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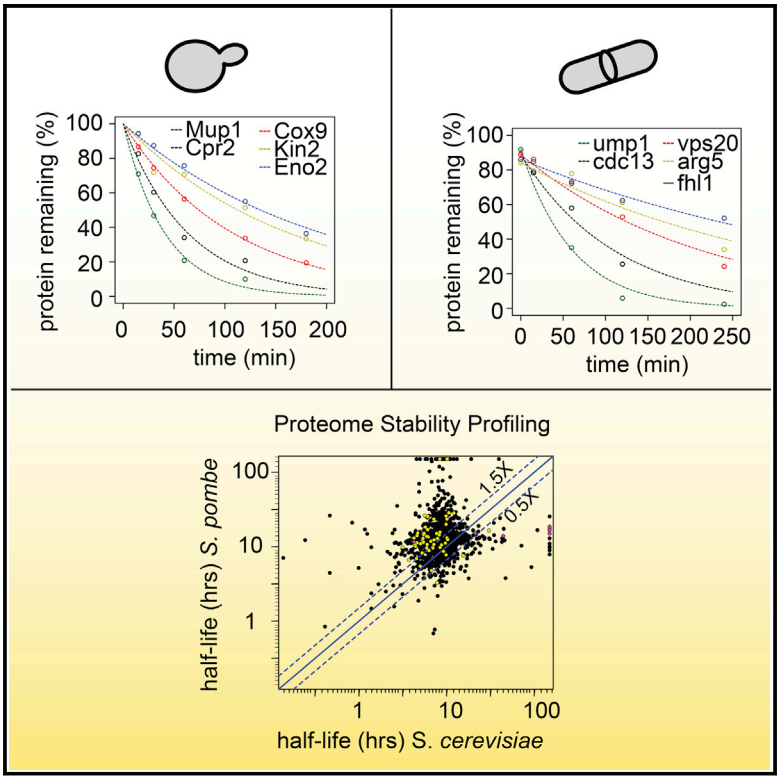
Global Proteome Turnover Analyses of the Yeasts *S. cerevisiae* and *S. pombe*

Graphical Abstract



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In Brief

Christiano et al. report protein turnover rates for *S. cerevisiae* and *S. pombe*. Overall, protein half-life is far less conserved than protein abundance. Protein turnover profiling of *hrd1Δ*, an endoplasmic-reticulum-associated degradation (ERAD)-defective mutant, identifies candidate substrates for this pathway in budding yeast.

Highlights

Proteome-wide turnover rates for two distant yeast species

The vast majority of proteins are long-lived in *S. cerevisiae* and *S. pombe*

Protein abundance, but not turnover rate, is well conserved

Identification of candidate substrates for ERAD degradation in *S. cerevisiae*

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Global Proteome Turnover Analyses of the Yeasts *S. cerevisiae* and *S. pombe*

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SUMMARY

How cells maintain specific levels of each protein and whether that control is evolutionarily conserved are key questions. Here, we report proteome-wide steady-state protein turnover rate measurements for the evolutionarily distant but ecologically similar yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. We find that the half-life of most proteins is much longer than currently thought and determined to a large degree by protein synthesis and dilution due to cell division. However, we detect a significant subset of proteins (~15%) in both yeasts that are turned over rapidly. In addition, the relative abundances of orthologous proteins between the two yeasts are highly conserved across the 400 million years of evolution. In contrast, their respective turnover rates differ considerably. Our data provide a high-confidence resource for studying protein degradation in common yeast model systems.

INTRODUCTION

The levels of proteins often determine phenotypes. Protein abundance is a result of their synthesis and degradation rates. At steady state, these rates are equal and characteristic for each protein under a set of conditions. In *S. cerevisiae*, bulk measurements of protein mass turnover indicate that protein half-lives can range two orders of magnitude (Gancedo et al., 1982). Analyses of individual proteins have revealed different proteasomal or lysosomal pathways for their degradation.

Most measurements of individual proteins' turnover rates were determined in experiments following a ^{35}S pulse label in a protein of interest isolated with specific antibodies during a chase. However, this approach is difficult to extend to the whole proteome. As an alternative, protein stability is assayed by blocking protein synthesis using cycloheximide and measuring the level of a protein of interest at different time points. Nevertheless, in this approach, cells are not kept at steady state, as blocking translation induces a stress response (MacGurn et al., 2011). In addition, performing such analyses systematically relies on tagging proteins, which may lead to changes in protein stability. Alternative approaches, such as imaging-based approaches in combination with photobleaching (Eden et al., 2011), chemical modification (Bojkowska et al., 2011), or mass spectrometry-based proteomics studies (Boisvert et al., 2012; Cambridge et al., 2011; Helbig et al., 2011; Pratt et al., 2002; Schwanhäusser et al., 2011; Toyama et al., 2013), have yielded important insights but have not been extended to a system-wide characterization of protein turnover.

Here, we overcome these limitations of globally determining protein turnover by capitalizing on recent advances in mass spectrometry-based proteomic technology (Bensimon et al., 2012; Fröhlich et al., 2013; Mann et al., 2013; Michalski et al., 2011; Zhang et al., 2013). We report protein turnover rates of nearly all proteins expressed under standard conditions for two model organisms, *S. cerevisiae* and *S. pombe.* We identify a subset of proteins (~15%) that turn over rapidly and provide evidence that protein stability is less evolutionary conserved than protein abundance.

RESULTS

Turnover Rate Measurements in *S. cerevisiae* and *S. pombe* by Pulse Stable Isotope Labeling by Amino Acids in Cell Culture

To determine protein turnover rates systematically, we metabolically labeled the yeasts with heavy isotopes containing lysine ("heavy" lysine, H) and diluted the cells in an excess of normal lysine ("light" lysine, L) at the beginning of the experiment (Figures 1A, S1A, and S1B). We analyzed the decay of the heavy lysine signal in the proteome over time by high-resolution mass spectrometry-based proteomics (Schwanhäusser et al., 2011) (Figure 1B). We identified 4,425 and 3,705 proteins with average sequence coverages of 11.2 and 8.9 peptides/proteins in *S. cerevisiae* and *S. pombe*, respectively (Table 1). Heavy to light (H/L) ratios from independent biological replicates were highly reproducible in both yeasts (R^2 = 0.98 and 0.96; Figures 1C and S1C). The abundance of proteins did not change significantly during the experiment, as expected at steady state (90% and 89% of 3,292 and 3,108 proteins identified in at least three time points in *S. cerevisiae* and *S. pombe,* respectively, have ≤5% variation of summed

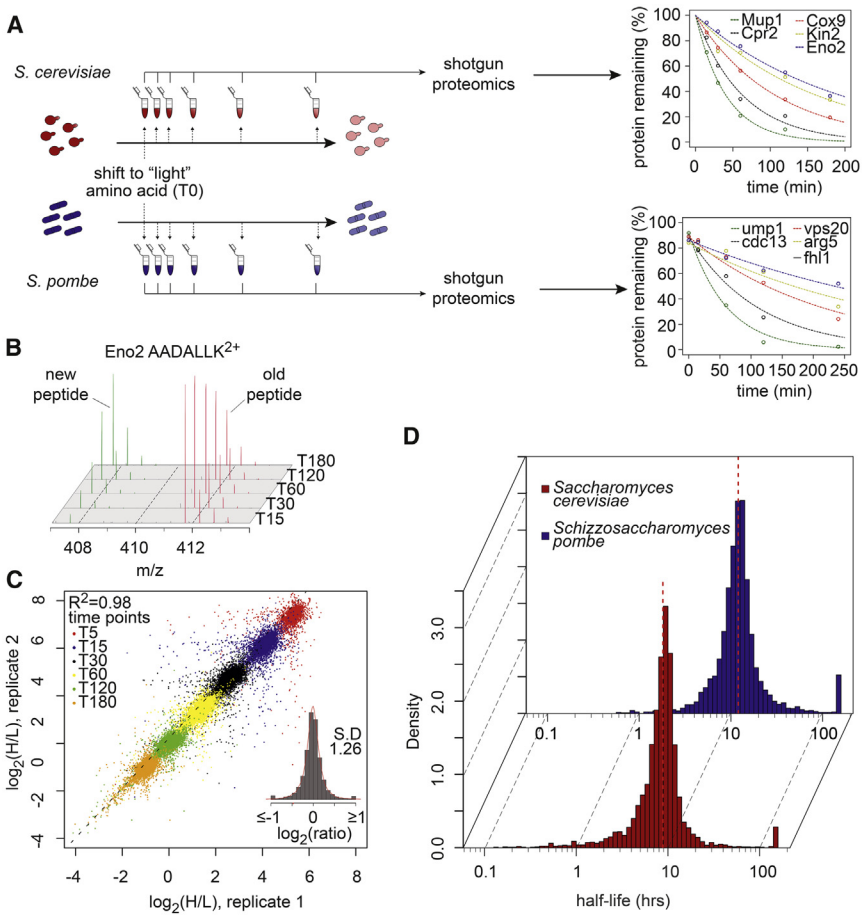


Figure 1. Quantifying Protein Turnover in *S. cerevisiae* and *S. pombe*

(A) Experimental design for turnover measurements in yeasts. Decay curves for the indicated proteins in *S. cerevisiae* and *S. pombe* are shown.

(B) Mass spectra after labeling of a peptide from Eno2 (AADALLK2+) in *S. cerevisiae*. The old peptide ("heavy," red) decays as the newly synthesized peptide ("light," green) increases in intensity during the time course of the experiment.

(C) Log_2(H/L) intensities from 40,452 peptides measured in two independent biological replicates. The insert shows the histogram distribution of log_2(H/L) (mean = −0.0018, SD = 1.26 expressed as a fold change from the mean).

(D) Histograms of protein half-lives in *S. cerevisiae* (red, dashed line indicates the median half-life = 8.8 hr) and *S. pombe* (blue, dashed line indicates the median half-life = 11.1 hr).

peptide intensities; Figures S1D and S1E). Curve fitting and stringent filtering (Experimental Procedures; Figures S1F and S1G) yielded high-confidence half-life measurements for 3,676 (85% of identified proteins) and 3,025 (81% of identified proteins) proteins for the two yeasts, respectively (Tables S1 and S2).

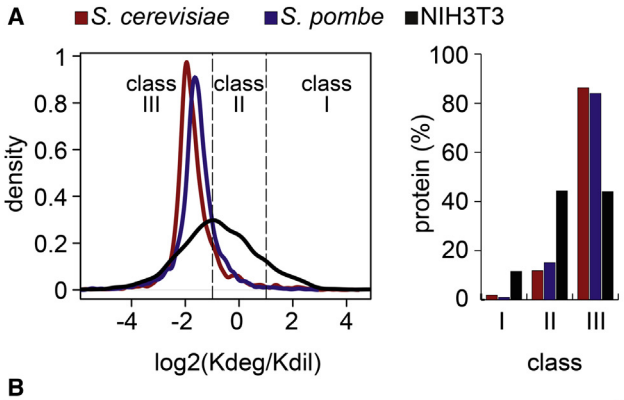
Surprisingly, the vast majority of proteins in both yeasts are very long-lived. The median half-lives in budding and fission yeast in the conditions of our experiments are 8.8 and 12.0 hr, respectively (Figure 1D). Remarkably, and in agreement with previous measurements on the turnover of bulk protein mass (Gancedo et al., 1982), distribution of half-lives span two orders of magnitude and range from a few minutes to more than 100 hr in both species (Figure 1D; Tables S1 and S2). As observed in other model systems (Doherty et al., 2009), the distribution of half-lives in both species does not follow a normal distribution but is skewed with more short-lived proteins (Kolmogorov-Smirnov test, $p < 2.2 \times 10^{-16}$). Our results were further validated by comparing our data set to known turnover rates determined by other experimental approaches, such as pulse chase with radioactively labeled amino acids (Table S3). In addition, comparative analysis of each protein's turnover rate also highlighted problems with measuring the half-lives of tagged proteins during cycloheximide treatment with significant deviations from the rates measured by our proteomics approach. This might be due to altered cell physiology during protein synthesis inhibition and/or the modification of proteins with tags (Belle et al., 2006) (Figures S2A and S2B).

Table 1. Description of Mass Spectrometry Data

Species	Unique Peptides Identified	Number of Proteins Identified	Mean Coverage (%)	Peptides per Protein	Number of Half-Lives
S. cerevisiae	48,448	4,425	30.45	11.24	3,748
S. pombe	32,980	3,705	29.9	8.9	3,016

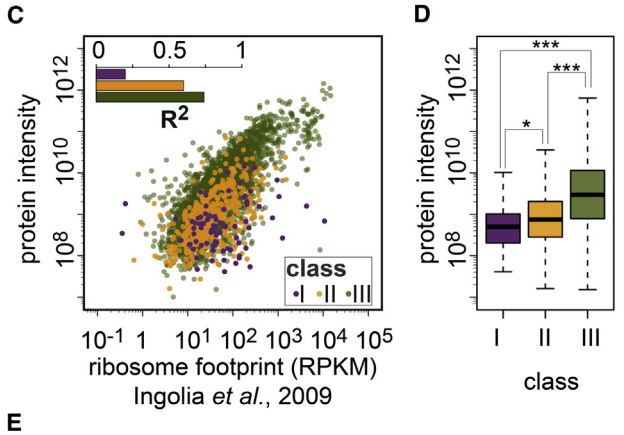
Three Regimes of Protein Abundance Control

Our analyses on the overwhelming majority of yeast proteins revealed three classes, representing three distinct regimes of protein abundance control (Figure 2A; Table S4). Two classes appear to mediate the rapid and competitive growth of the two yeasts. Class I contains a small fraction (~2% in *S. cerevisiae* and ~1% in *S. pombe*) of very-short-lived proteins, many driving the cell cycle ($p = 4.4 \times 10^{-3}$; Figure 2B), for which the degradation rates are at least twice the dilution rate due to cell growth ($t_{1/2}$ < 1.25 hr in *S. cerevisiae* and $t_{1/2}$ < 2 hr in *S. pombe*). Their abundance is likely primarily determined by their rapid degradation. A second class of very stable proteins predominantly driving growth and mass accumulation (class III) is defined by degradation rates less than half the dilution rate ($t_{1/2} \geq 5$ hr and $t_{1/2} \geq 8$ hr in *S. cerevisiae* and *S. pombe*, respectively) and contains most proteins in both yeasts (86% and 84% of proteins). The abundance of these proteins is predominantly determined by different synthesis rates but similar dilution rates due to cell division. Class II proteins show intermediate half-lives, where abundance is determined by the interplay of protein synthesis, degradation, and dilution (12.5% in *S. cerevisiae* and 15% in *S. pombe*). In *S. cerevisiae*, these proteins mediate many regulated processes,



B

Class I 67 proteins	
GO terms/KEGG (number of proteins)	p value
Cell Cycle	$4.4.10^{-3}$
Class II 426proteins	
GO terms/KEGG (number of proteins)	p value
intrinsinc component of membrane (135)	$7.8.10^{-3}$
mitohondrial ribosome (18)	1.110^{-2}
integral component of membrane (127)	$4.0.10^{-2}$
cell periphery (75)	$5.0.10^{-3}$
mitochondrial, respiratory chain (10)	$6.1.10^{-3}$
substrate-specific transmembrane transporter activity	8.10^{-3}
ion transmembrane transporter (26)	$7.2.10^{-3}$



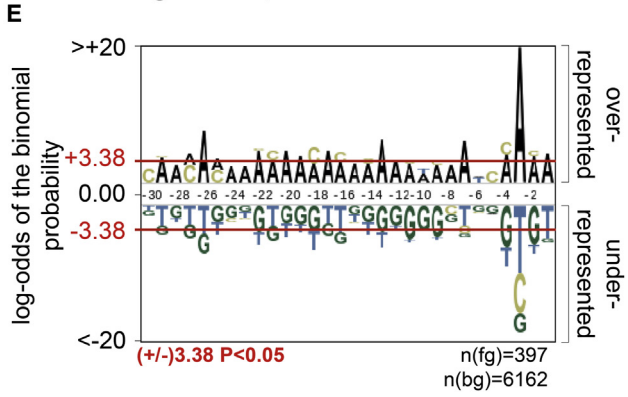


Figure 2. Protein Half-Life Differences in Distinct Sets of Proteins

(A) Contributions of degradation (K_{deg}) and protein dilution due to cell growth (K_{dil}) in *S. cerevisiae* and *S. pombe*. $Log_2(K_{deg}/K_{dil})$ ratios define three classes of protein abundance regulation: class I ($K_{deg} \geq 2xK_{dil}$), class II ($0.5xK_{dil} \leq K_{deg} \geq 2xK_{dil}$), and class III ($K_{deg} \leq 2xK_{dil}$).

(B) Gene Ontology (GO) analysis of class I and class II proteins in *S. cerevisiae*.

(C) Comparison of protein abundances with ribosome footprint data in *S. cerevisiae* for proteins of class I (purple), class II (orange), and class III (green).

(D) Comparison of protein abundances in class I, II, and III.

(E) Sequence analysis of the 5′ UTR (30-mer ahead of start codon) of the 10% most abundant proteins in *S. cerevisiae*.

such as nutrient transport across the plasma membrane (Figure 2B). Together, class I and II, with significant contribution of degradation to half-life, contain 14.5% of the analyzed proteins. As we do not have information on 20% of the proteins not passing our very stringent filtering criteria, this class could contain some more proteins.

The notion that the abundance of proteins in classes I and II is influenced by degradation is further supported by comparing the protein abundance in *S. cerevisiae* with available ribosome footprint data (Ingolia et al., 2009) (Figure 2C). These data reflect the amount of actively translating ribosomes on a message and thus serve as a proxy for measuring the protein synthesis rate of each protein. In agreement with a dominant contribution of protein synthesis and dilution to overall protein abundance, ribosome footprint experiments accurately predict the abundance of class III proteins ($R^2 = 0.73$ for class III). In contrast, ribosome footprint does not correlate as well with the abundance of class II proteins ($R^2 = 0.60$) and strikingly fails to predict the abundance of class I proteins ($R^2 = 0.20$). For the latter, degradation provides a significant additional contribution to determining protein abundance. Accordingly, the longer-lived proteins of class III are significantly more abundant than those of other classes, where protein degradation plays a significant role in determining overall levels ($p < 1 \times 10^{-15}$; Figure 2D). Remarkably, highly efficient translation to preferentially express large amounts of some proteins for cell growth and outcompete bacteria and other yeasts appears to contribute to this. We detected a significantly overrepresented sequence motif in the 30-mer nucleotide localized in the 5′ UTR of mRNAs encoding the 10% most abundant proteins of class III. Interestingly, similar sequences, albeit shorter, have been selected in vitro as mediating highly efficient translation (Dvir et al., 2013).

Protein Abundances, but Not Turnover Rates, Are Evolutionary Conserved

Abundance and turnover rate comparisons of 1,688 one-to-one orthologs between budding and fission yeast revealed surprising insights. Most importantly, the relative abundance of proteins was highly conserved and thus overall highly correlated between species (Pearson $R^2 = 0.64$; Figure 3A). In contrast, the specific turnover rates of conserved proteins in both organisms are remarkably different (Pearson $R^2 = 0.16$; Figures 3B and 3C). These findings suggest that relative protein abundances evolved under stronger evolutionary constraints across the 400 million years of evolution than the protein turnover rates that contribute to obtain these levels.

Our data set provides a rich resource for analysis of such divergent evolution. Proteins in *S. pombe* with half-lives at least twice

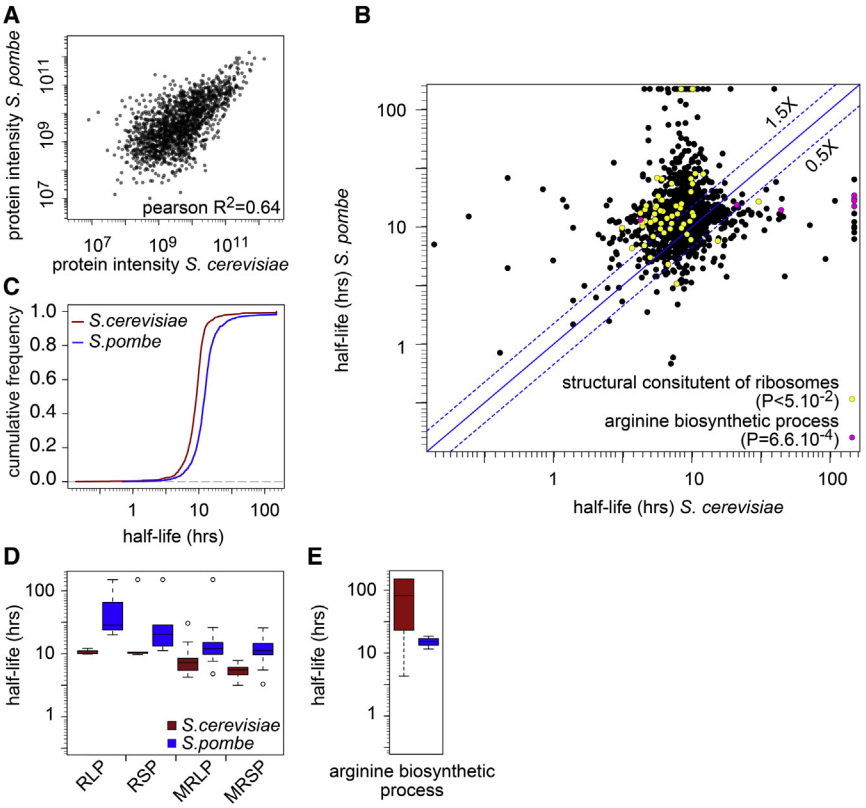


Figure 3. Protein Abundances, but Not Half-Lives, Are Evolutionarily Conserved between *S. cerevisiae* and *S. pombe*

(A) Scatterplot comparing homologous protein abundances.

(B) Scatterplot comparing homologous protein half-lives.

(C) Cumulative density distribution of protein half-lives.

(D) Half-life comparison of the large (RLP, MRLP) and the small (RSP, MRSP) subunits of the cytosolic and mitochondrial ribosomes.

(E) Half-life comparison of proteins involved in the arginine biosynthetic pathway.

as long as their counterparts in *S. cerevisiae* (329 proteins) are significantly enriched in factors for RNA processing (74 proteins, $p = 1.45 \times 10^{-6}$), ribosome biogenesis proteins (71 proteins, $p = 2.5 \times 10^{-13}$), and mitochondrial translation (27 proteins, $p = 3.66 \times 10^{-5}$). For example, the relative abundance of ribosomal proteins is very similar in *S. cerevisiae* and *S. pombe* ($R^2 = 0.64$; Figures S3A and S3B). However, turnover rates of the same proteins are very different in *S. cerevisiae* (Figure 3B). Protein turnover rates of the cytoplasmic ribosomal proteins fall within a very narrow range (Figure 3D). In contrast, we observed great variation of ribosomal protein turnover in *S. pombe (*Figure 3D). As ribosomal proteins in both organisms fall into class III of very-long-lived proteins, ribosomal protein synthesis rate, rather than assembly or degradation, is likely much more tightly coordinated in *S. cerevisiae* than in *S. pombe*. Interestingly, proteins with half-lives in *S. cerevisiae* twice as long as in *S. pombe* (64 proteins) are significantly enriched in factors playing a role in arginine metabolism (five proteins, $p = 6.6 \times 10^{-4}$; Figures 3B and 3E).

Evolutionary Divergence of Protein Degradation for Ergosterol Metabolic Enzymes

Divergence of gene expression regulation is thought to be important in evolution (Thompson et al., 2013). Using our data sets, we investigated whether evolution of protein turnover may also be important. We found that ergosterol synthetic enzymes provide examples for the evolution of protein levels governed by either gene expression or protein turnover in different species. Ergosterol synthesis enzymes have remarkably conserved relative abundances in both yeasts ($R^2 = 0.85$; Figure 4A). In *S. pombe,* most of the ergosterol synthetic enzymes have similar turnover rates (Figure 4B). The long half-lives of these proteins suggest that, during exponential growth in *S. pombe,* protein synthesis governs their abundance. In contrast, we found that *S. cerevisiae* has two groups of Erg enzymes with different turnover rates (Figure 4B). Compared with most ergosterol metabolic enzymes, the turnover of Erg1, Erg25, Erg5, Erg3, and Erg11 is much faster. Interestingly, their respective orthologs in *S. pombe* are anaerobically induced and regulated by the Sre1 transcription factor, which is absent in *S. cerevisiae* (Todd et al., 2006). This suggests that degradation of these enzymes, rather than their synthesis rate, has evolved to control protein levels in *S. cerevisiae.* Consistent with this notion, the mRNA abundance and ribosome footprint are similar for all Erg genes in *S. cerevisiae* (Figure 4C).

Protein Turnover Profiling Identifies Candidate Substrates for Hrd1-Dependent Pathway in *S. cerevisiae*

To test the applicability of our turnover approach to identify substrates of protein degradation pathways, we used proteome-wide turnover profiling in a mutant yeast strain. We reasoned that substrates of a degradation pathway have a longer half-life when the pathway is compromised, thus potentially increasing their abundance. Endoplasmic-reticulum-associated degradation (ERAD) regulates cholesterol metabolism by modulating turnover of the 3-hydroxy-3methylglutaryl-coenzyme A reductase (HMG-CoAR) and the squalene epoxidase (Erg1) in both mammals and budding yeast (Foresti et al., 2013; Ye and DeBose-Boyd, 2011). To test whether ERAD also mediates the fast turnover of the short-lived Erg enzymes, we quantitated changes in proteome turnover and abundance in *hrd1*Δ cells (Figure S4). Hrd1 is an E3 ligase core component of one of the two ERAD machines in budding yeast (Thibault and Ng, 2012). The turnover of Erg3, Erg25, and, to a lesser extent, Erg5 was significantly slower in *hrd1*Δ cells, thus leading to moderate but reproducible increases of their protein level (Figures 4D and 4E). In contrast, turnover and abundance of other

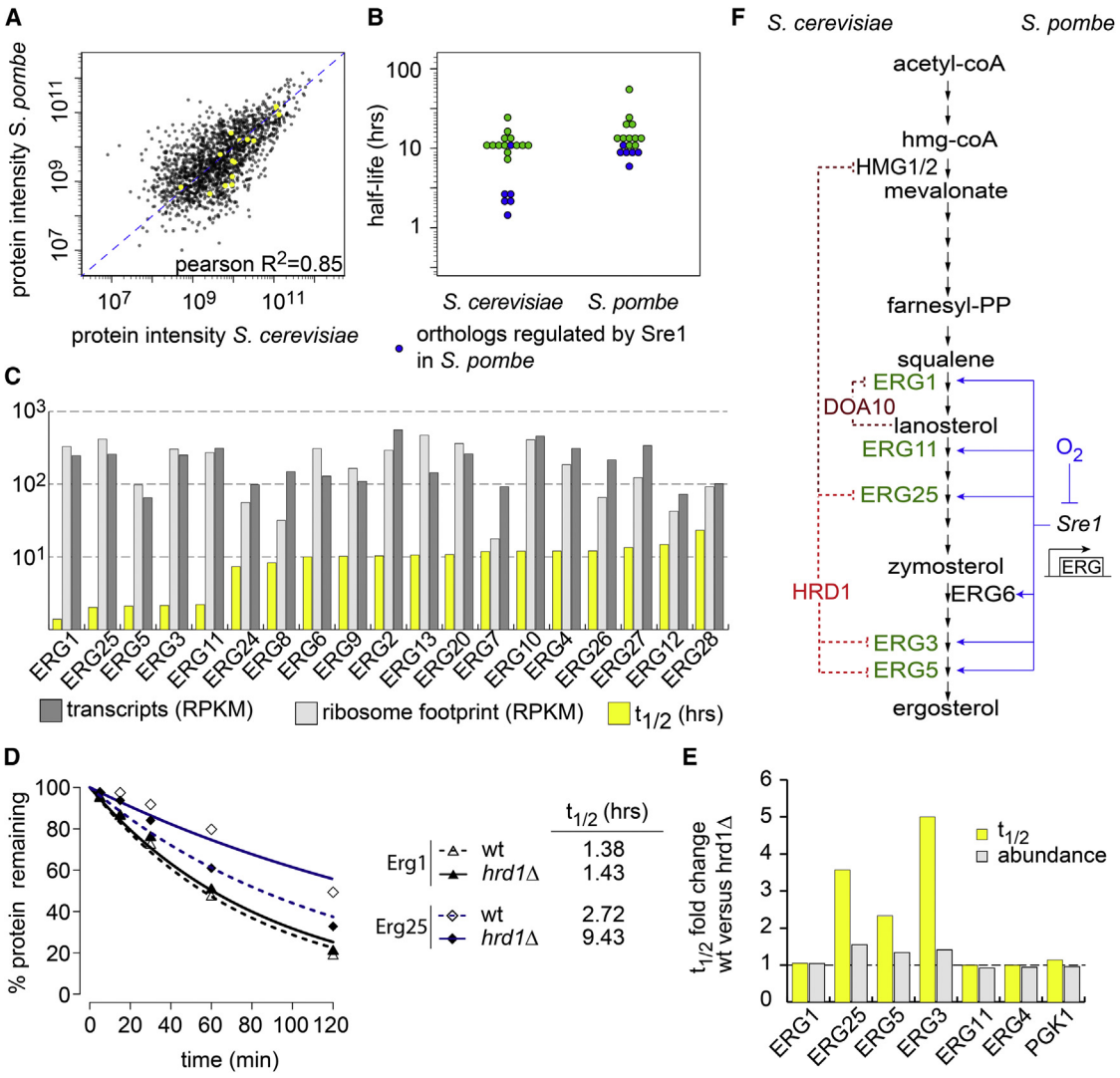


Figure 4. Quantitative Turnover Analysis Reveals the Evolution of Different Strategies to Control Ergosterol Metabolism Enzymes

(A) The abundance of ergosterol synthetic enzymes is conserved in *S. cerevisiae* and *S. pombe* (yellow).

(B) The half-lives of ergosterol synthesis enzymes are similar in *S. pombe*, whereas in *S. cerevisiae*, Erg1, Erg11, Erg3, Erg25, and Erg5 are short-lived proteins. In blue are anaerobically induced and Sre1-dependent genes in *S. pombe* and their counterparts in *S. cerevisiae*.

(C) Plot of half-lives (yellow), transcripts abundances (light gray), and ribosome footprint (dark gray) for ergosterol synthesis enzymes.

(D) Erg1 and Erg25 degradation followed by SILAC labeling decay in wild-type and *hrd1*Δ strains.

(E) Half-life (yellow) and abundance (gray) fold changes of the indicated proteins in *hrd1*Δ compared with wild-type strains.

(F) Representation of the regulation of the *S. cerevisiae*'s short-lived ergosterol metabolic enzymes (green, left) and their orthologs in S. pombe (right). ERAD ubiquitin ligases are in red, and previously characterized regulations are indicated in dashed dark red and new in dashed bright red. Previously described transcription factor regulation is indicated in blue.

Erg-enzymes, including the Doa10 E3-ubiquitin ligase substrate Erg1 (Foresti et al., 2013), Erg4, or Pgk1, was unchanged (Figure 4E).

DISCUSSION

In summary, we provide proteome-wide measurements of relative protein abundances and protein turnover for the two model systems *S. cerevisiae* and *S. pombe*. In comparison with other systematic approaches applied to investigate protein turnover in *S. cerevisiae* that rely on the perturbation of cells by inhibiting translation, our studies were performed at steady state with endogenous proteins using labeled amino acids. We find that the half-life of most proteins, especially those driving cell growth, is substantially longer than previously thought (Belle et al., 2006).

Complementary to ribosome profiling, which measures protein synthesis rates but does not predict the abundance of proteins that are degraded fast, our data set reveals two classes of short-lived proteins. These classes are enriched in cellular regulators and likely constitute a good set of candidates for studying

the different cellular protein degradation pathways. A comparison with available turnover data in mouse fibroblasts (Schwanhäusser et al., 2011) shows that the number of proteins in these classes, with significant protein degradation (classes I and II), expands with organismal complexity (Figure S2C; Table S4). Likely, this is explained by the presence of many more posttranslationally regulated processes in mammalian cells.

Comparison of the protein abundance and turnover rates from *S. cerevisiae* with those from *S. pombe,* where no prior global data set on protein turnover is available, highlights important commonalities and differences. Most strikingly, the relative abundance of one-to-one orthologous is remarkably conserved during roughly 400 million years of evolution. However, similar protein abundance is achieved differently for many orthologous proteins in both organisms.

Our data provide evidence for the evolution of different strategies governing protein abundance in the two yeasts. Specifically, ergosterol enzymes are regulated by protein synthesis in *S. pombe* and protein degradation in *S. cerevisiae*. Our approach further allowed us to identify candidate substrates for the Hrd1-dependent ERAD degradation pathway, such as Erg3, Erg25, and Erg5 (Table S5). Therefore, our findings have implications for understanding the evolution of phenotypic traits and the study of regulatory mechanisms of protein stability. The data provided here will also provide a reference resource, particularly for both yeast communities studying protein degradation pathways.

EXPERIMENTAL PROCEDURES

Strains and Cell Culture

All experiments with *S. cerevisiae* (wild-type) were performed with the BY4742 strain (Open Biosystems). *Hrd1*Δ were freshly prepared from sporulation of the heterozygous knockout collection BY4743, selected, and PCR confirmed to be isogenic to wild-type BY4742 genetic background (*his3*Δ1; *leu2*Δ; *lys2*Δ; *ura3*Δ). All experiments with *S. pombe* were performed with the lysine prototroph strain MKSP201 (*ade6-M21; ura4Δ18; leu1-32*).

S. cerevisiae strains were grown in synthetic medium containing 6.7 g/l yeast nitrogen base, 2 g/l dropout mix (US Biological) containing all amino acids except lysine and 2% glucose. For heavy prelabeling, heavy [13C6/15N2] L-lysine (Cambridge Isotope Labs) was added to a final concentration of 30 mg/l. Cells were precultured in 5 ml medium containing heavy lysine overnight at 30°C and repeated twice.

S. pombe strains were grown in Edinburgh minimal medium (Sunrise Science Products) supplemented with 75 mg/l leucine, histidine, uracil, and adenine. Native stable isotope labeling by amino acids in cell culture (nSILAC) protocol was followed for heavy prelabeling using heavy [13C6/15N2] L-lysine (Cambridge Isotope Labs) (Fröhlich et al., 2013).

Pulse SILAC

After preculture or nSILAC labeling, cells are cultured in biological duplicates up to optical density 600 = 0.4 in 500 ml. After three washes at 4°C with cold SILAC medium without lysine, cells are transferred to SILAC medium containing light lysine. For *S. cerevisiae*, cells are harvested at 0, 5, 15, 30, 60, 120, and 180 min. *S. pombe* cells are harvested at 0, 15, 60, 120, and 240 min. Mass spectrometry analysis was run for each time point in technical duplicates. Also, peptides from time 5 min and 0 min from *S. cerevisiae* and *S. pombe* pulse SILAC experiments, respectively, were fractionated in six fractions by strong anion chromatography.

Determination of Protein Half-Lives

Protein half-lives were determined mainly following a procedure described previously (Schwanhäusser et al., 2011), with slight modifications. For *S. cerevisiae*, which was fully labeled, a minimum of two time points was required to calculate decay rates (K_{deg}). For *S. pombe,* a minimum of three time points was required. A script written in R language extracted the raw (H/L) ratios (r) and as described in (Schwanhäusser et al., 2011), ln(r+1) were linearly fitted to get access to apparent degradation K_{deg} rates. Then, K_{deg} rates were further corrected for protein dilution due to cell growth rate (K_{dil}) using 2.5 and 4 hr as cells doubling time for *S. cerevisiae* and *S. pombe,* respectively, in the conditions of our experiments. Goodness-of-fit of linear regressions (R^2) were calculated and protein with $R^2 \geq 0.9$ were kept for half-life calculation. For subsequent comparison of half-lives between *S. cerevisiae* and *S. pombe* or *S. cerevisiae* wild-type and *S. cerevisiae hrd1*Δ, data sets were further filtered for degradation rates whose coefficient of variation of the slope analyzed by leave-one-out cross-validation (Schwanhäusser et al., 2011) was less than 10%.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and five tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.10.065.

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