquisition time for each fraction was 90 min and included column wash and equilibration.

Protein Identification, Protein Quantification, and Protein Half-Life Calculation

MS/MS spectra were searched with MaxQuant (version 1.4.1.2)[11] against the SGD yeast protein sequence database (downloaded January 2011, 6717 entries) with common contaminants added. The precursor mass tolerance was set to 4.5 ppm, and the fragment ion tolerance was set to 20 ppm. All searches used a fixed modification of cysteine carbamidomethylation (+57.0215 Da) and variable modifications of methionine oxidation (+15.9949 Da) and protein N-terminal acetylation (+42.0106 Da). LysC was the specified enzyme allowing for up to two missed cleavages. The false discovery rate (FDR) was estimated by searching against a concatenated forward–reverse database.[12] The maximum FDR was 0.01 on both the peptide and the protein level. The minimum required peptide length was seven residues. Proteins with at least two peptides (one of them unique to the protein) were considered identified. The “match between runs” option was enabled with a time window of 1 min to match identifications between replicates. The “requant option” of MaxQuant was disabled. We have deposited the raw files and identification files of the experiment for the prototroph strain to the ProteomeXchange Consortium (http://

proteomecentral.proteomexchange.org) via the PRIDE partner repository[13] and have the accession PXD001823. Peptide intensities were grouped into proteins according to MaxQuant standard procedures. Protein quantification of heavy-to-light intensity ratios from the MaxQuant protein groups output table were used for the calculation of heavy-label incorporation. At each time point and for each protein the relative isotope abundance (RIA) was calculated as follows:

$$\mathrm{RIA_t}=\mathrm{ratio\,H/L}/(1+\mathrm{ratio\,H/L}) \quad (1)$$

For derivation of the protein turnover parameters, the $\mathrm{RIA_t}$ values up to 10 h were used for nonlinear curve fitting to a plateau. According to median protein RIA values, the plateau was constrained to be 1 ($\mathrm{RIA_\infty}$= 1). We calculated the half-life ($t_{1/2}$) for each protein as the time when the protein is half-labeled (i.e., RIA = 0.5). For proteins showing exponential labeling trajectories half-life was calculated using one-parameter asymptotic exponential function:

$$\begin{aligned}\mathrm{RIA}&=\mathrm{RIA}_\infty(1-\exp(-t(\log_e 2/t_{1/2})))\\&=1-\exp(-t(\log_e 2/t_{1/2}))\end{aligned} \quad (2)$$

For proteins showing sigmoidal labeling trajectories, half-life was calculated using a logistic curve (where k is a constant):

$$\begin{aligned}\mathrm{RIA}&=\mathrm{RIA}_\infty/(1+\exp(-k(t-t_{1/2})))\\&=1/(1+\exp(-k(t-t_{1/2})))\end{aligned} \quad (3)$$

To automate and correctly assign each protein to one of the two groups, all protein labeling trajectories were fitted to both curves (eqs 2 and 3) with OriginPro software package version 9.0.0 (OriginLab, Northampton, MA), and half-life values showing best fitting parameters (R^2) were selected for data analysis. Only proteins with a significant goodness of fit (Pearson's χ^2 test, $p \leq 0.05$) were kept in the final data set. In order to properly compare protein half-lives across replicates, protein half-lives were normalized by the calculated doubling time for each replicate.

Absolute protein abundances were estimated by summing up the intensities of both heavy and light versions for each protein group and normalizing to the total intensity in each sample (sum of intensities from all protein groups excluding contaminants, reverse hits, and proteins identified only by modified peptides).

Data Analysis and Statistics

Comparisons between two groups were performed by using two-sample *t* test in Perseus. In all cases significant differences were established at the level of $p < 0.01$. Correlation coefficients and two-dimensional (2D) enrichment analysis ($p < 0.02$, Benjamini–Hochberg FDR for truncation) of the KEGG names (Uniprot Keywords) were performed in Perseus,

similarly to described.[14] GO analysis were performed using the Term Finder tool in the SGD database (www.yeastgenome.org).

RESULTS AND DISCUSSION

To test whether prototrophic yeast can be used for dynamic studies of protein turnover, we determined the half-lives of individual proteins through the incorporation of heavy-lysine (Lys8) labeling in a lysine prototrophic strain and compared the resulting values with those of its auxotrophic counterpart obtained from a separate study in our lab.[15] Yeast cells from an overnight culture were diluted down to OD 0.02 into a new media containing heavy lysine. Cell growth and incorporation of heavy lysine into proteins were monitored for 48 h, sampling at different time points (0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 24 and 48 h) (Figure 1). A replicate experiment was performed from a different culture at a different time. The resulting samples were lysed, digested with LysC, and eluted into a 96-well plate using a modified version of the iST method[10] for high-throughput proteomic sample processing. Peptide samples were analyzed by LC–MS/MS in a QExactive mass spectrometer resulting in the confident identification of >3300 proteins, accounting for about 60% of the yeast proteome.[16] The average number of identifications per run was 1885 ± 346 proteins (Figure 2, parts A and B).

The RIA at each time point was determined only for those proteins quantified with at least two unique or razor peptides. Growth curves and median protein labeling trajectories were very similar for both strains during the first 10 h growing in heavy medium, point at which full labeling was achieved (Figure 2C). Longer cultivation in heavy media results in a decay of protein labeling and RIA values, significantly more pronounced in the prototrophic strain. We attribute the appearance of light protein to the prototrophic strain engaging endogenous lysine biosynthesis when lysine becomes scarce in the culture medium, as it is expected to occur at the entrance into stationary phase. Furthermore, nutrient exhaustion differentiates yeast culture into two cell types: quiescent and nonquiescent.[17,18] Early unbudded quiescent cells would mostly retain the labeling, while for replicative nonquiescent cells the labeling would be diluting by synthesis of new proteins using endogenous (light) lysine. We observe this phenomenon in the prototroph strain as a heavy-labeling decay curve trending to an asymptotic RIA value ~0.4 (i.e., 40% of the protein pools are still heavy-labeled).

Surprisingly, a slight (<10%) decay in labeling was also observed in the auxotrophic strain on the last two time points (24 and 48 h), which could suggest a late metabolic switch and an alternative way to produce lysine. This could occur, for example, via a reverse mutation in some cells[19] or a bypass of the reaction catalyzed by Lys2. In this sense, it has been described that some LYS2 mutant yeasts are able to grow using metabolic compounds other than lysine as a single nitrogen source.[20] Identifying the exact mechanism, however, deserves an in-deep study to track the fate of the label that is beyond the scope of this work. Collectively, these results show that labeling of prototrophic yeast in exponential growth phase, but not stationary phase, goes to completion, and dynamic proteomic experiments are feasible under this state. In addition, these data highlight the importance of globally characterizing the degree of metabolic labeling in supposedly labeled samples, since

labeling loss can become an issue when proteomic studies are performed during the transition into stationary phase, even in auxotrophic strains.

Given the labeling profiles observed, we conducted our protein turnover study on exponential growth phase (up to 10 h after media pulse). During this phase individual protein abundances, estimated by the sum of heavy and light intensities for all peptides in a given protein, were very similar between both strains and time points (Figure S1, Supporting Information). However, when approaching stationary phase a significant change in the proteome is evidenced by a decrease on Pearson's correlation scores. These proteomic changes are consistent with the growth arrest and the diauxic shift from anaerobic to more aerobic metabolism that occur during the transition from exponential to stationary phase of growth.[18] As expected, for most proteins label incorporation fit a first-order exponential curve, with the exception of some proteins (10% in the auxotrophic and 13% in prototrophic strain), which followed a sigmoidal labeling trajectory (Figure 2D). To be able to compare protein turnover between both protein groups, we calculated protein half-lives as the time when the protein is half-labeled. Overall, we were able to calculate half-lives for 1722 proteins, about 50% of the total proteins identified (Figure 2E), comparing very favorably with other studies of protein turnover in yeast.[21–24] The remaining proteins did not pass our stringent data analysis criteria, demanding a significant curve fitting (p-value <0.05, Pearson's χ^2 test). Protein half-life calculations had an average of 6 time points, and most proteins (96%) showed a regression $R^2 > 0.9$, demonstrating the high quality of our data set (Table S1, Supporting Information). In general terms, proteins fitting a "sigmoidal" curve had longer half-lives than those fitting an "exponential" (Figure 2F) and were mostly related with mitochondrial functions (Figure 3). A heterogeneous protein turnover had also been observed by others in mice sperm, where proteins with sigmoidal labeling trajectories showed a delay in their synthesis.[25] In our experiment in *S. cerevisiae*, since most of these "sigmoidal" proteins are involved in functions characteristic of the aerobic-based stationary phase of growth, their synthesis arrest during the anaerobic-based exponential phase of growth is something to be expected.

These half-life values were very close to the calculated doubling times under the experimental growth conditions used (2.04 ± 0.11 h for the prototrophic strain and 1.80 ± 0.07 h for the auxotrophic). This is consistent with approximate doubling of the protein content as the cell divides and was also observed by others[6] when determining turnover rates in HeLa cells. Previous protein dynamic studies in yeast, however, indicated very different median half-life values at 43 min,[22] 8.8 h,[24] 10.8 h,[23] and 34 h.[21] These discrepancies are likely due to differences in strains, culture conditions, and methodologies employed for the quantification of protein half-lives in the different studies, as explained elsewhere.[15,23,24] In our study, we normalized protein half-lives according to the cellular doubling time to correct for experimental growth differences and properly compare the half-lives between the two strains. Thus, proteins whose turnover rate is faster than growth rate will have "relative" half-life ($rt_{1/2}$) values <1, while those with turnover rates slower than the growth rate will show $rt_{1/2} > 1$. The measurement of relative protein half-lives was highly reproducible (R = 0.95, Figure 4C; Figure S2, Supporting Information), and the values were very similar between both strains (R = 0.90, Figure 4D). The only significant differences were observed

in proteins involved in the lysine biosynthesis pathway (Figure 4, parts E and F). All enzymes belonging to this pathway showed a sigmoidal labeling trajectory in the prototrophic strain but not the auxotrophic strain and longer half-lives in the prototroph than in the auxotroph (Figure 5A–C). An exception to these trends was Aro8 (Figure 5E), an enzyme that also participates in the metabolism of other amino acids. Furthermore, protein levels of lysine biosynthesis enzymes dropped significantly during the first few hours of exponential growth in the prototrophic strain and then increased when approaching the low-nutrient stationary phase, whereas in the auxotrophic strain remained constant throughout the experiment (Figure 5, parts D and F). These proteins illustrate how a delay in their synthesis is associated with a sigmoidal labeling trajectory, which can be generally applicable. Together, these data reveal an adaptation of prototrophic yeast to the availability of lysine in the medium by modulating the expression of enzymes of the lysine biosynthetic pathway through an arrest in their synthesis, as evidenced by a change in their half-lives. This is consistent with previous findings showing that lysine metabolism is controlled by feedback inhibition, in which the presence of lysine decreases the synthesis and import capacity of this amino acid.[9] Our results, however, expand this knowledge by providing mechanistic insight with regards to protein dynamics.

Finally, we investigated if there are other biological processes for which protein stability differs between both strains. We conducted a 2D enrichment analysis of KEGG terms between the auxotrophic and prototrophic strains.[14] 2D enrichment analysis identifies those terms that have a significantly different distribution of paired half-life values compared to the total distribution. Each term gets a score between −1 and +1 on each dimension, and the scores are represented in a scatter plot. Terms with scores near +1 mean that many proteins belonging to this term are long-lived proteins, while values close to −1 represent terms containing mostly short-lived proteins. This analysis revealed that protein stability is consistently regulated in both strains, since the terms for most biological processes are located in the diagonal of the scatter plot, with the exception of lysine biosynthesis, as discussed above (Figure 4F). 2D enrichment analysis as performed here also shows which biological processes contain short-lived or long-lived proteins. In fact, we observed that ribosomes and nuclear functions were enriched in short-lived proteins, while proteins involved in mitochondrial-related functions (TCA, oxidative phosphorylation) as well as other energy production pathways were more stable. Ribosomal proteins were also described as unstable or rapidly degraded proteins in a previous study in yeast,[23] and some glycolytic enzymes (*Hxk1p* and *Fba1p*) and TCA cycle proteins (Lsc2p and Kgd1p) had also been reported as very stable. These results, besides confirming the validity of our approach, indicate that proteins with similar function tend to present similar half-lives, as has been previously observed.[23,26,27]

CONCLUSION

In the present study we have demonstrated the feasibility of using prototrophic yeast strains for SILAC-based studies of protein dynamics (npSILAC). Protein half-lives were highly correlated between the prototrophic and auxotrophic strains with the only exception of proteins involved in the lysine biosynthesis pathway. These proteins showed longer half-lives in the prototrophic strain due to a delayed synthesis during exponential growth in

lysine-enriched medium. The median protein half-life was 2 h, similar to estimated cellular doubling time. Ribosomal and nuclear proteins showed shorter half-lives, whereas mitochondrial proteins and other energy production related enzymes were more stable. The high quality of our study, evidenced by the high reproducibility of half-life estimations, together with the fact that our study was conducted under the same conditions as most yeast biology experiments (exponential growth in batch culture), makes our data set of protein half-lives a reliable and useful reference for the scientific community using yeast as study model.

Thus, npSILAC reveals as a promising tool for protein dynamic studies in prototrophic *S. cerevisiae*, overcoming the limitations of using auxotrophic mutant strains without compromising data quality. More importantly, npSILAC enables the analysis of protein turnover in a vast collection of yeast strains from the laboratories and the wild, which were largely excluded from dynamic analyses because of their genotype. However, two considerations should be taken into account. First, although most protein dynamic studies rely on the assumption that labeling trajectories follow a first-order kinetic model, here we report that some proteins may have different labeling trajectories. Second, the loss of labeling due to internal amino acid biosynthesis and the appearance of different cell subpopulations during the transition to the stationary phase of growth limit the application of this method to exponentially growing cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Gygi SP, Rochon Y, Franza BR, Aebersold R. Mol Cell Biol. 1999; 19:1720–1730. [PubMed: 10022859]
2. de Godoy LMF, Olsen JV, Cox J, Nielsen ML, Hubner NC, Fröhlich F, Walther TC, Mann M. Nature. 2008; 455:1251–1254. [PubMed: 18820680]
3. Selbach M, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Nature. 2008; 455:58–63. [PubMed: 18668040]
4. Doherty MK, Hammond DE, Clague MJ, Gaskell SJ, Beynon RJJ. Proteome Res. 2009; 8:104–112.
5. Schwanhäusser B, Gossen M, Dittmar G, Selbach M. Proteomics. 2009; 9:205–209. [PubMed: 19053139]
6. Boisvert FM, Ahmad Y, Gierlinski M, Charrière F, Lamont D, Scott M, Barton G, Lamond AI. Mol Cell Proteomics. 2012; 11:M111.011429.
7. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M. Mol Cell Proteomics. 2002; 1:376–386. [PubMed: 12118079]
8. Dilworth DJ, Saleem RA, Rogers RS, Mirzaei H, Boyle J, Aitchison JD. J Am Soc Mass Spectrom. 2010; 21:1417–1422. [PubMed: 20451407]

9. Fröhlich F, Christiano R, Walther TC. Mol Cell Proteomics. 2013; 12:1995–2005. [PubMed: 23592334]
10. Kulak NA, Pichler G, Paron I, Nagaraj N, Mann M. Nat Methods. 2014; 11:319–324. [PubMed: 24487582]
11. Cox J, Mann M. Nat Biotechnol. 2008; 26:1367–1372. [PubMed: 19029910]
12. Elias JE, Gygi SP. Nat Methods. 2007; 4:207–214. [PubMed: 17327847]
13. Vizcaíno JA, Deutsch EW, Wang R, Csordas A, Reisinger F, Ríos D, Dianes JA, Sun Z, Farrah T, Bandeira N, Binz PA, Xenarios I, Eisenacher M, Mayer G, Gatto L, Campos A, Chalkley RJ, Kraus HJ, Albar JP, Martinez-Bartolomé S, Apweiler R, Omenn GS, Martens L, Jones AR, Hermjakob H. Nat Biotechnol. 2014; 32:223–226. [PubMed: 24727771]
14. Cox J, Mann M. BMC Bioinf. 2012; 13(Suppl 16):S12.
15. Martin-Perez, M.; Villen, J. University of Washington; Seattle, WA: Apr. 2014 Unpublished work
16. Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS. Nature. 2003; 425:737–741. [PubMed: 14562106]
17. Allen C, Büttner S, Aragon AD, Thomas JA, Meirelles O, Jaetao JE, Benn D, Ruby SW, Veenhuis M, Madeo F, Werner-Washburne M. J Cell Biol. 2006; 174:89–100. [PubMed: 16818721]
18. Davidson GS, Joe RM, Roy S, Meirelles O, Allen CP, Wilson MR, Tapia PH, Manzanilla EE, Dodson AE, Chakraborty S, Carter M, Young S, Edwards B, Sklar L, Werner-Washburne M. Mol Biol Cell. 2011; 22:988–998. [PubMed: 21289090]
19. Simchen G, Winston F, Styles CA, Fink GR. Proc Natl Acad Sci USA. 1984; 81:2431–2434. [PubMed: 6326126]
20. Zabriskie TM, Jackson MD. Nat Prod Rep. 2000; 17:85–97. [PubMed: 10714900]
21. Pratt JM, Petty J, Riba-Garcia I, Robertson DHL, Gaskell SJ, Oliver SG, Beynon RJ. Mol Cell Proteomics. 2002; 1:579–591. [PubMed: 12376573]
22. Belle A, Tanay A, Bitincka L, Shamir R, O'Shea EK. Proc Natl Acad Sci USA. 2006; 103:13004–13009. [PubMed: 16916930]
23. Helbig AO, Daran-Lapujade P, van Maris AJA, de Hulster EAF, de Ridder D, Pronk JT, Heck AJR, Slijper M. Mol BioSyst. 2011; 7:3316–3326. [PubMed: 21984188]
24. Christiano R, Nagaraj N, Fröhlich F, Walther TC. Cell Rep. 2014; 9:1959–1965. [PubMed: 25466257]
25. Claydon AJ, Ramm SA, Pennington A, Hurst JL, Stockley P, Beynon R. Mol Cell Proteomics. 2012; 11:M111.014993. [PubMed: 22331477]
26. Kristensen AR, Gsponer J, Foster LJ. Mol Syst Biol. 2013; 9:689. [PubMed: 24045637]
27. Nolte H, Hölper S, Selbach M, Braun T, Krüger M. Anal Chem. 2014; 86:11033–11037. [PubMed: 25347402]

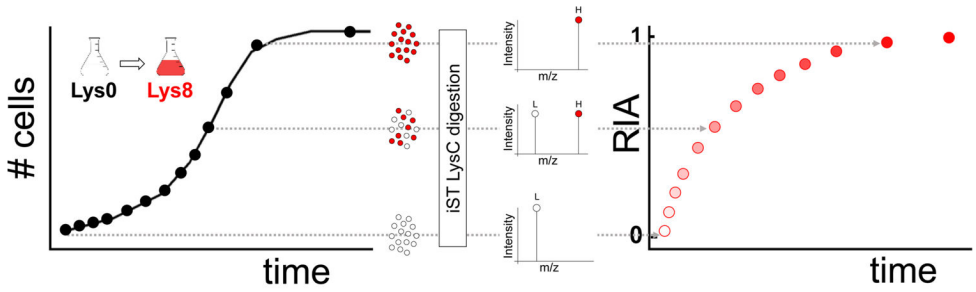

Figure 1.

Experimental design. Yeast cells were grown in minimal medium containing light (L) lysine (Lys0), pulsed into heavy (H) lysine medium (Lys8), and harvested at different time points. Incorporation of Lys8 into proteins was monitored by LC–MS/MS analysis of LysC peptides and expressed as “relative isotope abundance” [RIA = intensity H/(intensity H + intensity L)].

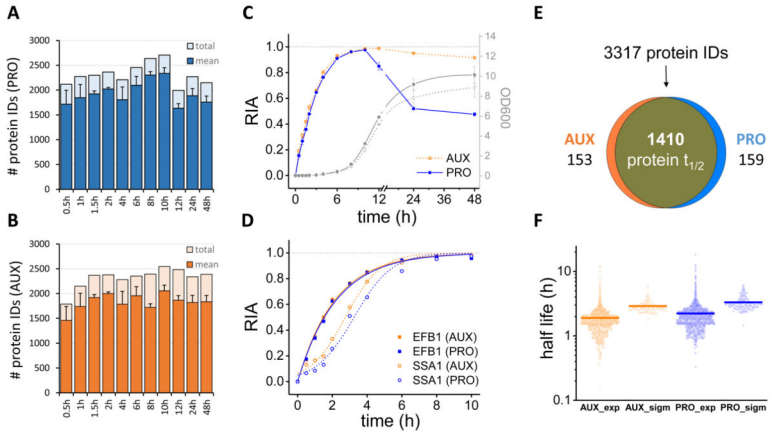

Figure 2.

Protein identification and calculation of protein half-lives. (A) Number of proteins identified in the prototroph and auxotroph (B) strains. Dark bars indicate mean between two replicates, and light bars represent totals. Error bars represent SD. (C) Median incorporation of heavy lysine into proteins (left axis) during different phases of cell culture growth in both auxotrophic (AUX, dotted green line) and prototrophic (PRO, solid blue line) *S. cerevisiae* strains. Cell growth represented by OD_{600} values is shown on the right axis. Each point corresponds to the mean values ± SD of two biological replicates. (D) Isotopic label incorporation into individual proteins during exponential phase of growth. Protein heavy-label incorporation curves can follow an exponential or a sigmoidal pattern as observed in EFB1 and SSA1, respectively. In order to compare turnover rates between proteins with different incorporation pattern, we calculate the protein half-life as the time when the protein reaches 50% of labeling (i.e., RIA = 0.5). (E) Number of proteins identified and half-lives calculated in the experiment. From 3317 proteins identified across all experimental time points, we were able to confidently calculate half-lives for more than 1700 proteins in both the prototrophic and auxotrophic strains. (F) Plot for half-lives of the two protein groups (exponential and sigmoidal) in two strains (auxotrophic and prototrophic). Each data point represents an individual protein, and horizontal lines represent group means. Number of proteins for each group (in order of appearance) are 1400, 163, 1361, and 209.

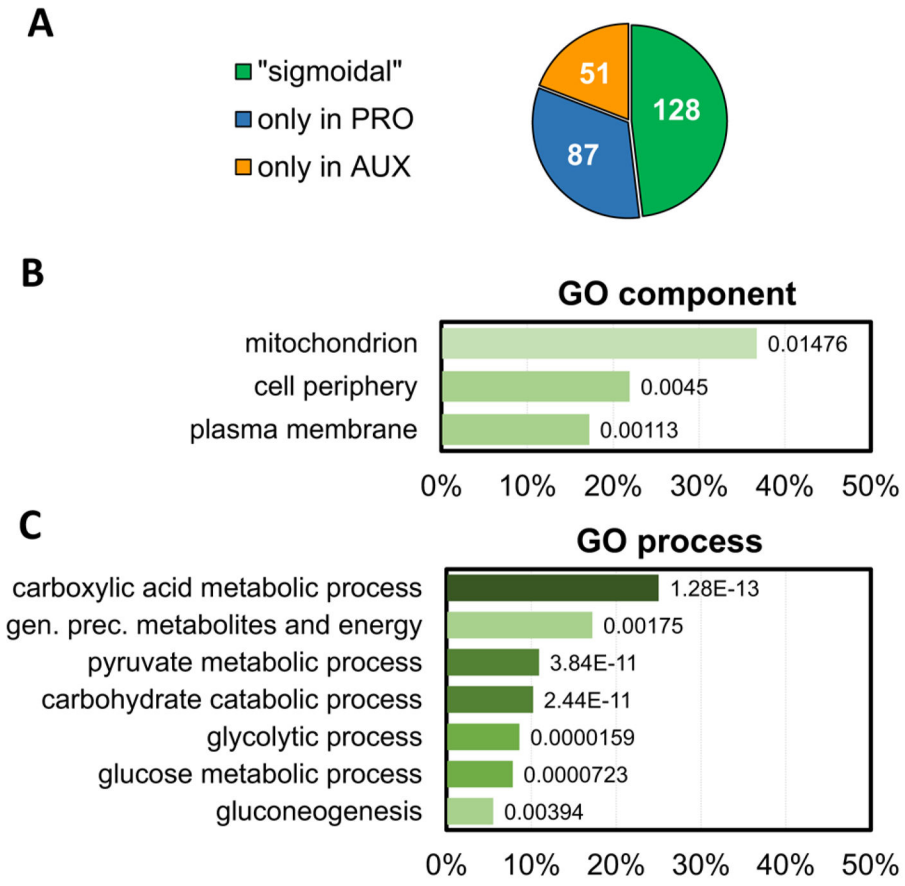

Figure 3.

Descriptive analysis of sigmoidal proteins. (A) Distribution of sigmoidal proteins in both strains. “Sigmoidal” term is attributed to those proteins with a sigmoidal trajectory of label incorporation in both strains or only determined in one strain. “Only in PRO” and “only in AUX” denote those proteins with sigmoidal behavior in one strain, but not in the other. Distribution of cellular compartment (B) and biological process (C) GO terms for sigmoidal proteins (numbers to the right of the bar indicate term enrichment *p*-value, using the set of proteins with calculated half-lives as background, and bars are colored accordingly, where darker colors indicate a lower *p*-value).

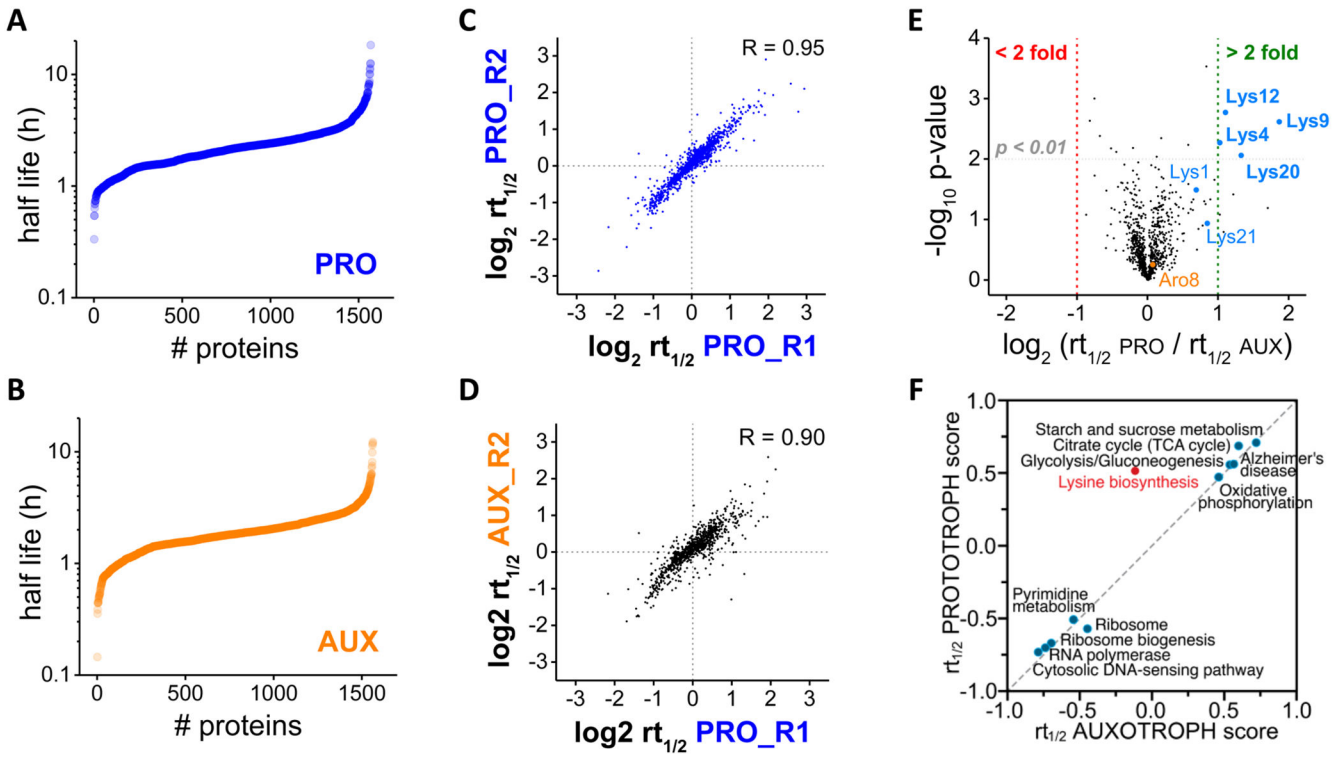

Figure 4.

Analysis of protein half-lives in prototrophic and auxotrophic *S. cerevisiae* strains. (A) Distribution of protein half-lives in the prototrophic (n = 1569) and (B) auxotrophic (n = 1563) strains, showing median values of 2.13 and 1.84 h, respectively. (C) Reproducibility of relative half-life ($rt_{1/2}$) measurements (half-life normalized by cell growth) between two replicates (n = 1162). (D) Correlation of protein half-lives between prototrophic and auxotrophic strains (n = 1027). (E) Volcano plot showing significant differences ($p < 0.01$ and 2-fold change) in $rt_{1/2}$ between the prototrophic and the auxotrophic strain (n = 932). Colored dots are proteins belonging to the aminoadipate lysine biosynthesis pathway of *S. cerevisiae* (in blue are proteins exclusively used in that pathway). (F) 2D enrichment analysis of KEGG annotation terms between the $rt_{1/2}$ of the prototrophic and auxotrophic cell strains.

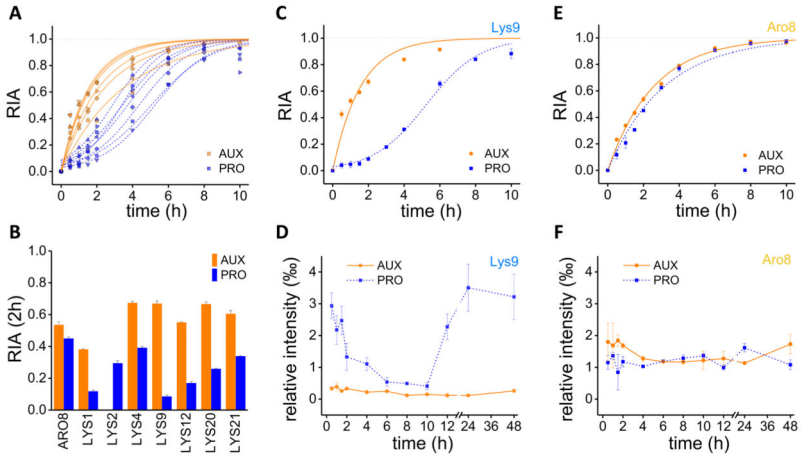

Figure 5.

Analysis of incorporation and abundance of proteins in the lysine biosynthesis pathway. (A) Labeling trajectory of proteins in the lysine biosynthesis pathway (except Aro8) in auxotroph (orange) and prototroph (blue) *S. cerevisiae* strains. (B) RIA values at a representative time point (2 h) after SILAC pulse. (C) Labeling trajectory of Lys9, a representative protein functioning exclusively in the lysine biosynthetic pathway. (D) Relative abundance dynamics of Lys9. (E) Labeling trajectory of Aro8, a protein involved in lysine biosynthesis and other pathways. (F) Relative abundance dynamics of Aro8. Values represent mean ± SD of two replicates.