Dynamics of Protein Turnover, a Missing Dimension in Proteomics*

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Functional genomic experiments frequently involve a comparison of the levels of gene expression between two or more genetic, developmental, or physiological states. Such comparisons can be carried out at either the RNA (transcriptome) or protein (proteome) level, but there is often a lack of congruence between parallel analyses using these two approaches. To fully interpret protein abundance data from proteomic experiments, it is necessary to understand the contributions made by the opposing processes of synthesis and degradation to the transition between the states compared. Thus, there is a need for reliable methods to determine the rates of turnover of individual proteins at amounts comparable to those obtained in proteomic experiments. Here, we show that stable isotope-labeled amino acids can be used to define the rate of breakdown of individual proteins by inspection of mass shifts in tryptic fragments. The approach has been applied to an analysis of abundant proteins in glucose-limited yeast cells grown in aerobic chemostat culture at steady state. The average rate of degradation of 50 proteins was 2.2%/h, although some proteins were turned over at imperceptible rates, and others had degradation rates of almost 10%/h. This range of values suggests that protein turnover is an important missing dimension in proteomic experiments and needs to be considered when assessing protein abundance data and comparing it to the relative abundance of cognate mRNA species. Molecular & Cellular Proteomics 1:579–591, 2002.

EXPERIMENTAL PROCEDURES

Yeast Strain and Growth Conditions—The diploid yeast strain BY4743 (EUROSCARF accession number Y23935, www.uni-frankfurt.de/lbf15/mikro/euroscarf/index.html) (9), a leucine auxotroph, was used throughout. Yeast were grown in glucose-limited chemostat culture as described previously (10) in a medium (Table I) containing 100 mg/liter L-[1-3H]leucine (98.5 atom % excess) at a dilution rate of 0.1 h⁻¹. After a minimum of seven doubling times, sufficient to ensure that cells were fully labeled, unlabeled L-leucine (1 g in 50 ml) was added, and the incoming medium was changed to one containing unlabeled L-leucine at 50 mg/liter. Sampling was at 0, 1.5, 3, 4.5, 6, 8, 10, 12, 24.5, and 51 h into the chase. This sampling frequency served to reduce the true dilution rate in the chase phase from a nominal 0.1 h⁻¹ to an actual 0.086 h⁻¹. At each time point, cells were collected directly into ice-cold tubes containing cycloheximide (final concentration 100 μg/ml). Cells (40 ml at an A600 of ~1.6) were harvested and centrifuged at 5000 rpm for 5 min at 4 °C. The pellet was resuspended in 1 ml of ice-cold double distilled H2O and transferred to a 1.5-ml microcentrifuge tube. Cells were pelleted by centrifugation at 10,000 rpm, the supernatant was dis-
was the number of leucine residues in the peptide. The protein was identified by recording the masses of peptides in the 51-h spectrum (fully unlabeled) and including the leucine composition of each peptide (derived from comparison of the 0- and 51-h spectra) in a manual search of the yeast data base using MASCOT (www.matrixscience.co.uk), which allows inclusion of composition data in its search (11).

Data Analysis—The monoisotopic peak intensities of the heavy and light tryptic peptides (A_0 and A_0, respectively) were obtained and were used to calculate the relative isotope abundance at each time, t, (RIA),^1, as the ratio:

\[ \text{RIA} = \frac{A_t}{A_0 + A_0} \]

The value of RIA, changes over time as the proteins, prelabeled with heavy leucine, are replaced by those labeled with light leucine. This is a consequence of two processes, namely loss of cells from the chemostat and loss through intracellular protein turnover. The generic form of the exponential equation relates the RIA at any time, t, to the values for RIA at t = 0 (RIA_0) and t = ∞ (RIA_∞, in practice, t = 51 h):

\[ \text{RIA}_t = \text{RIA}_0 + (\text{RIA}_∞ - \text{RIA}_0) \exp(-k_{\text{loss}} \times t) \]

Rather than use non-linear curve fitting to recover the values of three parameters (RIA_0, RIA_∞, and k_{\text{loss}}), RIA_0 was measured for 26 peptides derived from a total of 18 different proteins and yielded a value of 0.985 ± 0.001 (mean ± S.E., n = 26). The variance in this experimentally determined parameter was so low that it was fixed as the mean value in the non-linear curve fitting. Similarly, the value for RIA_∞, was set to zero since after 51 h, equivalent to seven doubling times, over 99% of the heavy labeled cells in the vessel at t = 0 h would have been lost from the vessel. By fixing these parameters, we also removed some of the error inherent in determination of the RIA at the start and end of the experiment where either the heavy or the light peak was small relative to the other and therefore the data were sometimes compromised by chemical noise in the mass spectrum. The fitted equation simplified to:

\[ \text{RIA}_t = 0.985 \exp(-k_{\text{loss}} \times t) \]

The curve of this form was fitted to the (t, RIA_0) data using non-linear curve fitting to obtain k_{\text{loss}}, the error in the parameter estimate and the confidence limits for the fitted curve. In single time point experiments, k_{\text{loss}} was calculated from the value of RIA_0 determined at a single time, t, according to the equation:

\[ k_{\text{loss}} = -\ln(\text{RIA}_0/0.985)/t \]

Finally, the true rate of degradation (k_{\text{degr}}) was calculated by simple subtraction of the constant dilution rate D from k_{\text{loss}} (the subtraction of a constant does not affect the error of the parameter estimate).

RESULTS

Determination of the rate of turnover of specific proteins is fraught with difficulty, and our strategy was designed to yield turnover rates under carefully controlled conditions. Our approach measures the kinetics of labeling of proteins with stable isotope-labeled amino acids and uses mass spectrometry to determine the presence of those labeled amino acids in tryptic peptides. In this respect it differs from other studies.

^1 The abbreviations used are: RIA, relative isotope abundance; 2DGE, two-dimensional gel electrophoresis; CHAPS, 3-[3-cholamidopropyl(dimethylammonio)]-1-propanesulfonic acid; MALDI-TOF, matrix-assisted laser desorption ionization-time of flight.
that have prelabeled proteins with stable isotope-labeled amino acids either to compare expression levels (12, 13), to determine the numbers of specific amino acids to aid in protein identification by peptide mass fingerprinting (11, 14–17), or to determine post-translational modifications (18). We measured the rate of turnover of several *Saccharomyces cerevisiae* proteins in glucose-limited aerobic continuous culture at the steady state. In continuous culture, the cells are maintained in a constant metabolic state, and the gain in biomass through growth is balanced by physical loss of cells as the cell suspension is displaced by incoming fresh medium. In this respect, continuous culture is superior to growth in "batch" culture in which nutrients are depleted, cell number increases, medium pH can fall, and the rate of growth declines.

The yeast cells were grown at a fixed dilution (doubling) rate, and proteins were uniformly labeled with a deuterated amino acid provided in the incoming growth medium. Subsequently a large excess of unlabeled amino acid was added instantaneously to the culture medium, and at the same time, the medium reservoir was switched to one containing the unlabeled amino acid. Because the cells are glucose-limited, the addition of an excess of the unlabeled amino acid does not affect the growth rate, but the labeled proteins are degraded and/or diluted into the daughter cells. The chosen precursor was decadeuterated leucine, labeled at all positions other than the α-amino and α-carboxyl groups and chosen because leucine is present in the great majority of tryptic peptides derived from the yeast proteome (11). Use of a leucine auxotrophic mutant of *S. cerevisiae* ensured that dilution of the label by endogenous leucine would be minimized. Finally, we have shown that the deuterium atom bonded to the α-carbon atom is metabolically labile (probably through transamination), and the relative incorporation of decadeuterated or nondecadeuterated leucine provides a valuable insight into the metabolic lability of the precursor pool, important information in establishing the effectiveness of the labeling strategy (11, 14).

Proteins were prelabeled with heavy leucine for approximately 50 h, more than seven doubling times at a dilution rate of 0.1 h$^{-1}$. Thus, over 99% of the leucine in the cells would be the stable isotope-labeled form. Replacement of the entire leucine pool in this way had no effect on growth rate or on the overall pattern of proteins in a two-dimensional gel (results not shown). The chemostat was then injected with a large excess (20-fold) of unlabeled L-leucine to rapidly reduce the isotope abundance of the precursor pool. The feedstock was switched to unlabeled leucine, and samples of cells were taken from the culture vessel over the next 51 h. The unlabeled leucine pulse added to the growth vessel had no effect on CO2 production, demonstrating that leucine was not being used as an alternative carbon source. Further, there were no quantitative differences in 2DGE patterns from cells before or after the chase with unlabeled leucine (results not shown). During the chase period, all newly synthesized proteins would only incorporate unlabeled leucine. The rate of loss of labeled protein ($k_{\text{loss}}$) is a composite term reflecting the sum of losses through dilution of the cells (synthesis de novo) or through degradation (Fig. 1).

At each sampling time before and throughout the chase period, cells were lysed, and proteins from the cleared lysate
were separated by 2DGE. The pattern of spots on the gels was very consistent (although this is not a prerequisite of the approach), and we could recover the same protein from each gel, which was then subjected to in-gel tryptic digestion followed by MALDI-TOF mass spectrometry (19). The profiles for individual peaks in the trypsin peptide mass fingerprint tracked the replacement of the labeled protein by the unlabeled protein as the cells continued to grow in culture. A representative set of MALDI-TOF data over 51 h, expanded to emphasize the behavior of single peptides (containing one and three leucine residues). The spectra on the right are amplified to show the behavior of single peptides (containing one and three leucine residues). The spectra on the left cover the m/z range from 1000 to 2500 Th. The arrow indicates a peptide that contains no leucine residues and therefore remains at the same mass throughout the chase.

Fig. 2. Changes in peptide mass fingerprint during unlabeled chase period. Yeast cells, prelabeled in the presence of decadeuterated leucine, were subjected to a 51-h chase phase in the presence of excess unlabeled leucine. At different times, samples of the cell suspension were recovered, lysed, and resolved by 2DGE. Following staining with Coomassie Blue the same spot was recovered from each gel and subjected to in-gel tryptic digestion and MALDI-TOF mass spectrometry. The spectra on the right are amplified to show the behavior of single peptides (containing one and three leucine residues). The spectra on the left cover the m/z range from 1000 to 2500 Th. The arrow indicates a peptide that contains no leucine residues and therefore remains at the same mass throughout the chase.

were separated by 2DGE. The pattern of spots on the gels was very consistent (although this is not a prerequisite of the approach), and we could recover the same protein from each gel, which was then subjected to in-gel tryptic digestion followed by MALDI-TOF mass spectrometry (19). The profiles for individual peaks in the trypsin peptide mass fingerprint tracked the replacement of the labeled protein by the unlabeled protein as the cells continued to grow in culture. A representative set of MALDI-TOF data over 51 h, expanded to emphasize the behavior of individual peptides with one or multiple leucine residues, shows that the transition from fully labeled to fully unlabeled peptides was readily apparent (Fig. 2). Proteins were identified by peptide mass fingerprinting supplemented by the data on the leucine composition of each peptide derived from the separation between the heavy and light peaks (see “Experimental Procedures”). Incidentally, if the precursor pool had not been effectively “chased,” peptides of intermediate masses, distributed binomially, would be expected (20). However, for peptides containing more than one leucine residue, the lack of peaks of mass values intermediate between the fully labeled and fully unlabeled forms is convincing proof that the relative isotope abundance of the precursor pool had been efficiently reduced to zero, a prerequisite of this approach. Of course, the lower limit on detection of these peptides of intermediate mass is influenced by the background noise in the spectrum. However, the lack of any such peaks above the noise floor is good evidence for an effective chase. The natural isotope abundance profiles of labeled peptides also confirmed that virtually all of the [2H10]leucine supplied in the medium was converted to
[\textsuperscript{[\textsuperscript{2}H\textsubscript{9}]}leucine in vivo (probably through transamination).

The intensity of the monoisotopic peaks of the heavy and light tryptic fragments were measured for each sampling time point in the chase phase. The transition in intensity between the fully labeled and the fully unlabeled leucine-containing peptides is most simply defined by a single exponential curve that yields the first order rate constant for loss of the label from the protein \(k_{\text{loss}}\). To determine this rate constant, a single exponential curve was fitted to the set of \(RIA_t\) data for each leucine-containing tryptic peptide. Since multiple peptides in a single peptide mass fingerprint would be expected to contain leucine, each peptide should deliver an independent measure of the rate of turnover of the parent protein. For each protein, the first order rate constant was remarkably consistent whether derived from peptides with one or more than one leucine residue (Fig. 3). The errors in the fitted rate constants were typically less than 10\% of the parameter value, and the concordance between the degradation rates, defined by multiple peptides derived from a single protein, was high. For all further analyses, we pooled the data from different peptides to yield a single fitted curve based on multiple determinations of \(RIA_t\) at each time point (Table II, spots 1–31).

Accurate measurement of the degradation rate of a single protein is of particular value in the elucidation of structural parameters that dictate intracellular stability. However, when applied to a global population of proteins in a proteome, multipoint determination of degradation rate would be extremely laborious. A higher throughput approach, more appropriate to global proteomic surveys, might be derived by analysis of a single time point during the chase period. For each protein, analysis of several (leucine-containing) peptides would still yield a statistical estimate of \(RIA_t\) at one value of \(t\) which is related to an estimate of \(k_{\text{loss}}\) by the simple relationship \(k_{\text{loss}} = -\ln(RIA_t/RIA_0)/t\).

We measured \(k_{\text{loss}}\) for several proteins recovered from 2DGE (Fig. 4 and Table II). For almost all proteins in this set, we were able to use data from multiple peptides containing between one and three leucine residues. For protein spots numbered 33 onward, the values of \(k_{\text{loss}}\) were recovered by sampling the cells at two time points (4 and 8 h), the degradation rates were calculated directly, and data from multiple peptides were combined to yield a statistical estimate of the certainty of the rate constant. For spots 1–31, the degradation rate constants had previously been assessed by non-linear curve fitting (FigP, Biosoft, Cambridge, UK). The different symbols indicate the \(RIA_t\) values determined for peptides derived from the same protein, and the solid lines are the best-fit curves for these different data sets. In most instances, the lines are so closely superimposable that the individual traces are not discernible. In the lower panel, the measured rates of loss of label are plotted for the individual peptides. Note that the true degradation rates must be corrected for the base-line rate of loss of protein from the chemostat at 0.086 h\(^{-1}\).

Rate, from cell breakage onward, for a limited number of proteins \((n = 15)\). Again, the correlation between the \(k_{\text{loss}}\) calculated in each experiment was very high \((r^2 = 0.86, p < 0.001; \text{Fig. 5, panel B})\).

Because the cells are in true steady state in the chemostat, the rate of loss of label includes irreversible losses by exit of cells from the system at a true rate of 0.086 h\(^{-1}\) (corrected for sampling). Thus, the intracellular degradation rates of the proteins should be corrected for this loss by simple subtraction of the dilution rate (Fig. 6). Of the ~50 proteins analyzed in this study, one-quarter are degraded at rates less than 0.01 h\(^{-1}\). Two of these (spots 3 and 11, glutamate dehydrogenase and ketol-acid isomerase) are not significantly degraded at all and are only lost by dilution into daughter cells. At the other extreme, one protein (spot 41, methionine synthase) was de-
Proteomic Strategy for Protein Turnover

**TABLE II**

Rates of degradation of yeast proteins

Rates of degradation were measured for approximately 50 spots on a two-dimensional gel. For spots 1–31, gels were run under identical conditions for 12 time points. The same spots were then excised and analyzed for the RIA of heavy leucine in specific peptides; only data from good quality mass spectra are included. For each set of (RIA, t) data, non-linear curve fitting was used to acquire the first order rate constant for loss of label from the protein. This is a composite value representing the sum of the true intracellular degradation rate and the loss of protein due to exit of cells from the chemostat. Finally, the (RIA, t) data from all peptides were combined to generate a single, fitted value for the parameter estimate (all peptides). For spot 33 onward, only cells harvested at 4 and 8 h postchase were analyzed by the single point method.

| Spot no. | Protein ID | Peptide (observed [M + H]+) [number of Leu residues] | Isotope removal (kloss) (h⁻¹, curve fitting analysis $k_{loss} \pm \text{S.E.} (n)$) | Isotope removal (kloss), single point determination (mean $k_{loss} \pm \text{S.E.} (n)$) |
|----------|------------|--------------------------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| 1        | Heat shock protein SSA1 (HS71) | 1552.98 [2] | 0.0972 ± 0.0028 (12) | 0.0972 ± 0.0062 (10) |
|          |            | 2130.22 [2] | 0.1035 ± 0.0040 (12) |                                                                       |
|          |            | 1199.94 [2] | 0.1117 ± 0.0081 (12) |                                                                       |
|          |            | 1431.01 [1] | 0.1012 ± 0.0078 (12) |                                                                       |
|          |            | All peptides | 0.1032 ± 0.0051 (48) |                                                                       |
| 2        | Pyruvate dehydrogenase | 1997.98 [1] | 0.0901 ± 0.0021 (10) | 0.1221 ± 0.0033 (10) |
|          |            | 1597.01 [1] | 0.0930 ± 0.0029 (10) |                                                                       |
|          |            | 1689.90 [1] | 0.0897 ± 0.0035 (10) |                                                                       |
|          |            | All peptides | 0.0919 ± 0.0024 (30) | 0.0976 ± 0.0062 (10) |
| 3        | NADP-specific glutamate dehydrogenase | 1188.80 [1] | 0.0717 ± 0.0024 (10) | 0.0633 ± 0.0024 (6) |
|          |            | 1605.14 [1] | 0.0688 ± 0.0059 (10) |                                                                       |
|          |            | 1468.86 [1] | 0.0721 ± 0.0041 (10) |                                                                       |
|          |            | All peptides | 0.0709 ± 0.0025 (30) |                                                                       |
| 4        | Heat shock protein SSA1 or -2 (HS71/72) (fragment) | 1552.98 [1] | 0.1033 ± 0.0034 (12) |                                                                       |
|          |            | 1675.90 [1] | 0.1100 ± 0.0076 (12) |                                                                       |
|          |            | 1431.03 [1] | 0.1074 ± 0.0057 (12) |                                                                       |
|          |            | 1816.07 [2] | 0.1167 ± 0.0083 (11) |                                                                       |
|          |            | All peptides | 0.1092 ± 0.0049 (47) | 0.1221 ± 0.0033 (10) |
| 5        | Enolase II | 1876.14 [1] | 0.0865 ± 0.0038 (12) |                                                                       |
|          |            | 1431.03 [2] | 0.0906 ± 0.0069 (12) |                                                                       |
|          |            | 2741.17 [2] | 0.0909 ± 0.0023 (12) |                                                                       |
|          |            | All peptides | 0.0893 ± 0.0041 (36) | 0.0851 ± 0.0019 (10) |
| 6        | Enolase I pl ~7.5 | 1876.11 [1] | 0.0933 ± 0.0027 (12) |                                                                       |
|          |            | 1856.98 [1] | 0.0993 ± 0.0103 (12) |                                                                       |
|          |            | 2441.11 [2] | 0.1048 ± 0.0075 (11) |                                                                       |
|          |            | All peptides | 0.0950 ± 0.0058 (35) |                                                                       |
| 7        | Enolase I pl ~7.7 | 1822.08 [2] | 0.1130 ± 0.0077 (11) |                                                                       |
|          |            | 2441.47 [2] | 0.0987 ± 0.0024 (11) |                                                                       |
|          |            | 1578.98 [2] | 0.1190 ± 0.0128 (11) |                                                                       |
|          |            | 1373.87 [1] | 0.0998 ± 0.0025 (11) |                                                                       |
|          |            | All peptides | 0.1034 ± 0.0029 (44) | 0.0952 ± 0.0036 (10) |
| 8        | Phosphoglycerate kinase | 1440.00 [3] | 0.1145 ± 0.0025 (11) |                                                                       |
|          |            | 1768.14 [3] | 0.1079 ± 0.0026 (11) |                                                                       |
|          |            | 1668.04 [2] | 0.1058 ± 0.0025 (11) |                                                                       |
|          |            | 2327.27 [1] | 0.1005 ± 0.0042 (11) |                                                                       |
|          |            | 2039.14 [2] | 0.1005 ± 0.0051 (11) |                                                                       |
|          |            | All peptides | 0.1058 ± 0.0027 (55) | 0.1085 ± 0.0045 (10) |
| 9        | Yol 154wp | 1617.94 [2] | 0.1273 ± 0.0050 (12) |                                                                       |
|          |            | 1961.58 [1] | 0.1155 ± 0.0111 (11) |                                                                       |
|          |            | 1228.75 [1] | 0.1202 ± 0.0027 (10) |                                                                       |
|          |            | All peptides | 0.1224 ± 0.0048 (33) | 0.1241 ± 0.0026 (8) |
| 10       | Fructose-bisphosphate aldolase | 2160.21 [1] | 0.1209 ± 0.0073 (12) |                                                                       |
|          |            | 1794.99 [1] | 0.1157 ± 0.0053 (12) |                                                                       |
|          |            | 1863.04 [1] | 0.1273 ± 0.0069 (12) |                                                                       |
|          |            | 2035.05 [1] | 0.1178 ± 0.0039 (12) |                                                                       |
|          |            | All peptides | 0.1203 ± 0.0054 (48) | 0.1270 ± 0.0035 (8) |
| Spot no. | Protein ID                                      | Peptide (observed [M + H]+) [number of Leu residues] | Isotope removal (k_{loss}) (h^{-1}, curve fitting analysis (k_{loss} ± S.E. (n))) | Isotope removal (k_{loss}) single point determination (mean k_{loss} ± S.E. (n)) |
|---------|------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| 11      | Ketol-acid isomerase                            | 1355.99 [1]                                         | 0.0760 ± 0.0019 (11)                                                           | 0.0038 (9)                                                                   |
|         |                                                | 1170.06 [1]                                         | 0.0790 ± 0.0039 (12)                                                           |                                                                              |
|         |                                                | 1181.94 [1]                                         | 0.0834 ± 0.0068 (12)                                                           |                                                                              |
|         |                                                | 1300.09 [1]                                         | 0.0781 ± 0.0024 (11)                                                           |                                                                              |
|         |                                                | 1530.13 [1]                                         | 0.0772 ± 0.0020 (11)                                                           |                                                                              |
|         |                                                | 1753.27 [2]                                         | 0.0857 ± 0.0041 (11)                                                           |                                                                              |
|         |                                                | 2099.52 [3]                                         | 0.0902 ± 0.0068 (11)                                                           |                                                                              |
|         |                                                | 2145.45 [1]                                         | 0.0801 ± 0.0057 (11)                                                           |                                                                              |
|         |                                                | All peptides                                       | 0.0805 ± 0.0033 (90)                                                          | 0.0680 ± 0.0021 (14)                                                        |
| 12      | Enolase I fragment                              | 1159.82 [1]                                         | 0.1091 ± 0.0174 (9)                                                            |                                                                              |
|         |                                                | 1856.97 [1]                                         | 0.1029 ± 0.0036 (10)                                                           |                                                                              |
|         |                                                | 1578.91 [2]                                         | 0.1149 ± 0.0128 (9)                                                            |                                                                              |
|         |                                                | 1373.78 [1]                                         | 0.1053 ± 0.0077 (9)                                                            |                                                                              |
|         |                                                | 1756.03 [1]                                         | 0.1084 ± 0.0056 (9)                                                            |                                                                              |
|         |                                                | All peptides                                       | 0.1075 ± 0.0042 (37)                                                          |                                                                              |
| 13      | Phosphoglycerate kinase fragment                | 1440.01 [3]                                         | 0.1113 ± 0.0035 (9)                                                            |                                                                              |
|         |                                                | 1668.06 [2]                                         | 0.1034 ± 0.0037 (9)                                                            |                                                                              |
|         |                                                | All peptides                                       | 0.1073 ± 0.0034 (18)                                                          |                                                                              |
| 14      | Glyceraldehyde-3-phosphate dehydrogenase 3      | 1752.78 [1]                                         | 0.0891 ± 0.0021 (8)                                                            |                                                                              |
|         |                                                | 2591.20 [2]                                         | 0.0938 ± 0.0078 (9)                                                            |                                                                              |
|         |                                                | All peptides                                       | 0.0908 ± 0.0045 (17)                                                          |                                                                              |
| 15      | Enolase I fragment                              | 1856.96 [1]                                         | 0.1003 ± 0.0063 (12)                                                           |                                                                              |
|         |                                                | 1578.89 [2]                                         | 0.1164 ± 0.0150 (11)                                                           |                                                                              |
|         |                                                | 1373.78 [1]                                         | 0.1061 ± 0.0112 (11)                                                           |                                                                              |
|         |                                                | All peptides                                       | 0.1071 ± 0.0100 (34)                                                          |                                                                              |
| 16      | Malate dehydrogenase                            | 1752.87 [1]                                         | 0.0897 ± 0.0028 (12)                                                           |                                                                              |
| 17      | Glyceraldehyde-3-phosphate dehydrogenase 3      | 1749.49 [1]                                         | 0.0945 ± 0.0017 (7)                                                            |                                                                              |
| 18      | Glyceraldehyde-3-phosphate dehydrogenase 3      | 1750.67 [1]                                         | 0.0933 ± 0.0013 (9)                                                            |                                                                              |
| 19      | Heat shock protein 26                           | 1806.21 [1]                                         | 0.1262 ± 0.0150 (7)                                                            |                                                                              |
|         |                                                | 2040.57 [2]                                         | 0.1265 ± 0.0228 (7)                                                            |                                                                              |
|         |                                                | All peptides                                       | 0.1263 ± 0.0187 (14)                                                          | 0.1260 ± 0.0083 (6)                                                         |
| 20      | Fructose-bisphosphate aldolase                  | 2390.20 [1]                                         | 0.0998 ± 0.0058 (6)                                                            |                                                                              |
|         |                                                | 1863.07 [1]                                         | 0.0958 ± 0.0066 (6)                                                            |                                                                              |
|         |                                                | 2035.13 [1]                                         | 0.0936 ± 0.0024 (6)                                                            |                                                                              |
|         |                                                | All peptides                                       | 0.0964 ± 0.0044 (18)                                                          |                                                                              |
| 21      | Adenylate kinase                               | 1456.01 [2]                                         | 0.1146 ± 0.0021 (7)                                                            |                                                                              |
|         |                                                | 1994.30 [2]                                         | 0.1166 ± 0.0080 (8)                                                            |                                                                              |
|         |                                                | All peptides                                       | 0.1152 ± 0.0055 (15)                                                          | 0.1299 ± 0.0139 (10)                                                        |
| 22      | Triosephosphate isomerase                       | 1096.83 [1]                                         | 0.1019 ± 0.0017 (12)                                                           |                                                                              |
|         |                                                | 1252.86 [1]                                         | 0.0932 ± 0.0042 (12)                                                           |                                                                              |
|         |                                                | 2763.38 [2]                                         | 0.0954 ± 0.0038 (8)                                                            |                                                                              |
|         |                                                | All peptides                                       | 0.0966 ± 0.0019 (32)                                                          | 0.1035 ± 0.0039 (10)                                                        |
| 23      | Glyceraldehyde-3-phosphate dehydrogenase 3      | 1753.06 [1]                                         | 0.0928 ± 0.0038 (10)                                                           | 0.1046 ± 0.0066 (3)                                                         |
| 25      | Glyceraldehyde-3-phosphate dehydrogenase 3      | 1820.08 [2]                                         | 0.0991 ± 0.0043 (10)                                                           |                                                                              |
| 26      | No ID                                          | 1752.47 [1]                                         | 0.1055 ± 0.0084 (6)                                                            |                                                                              |
| 27      | Glyceraldehyde-3-phosphate dehydrogenase 2      | 1752.94 [1]                                         | 0.1046 ± 0.0067 (11)                                                           |                                                                              |
| 27      | No ID                                          | 1785.86 [2]                                         | 0.1098 ± 0.0090 (5)                                                            |                                                                              |
| 28      | No ID                                          | 1737.86 [2]                                         | 0.1130 ± 0.0050 (5)                                                            |                                                                              |
| 29      | Cpn10                                          | 1591.96 [1]                                         | 0.1087 ± 0.0078 (7)                                                            |                                                                              |
### Table II — continued

| Spot no. | Protein ID | Peptide (observed $[M + H]^+$) [number of Leu residues] | Isotope removal ($k_{loss}$) (h$^{-1}$), curve fitting analysis ($k_{loss}$ ± S.E. (n)) | Isotope removal ($k_{loss}$), single point determination (mean $k_{loss}$ ± S.E. (n)) |
|----------|------------|--------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| 30       | Peptidylprolyl cis-trans isomerase            | 1525.93 [2] 0.1112 ± 0.0078 (7) 0.0978 ± 0.0110 (14) 0.1155 ± 0.0112 (7) 0.089 (1) | All peptides 0.0978 ± 0.0110 (14) 0.1155 ± 0.0112 (7) 0.089 (1) |
| 31       | Heat shock protein 12                  | 1437.80 [1] 0.1126 ± 0.0076 (7) 0.1094 ± 0.0092 (8) | All peptides 0.1111 ± 0.0080 (15) 0.1037 ± 0.0222 (4) |
| 33       | Microsomal protein of CDC48/PAS1/SEC18 family of ATPases | 1756.11 [3] 0.1612, 0.1462 0.1385 ± 0.0061 (6) | All peptides 0.0902 ± 0.0035 (10) |
| 34       | Actin binding protein; Abp1p          | 1583.22 [1] 0.0859, 0.0921 0.0730, 0.0907 0.0719, 0.0936 0.1385 ± 0.0061 (6) | All peptides 0.0902 ± 0.0035 (10) |
| 35       | Kar 2p                                  | 1199.88 [2] 0.0773, 0.08187 0.0985, 0.0805 0.1046, 0.0894 0.0877, 0.0771 0.1176, 0.0909 0.1437, 0.1039 0.1007, 0.0838 0.0855, 0.1027 | All peptides 0.0945 ± 0.0058 (16) |
| 36       | Chain A proteinase A or vacuolar proteinase A Pep 4p | 1700.05 [1] 0.1036, 0.1193 0.0890, 0.0972 0.1013, 0.1415 0.0874, 0.1045 0.1181, 0.1379 0.0871, 0.0973 | All peptides 0.0945 ± 0.0058 (16) |
| 37       | Sse1p; HSP70 family (homologous to Ssa1p and Sse2p) | 1377.88 [1] 0.1216, 0.1253 0.1515, 0.1189 0.1267, 0.0998 0.1182, 0.1163 0.1077, 0.1271 | All peptides 0.1100 ± 0.0006 (10) |
| 38       | Nuclear encoded mitochondrial protein; member of HSP70 family, most similar to Escherichia coli DnaK | 1537.94 [1] 0.0943, 0.1036 0.1515, 0.1189 0.1267, 0.0998 0.1182, 0.1163 0.1077, 0.1271 0.1217 ± 0.0020 (14) | All peptides 0.1217 ± 0.0020 (14) |
| 39       | YDL184w-b                               | 1307.90 [1] 0.1083, 0.1009 0.1040, 0.1018 0.0901, 0.0913 0.0815, 0.1135 | All peptides 0.1186 ± 0.0123 (8) |
| Spot no. | Protein ID | Peptide (observed [M + H]+) | Degradation rate (h⁻¹), single point determination, all peptides (mean ± S.E. (n)) |
|----------|------------|-----------------------------|----------------------------------------------------------------------------------|
| 40       | Ssb2p; stress-seventy subfamily B/Ssb1p; stress-seventy subfamily B, involved in translation | All peptides 1459.95 [1] | 0.1123, 0.1163 0.0989 ± 0.0026 (8) |
|          |            | 1394.86 [2]                 | 0.1364, 0.1288 0.1255 ± 0.0062 (11) |
|          |            | 1185.79 [1]                 | 0.1545, 0.1348 0.1255 ± 0.0062 (11) |
|          |            | 1242.76 [2]                 | 0.1567, 0.1002 0.1255 ± 0.0062 (11) |
|          |            | 1730.07 [1]                 | 0.0917, 0.1347 0.1255 ± 0.0062 (11) |
|          |            | 2071.14 [1]                 | 0.1145 0.1255 ± 0.0062 (11) |
| 41       | Methionine synthase | All peptides 1710.14 [1] | 0.2059, 0.1525 0.1769 ± 0.0119 (4) |
|          |            | 1517.83 [1]                 | 0.1859, 0.1633 0.1769 ± 0.0119 (4) |
| 42       | Tkl1p; transketolase 1/yeast transketolase | All peptides 1230.81 [2] | 0.1331, 0.1172 0.1769 ± 0.0119 (4) |
|          |            | 1094.74 [1]                 | 0.1105, 0.1016 0.1769 ± 0.0119 (4) |
|          |            | 1485.93 [2]                 | 0.1209, 0.1206 0.1769 ± 0.0119 (4) |
|          |            | 1708.10 [1]                 | 0.1372, 0.1004 0.1769 ± 0.0119 (4) |
|          |            | 1869.00 [1]                 | 0.1092, 0.1023 0.1769 ± 0.0119 (4) |
|          |            | 2315.33 [1]                 | 0.1107, 0.1004 0.1769 ± 0.0119 (4) |
| 43       | Acetyl-CoA deacylase, mannose-containing glycoprotein that binds concanavalin A; Ach1p | All peptides 1259.89 [1] | 0.0996, 0.1111 0.1137 ± 0.0034 (10) |
|          |            | 1608.03 [1]                 | 0.0996, 0.1122 0.1137 ± 0.0034 (10) |
|          |            | 1409.00 [1]                 | 0.1132, 0.1059 0.1137 ± 0.0034 (10) |
|          |            | 1424.94 [1]                 | 0.1082, 0.0977 0.1137 ± 0.0034 (10) |
|          |            | 1769.12 [1]                 | 0.0754, 0.1145 0.1137 ± 0.0034 (10) |
| 44       | Pyruvate kinase Cdc19p; required for start of cell cycle | All peptides 1501.98 [1] | 0.0876, 0.0879 0.1037 ± 0.0020 (10) |
|          |            | 1315.89 [1]                 | 0.0973, 0.0959 0.1037 ± 0.0020 (10) |
|          |            | 1227.75 [2]                 | 0.0981, 0.1041 0.1037 ± 0.0020 (10) |
|          |            | 2001.22 [3]                 | 0.0867, 0.1024 0.1037 ± 0.0020 (10) |
|          |            | 1761.03 [1]                 | 0.0893, 0.0781 0.1037 ± 0.0020 (10) |
| 45       | Lipoamide dehydrogenase, chain A, FAD flavoprotein | All peptides 1174.67 [1] | 0.1147, 0.1102 0.1142 ± 0.0025 (8) |
|          |            | 1564.48 [1]                 | 0.1115, 0.1172 0.1142 ± 0.0025 (8) |
|          |            | 1496.43 [1]                 | 0.1284, 0.1094 0.1142 ± 0.0025 (8) |
|          |            | 1801.00 [1]                 | 0.1077, 0.1145 0.1142 ± 0.0025 (8) |
| 46       | ATPase1 α su | All peptides 1553.84 [1] | 0.1037, 0.1160 0.1117 ± 0.0020 (16) |
|          |            | 1325.80 [2]                 | 0.1166, 0.1267 0.1117 ± 0.0020 (16) |
|          |            | 1350.83 [1]                 | 0.1103, 0.1114 0.1117 ± 0.0020 (16) |
|          |            | 1458.84 [1]                 | 0.1016, 0.1108 0.1117 ± 0.0020 (16) |
|          |            | 1438.93 [1]                 | 0.1077, 0.1150 0.1117 ± 0.0020 (16) |
|          |            | 1273.75 [2]                 | 0.1032, 0.1176 0.1117 ± 0.0020 (16) |
|          |            | 1602.91 [1]                 | 0.1155, 0.1131 0.1117 ± 0.0020 (16) |
|          |            | 1935.08 [1]                 | 0.1037, 0.1153 0.1117 ± 0.0020 (16) |
| 47       | Shm2p serine hydroxymethyltransferase | All peptides 1196.70 [1] | 0.1607, 0.1305 0.1376 ± 0.0060 (11) |
|          |            | 1168.69 [1]                 | 0.1471, 0.1126 0.1376 ± 0.0060 (11) |
|          |            | 1572.99 [1]                 | 0.1646, 0.1318 0.1376 ± 0.0060 (11) |
|          |            | 1728.05 [3]                 | 0.1305, 0.1163 0.1376 ± 0.0060 (11) |
|          |            | 1690.00 [1]                 | 0.1545, 0.1147 0.1376 ± 0.0060 (11) |
|          |            | 1707.02 [1]                 | 0.1501 0.1376 ± 0.0060 (11) |
| 48       | G4p1 ± Arc1p; associated with tRNA and aminoaeryl-tRNA synthetases | All peptides 1259.64 [1] | 0.1049, 0.1067 0.1376 ± 0.0060 (11) |
graded intracellularly at a rate of over 9%/h. Even allowing the caveat that we selected high abundance proteins, the range of degradation rates is over 9-fold in cells that are in balanced exponential growth.

**DISCUSSION**

We deliberately focused on the more abundant proteins to develop the approach and to assess the dynamics of bulk proteins in exponentially growing cells. Although such abundant proteins might be expected to be long-lived, the degradation rates are remarkably heterogeneous, which raises important issues of selectivity of degradation of this class of proteins. For example, a simple process of vacuolar internalization cannot impose any heterogeneity on degradation rates, and whatever the mechanism of intracellular proteolysis, selective mechanisms must operate. A previous study of selected yeast proteins (including several analyzed here) implied that they were not degraded in exponential growth (21), but the labeling/chase time periods were short, which serves to emphasize the importance of the extended labeling/chase protocol in accessing lower turnover rates. Moreover, the radiolabeling approach used previously (21, 22) cannot readily be combined with the identification step, and autoradiography of the entire protein spot means that the enhanced statistical certainty deriving from multiple peptides is inaccessible.

The ability to determine, with a high degree of accuracy and precision, the rate of degradation of individual proteins opens up an additional dimension in proteomics. The "single point" method yields a reliable parameter estimate and a statistical certainty of high quality and would be particularly suited to studies of relative rates of protein degradation. For more detailed analyses of the routes and rates of degradation of individual proteins, serial sampling and multiple time point determination of isotope abundance creates a data set that is amenable to non-linear curve fitting. The labeling strategy used here works optimally when cells are maintained in steady-state culture, but there will be other labeling protocols that are more amenable to batch culture, organ culture, or intact animals. It would, for example, be feasible to measure the rate of synthesis of each protein by transient exposure to the precursor label, although this would require knowledge of the precursor isotope abundance, and unlike radioisotope labeling, the degree of incorporation of the stable isotope label has to be substantial (>20%) for reliable estimation of the abundance of mass shifted peptides.

**TABLE II—continued**

| Spot no. | Protein ID | Peptide (observed \([M + H]^+\) | Degradation rate (h\(^{-1}\)) single point determination (4 h, 8 h) | Degradation rate (h\(^{-1}\)), single point all peptides | Degradation rate (h\(^{-1}\)), single point all peptides (mean ± S.E. (n)) |
|----------|------------|----------------------------------|---------------------------------------------------------------|---------------------------------------------------|---------------------------------------------------------------|
| 49       | Idh2p NAD\(^+\)-dependent isocitrate dehydrogenase | 1243.64 [1] | 0.1155, 0.1082 | 0.1025 ± 0.0026 (10) |
| 50       | Tpm1p; tropomyosin I | 1435.73 [2] | 0.1113, 0.1241 | 0.1070 ± 0.0038 (6) |
| 51       | Bm2p; brain modulosignalin homologue/protein from family 14.3.3 | 1644.89 [1] | 0.1013, 0.1440 | 0.1246 ± 0.0087 (7) |
| 52       | Brain modulosignalin homologue or BMHI | 1834.04 [1] | 0.1000, 0.1066 | 0.1074 ± 0.0020 (16) |

graded intracellularly at a rate of over 9%/h. Even allowing the caveat that we selected high abundance proteins, the range of degradation rates is over 9-fold in cells that are in balanced exponential growth.
The method, in common with all other methods to measure protein turnover rates, is limited in the ability to define the rate of high turnover proteins. But high turnover proteins would be manifest by a very rapid loss of heavy label, and even if the precise rate of degradation was not quantifiable, the protein would still be classified as “high turnover.” By the same arguments, the proteins for which the rate of loss of label is equal to dilution rate can be classified as “extremely low turnover,” and two such proteins have been identified in this set. The mechanism whereby a protein evades the degradative machinery of the cell can cast as much light on the molecular recognition and enzymology of the process as can the study of high turnover proteins.

Although it is most simple to obtain multiple measures of turnover rate from the peptides derived from an excised spot, there is no reason why this method could not be applied to peptides isolated by liquid chromatography. A liquid chromatography/mass spectrometry experiment would yield $R_{IA}$, but this would be as a single value. Further improvement in the statistical certainty of the measure would be obtained either from the use of multiple time points, to which the curve fitting approach could be applied, or by sufficiently exhaustive coverage of a liquid chromatography profile to recover turnover rates from multiple unique peptides deriving from one protein. In this respect, the choice of leucine is important as it is the...
most abundant amino acid in the proteome (11).

We plan to extend this analysis to the study of proteins commonly occurring in supramolecular complexes, the assembly and conformation of which have profound effects on function and stability. In this study we measured the turnover rate of a protein species that migrates to a single spot. However these protein molecules could have been part of widely different functional complexes with other proteins, and the turnover rate could just be an average of a number of distinct rates that operate at the level of supramolecular complexes. Including a fractionation step that preserves protein-protein interactions prior to analysis would separate proteins that are involved in multiple tasks into different fractions. Subsequent analysis of turnover could reveal the same protein to exhibit a different turnover rate depending on the function of the complex from which it derived.

Despite the importance of protein degradation in maintenance of the proteome, the process remains largely elusive. The discovery of the ubiquitin conjugation and proteasome systems has identified the marking and proteolytic mechanisms that are responsible for degradation of many intracellular proteins (23, 24). The key area that is poorly understood is that of the selectivity of the process whereby different proteins are committed to degradation at dramatically different rates. The accurate determination of degradation rate is an essential parameter in the study of the regulation and manipulation of protein turnover, for instance in the industrial production of recombinant proteins. Further, the single peptide approach can provide a rapid overview of the range and scope of protein degradation for large numbers of constituents of the proteome. With the development of such approaches, the dynamics of the proteome need no longer be considered inaccessible, and the relationship between transcriptome and proteome should be better understood.

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Fig. 6. True degradation rates of soluble yeast proteins. For each of the proteins indicated on the two-dimensional gel (Fig. 4), the true intracellular degradation rate was calculated by correction for the cell dilution rate. Data are presented with the error indicated (spots 1–31, S.E. for the fitted value of the parameter estimate, spots 33 onward, mean ± S.E. for multiple peptides). For values of n, see Table II. The inset shows a distribution profile of degradation rates for these ~50 relatively high abundance yeast proteins.
REFERENCES

1. Miklos, G. L., and Maleszka, R. (2001) Protein functions and biological contexts. Proteomics 1, 169–178
2. Gottesman, S., and Maurizi, M. R. (1992) Regulation by proteolysis: energy-dependent proteolysis and their targets. Microbiol. Rev. 56, 592–621
3. Hochstrasser, M., Johnson, P. R., Arendt, C. S., Amerik, A., Swaminathan, S., Swanson, R., Li, S. J., Laney, J., Pals-Pylaarsdam, R., Nowak, J., and Connelly, P. L. (1999) The Saccharomyces cerevisiae ubiquitin-proteasome system. Philos. Trans. R. Soc. Lond. B Biol. Sci. 354, 1513–1522
4. Benardoudj, N., Tarsca, E., Cascio, P., and Goldberg, A. L. (2001) The unfolding of substrates and ubiquitin-independent protein degradation by proteasomes. Biochimie (Paris) 83, 311–318
5. Gygi, S. P., Rocchon, Y., Franza, B. R., and Aebersold, R. (1999) Correlation between protein and mRNA abundance in yeast. Mol. Cell. Biol. 19, 1720–1730
6. Ideker, T., Thorsson, V., Ranish, J. A., Christmas, R., Bhuler, J., Eng, J. K., Bumgarner, R., Goodlett, D. R., Aebersold, R., and Hood, L. (2001) Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. Science 292, 929–934
7. Griffin, T. J., Gygi, S. P., Ideker, T., Rist, B., Eng, J., Hood, L., and Aebersold, R. (2002) Complementary profiling of gene expression at the transcriptome and proteome levels in Saccharomyces cerevisiae. Mol. Cell. Proteomics 1, 323–333
8. Chen, G., Gharib, T. G., Huang, C. C., Taylor, J. M., Misek, D. E., Kardia, S. L., Giordano, T. J., Iannettoni, M. D., Orringer, M. B., Hanash, S. M., and Beer, D. G. (2002) Discordant protein and mRNA expression in lung adenocarcinomas. Mol. Cell. Proteomics 1, 304–313
9. Brachmann, C. B., Davies, A., Cost, G. J., Caputo, E., Li, J., Hieter, P., and Boeke, J. D. (1998) Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115–132
10. Baganz, F., Hayes, A., Farquhar, R., Butler, P. R., Gardner, D. C., and Oliver, S. G. (1998) Quantitative analysis of yeast gene function using competition experiments in continuous culture. Yeast 14, 1417–1427
11. Pratt, J. M., Robertson, D. H., Gaskell, S. J., Riba-Garcia, I., Hubbard, S. J., Sidhu, K., Oliver, S. G., Butler, P., Hayes, A., Petty, J., and Beynon, R. J. (2002) Stable isotope labelling in vivo as an aid to protein identification in peptide mass fingerprinting. Proteomics 2, 157–163
12. Ong, S. E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., and Mann, M. (2002) Stable isotope labeling by amino acids in cell culture, SILAC as a simple and accurate approach to expression proteomics. Mol. Cell. Proteomics 1, 376–386
13. Jiang, J., and English, A. M. (2002) Quantitative analysis of the yeast proteome by incorporation of isotopically labeled leucine. J. Proteome Res. 1, 345–350
14. Hirayama, K., Yuji, R., Yamada, N., Naguchi, K., Yamaguchi, Y., Enokizono, J., Kato, K., Arata, Y., and Shimada, I. (1998) Convenient peptide mapping of immunoglobulin g2b and determination between leucine and isoleucine residues by mass spectrometry using 2h-labeled leucine. J. Mass. Spectrom. Soc. Jpn. 46, 83–89
15. Chen, X., Smith, L. M., and Bradbury, E. M. (2000) Site-specific mass tagging with stable isotopes in proteins for accurate and efficient protein identification. Anal. Chem. 72, 1134–1143
16. Hunter, T. C., Yang, L., Zhu, H., Majidi, V., Bradbury, E. M., and Chen, X. (2001) Peptide mass mapping constrained with stable isotope-tagged peptides for identification of protein mixtures. Anal. Chem. 73, 4891–4902
17. Engen, J. R., Bradbury, E. M., and Chen, X. (2002) Using stable-isotope-labeled proteins for hydrogen exchange studies in complex mixtures. Anal. Chem. 74, 1680–1686
18. Zhu, H., Hunter, T. C., Pan, S., You, P. M., Bradbury, E. M., and Chen, X. (2002) Residue-specific mass signatures for the efficient detection of protein modifications by mass spectrometry. Anal. Chem. 74, 1687–1694
19. Shevchenko, A., Jensen, O. N., Pontelectniov, A. V., Sagioloco, F., Wilm, M., Vor, O., Mortsensen, P., Boucherie, H., and Mann, M. (1996) Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels. Proc. Natl. Acad. Sci. U. S. A. 93, 14440–14445
20. Papageorgopoulos, C., Caldwell, K., Shackleton, C., Schweingrubner, H., and Hellerstein, M. K. (1999) Measuring protein synthesis by mass isotopomer distribution analysis (MIDA). Anal. Biochem. 267, 1–16
21. Futter, B., Latter, G. I., Monardo, P., McLaughlin, C. S., and Garrels, J. I. (1999) A sampling of the yeast proteome. Mol. Cell. Biol. 19, 7357–7368
22. Grunenfelder, B., Rummel, G., Vohnadsky, J., Roden, D., Langen, H., and Jenal, U. (2001) Proteomic analysis of the bacterial cell cycle. Proc. Natl. Acad. Sci. U. S. A. 98, 4681–4686
23. Varshavsky, A. (1996) The N-end rule: functions, mysteries, uses. Proc. Natl. Acad. Sci. U. S. A. 93, 14140–14145
24. DeMartino, G. N., and Slaughter, C. A. (1999) The proteasome, a novel protease regulated by multiple mechanisms. J. Biol. Chem. 274, 22123–22126