Proteome-wide Changes in Protein Turnover Rates in *C. elegans* Models of Longevity and Age-Related Disease

Highlights

- Proteome-wide protein turnover rates are affected in worm models of aging
- Protein turnover rate is regulated at the level of the entire proteome
- Local expression of an aggregation-prone protein affects global proteome turnover

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In Brief

Visscher et al. use pulsed labeling and quantitative mass spectrometry to estimate and compare proteome-wide protein turnover rates in worm models of aging. The study shows that long-lived *daf-2* worms have a faster proteome-wide protein turnover at old age, whereas a Parkinson’s model has a much slower protein turnover compared to control.

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Proteome-wide Changes in Protein Turnover Rates in *C. elegans* Models of Longevity and Age-Related Disease

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**SUMMARY**

The balance between protein synthesis and protein breakdown is a major determinant of protein homeostasis, and loss of protein homeostasis is one of the hallmarks of aging. Here we describe pulsed SILAC-based experiments to estimate proteome-wide turnover rates of individual proteins. We applied this method to determine protein turnover rates in *Caenorhabditis elegans* models of longevity and Parkinson’s disease, using both developing and adult animals. Whereas protein turnover in developing, long-lived *daf-2(e1370)* worms is about 30% slower than in controls, the opposite was observed in day 5 adult worms, in which protein turnover in the *daf-2(e1370)* mutant is twice as fast as in controls. In the Parkinson’s model, protein turnover is reduced proportionally over the entire proteome, suggesting that the protein homeostasis network has a strong ability to adapt. The findings shed light on the relationship between protein turnover and healthy aging.

**INTRODUCTION**

To ensure integrity of the soma and to adapt to different stages of life, tissues need to grow, renew, and be maintained in good condition. The balance between protein synthesis and protein breakdown (i.e., protein turnover) plays an essential role in these processes. Protein turnover is a major determinant of protein homeostasis (sometimes dubbed proteostasis) (Balch et al., 2008). The loss of protein homeostasis is one of the hallmarks of aging (Balch et al., 2008; López-Otín et al., 2013), and protein aggregation is implicated in the pathology of age-related diseases like Parkinson’s disease, Huntington’s disease, cancer, and Alzheimer’s disease (Powers et al., 2009). The idea that loss of protein homeostasis is implicated in aging comes mostly from studies that measure global protein synthesis and translation rates (by $^{35}$S-methionine [$^{35}$S-Met] incorporation and polysome profiling, respectively) during normal aging and in models of age-related disease (Depuydt et al., 2013, 2016; Hansen et al., 2007; Kirstein-Miles et al., 2013; Pan et al., 2007; Stout et al., 2013). Other studies used quantitative mass spectrometry or SDS-PAGE and western blotting to measure changes in the relative abundance of proteins in the proteome of aging model systems (Depuydt et al., 2013, 2016; Kirstein-Miles et al., 2013; Walther et al., 2015). In addition to loss of protein homeostasis during aging, it has been proposed that eventual lifespan is partly determined by the balance of allocation of resources to either maintenance of the soma or reproduction. If this were the case, we hypothesized that this would be reflected by subsets of the proteome having different rates of protein turnover.

The relatively short lifespan of *C. elegans* makes it a convenient aging model system, and many of the preceding observations regarding changes in protein synthesis with aging come from work using this model organism (Depuydt et al., 2013, 2016; Hansen et al., 2007; Kirstein-Miles et al., 2013; Pan et al., 2007; Stout et al., 2013; Walther et al., 2015). One of the best-characterized strains is the long-lived insulin receptor *daf-2* mutant, which is generally found to have decreased protein synthesis or translation activity (Depuydt et al., 2013, 2016; Kirstein-Miles et al., 2013; Pan et al., 2007; Stout et al., 2013). Loss of *daf-2* is known to extend the lifespan of *C. elegans* more than 2-fold, which is, by epistasis analysis, attributed to DAF-16 function (Kenyon et al., 1993).

*C. elegans* is also an established model to study age-related diseases like Parkinson’s disease. For instance, overexpression of human α-synuclein coupled to yellow fluorescent protein (YFP) in the body-wall muscles of *C. elegans* leads to age-dependent...
accumulation and aggregate formation of this protein (van Ham et al., 2008).

However, the observed changes in protein translation rates and proteome composition when comparing, e.g., long-lived daf-2 C. elegans mutants with the slightly short-lived daf-2; daf-16 mutant do not necessarily mean that the combined rate of synthesis and degradation (i.e., protein turnover) is changed. The level of a protein remains de facto unaffected when a slower rate of translation is compensated for by a similar reduction in protein degradation. To get better insight into the changes in proteome-wide protein turnover, we have applied a quantitative mass spectrometry-based method that allows for the estimation of the turnover rate of each protein in the proteome and is suitable for use in C. elegans. This method uses a pulsed form of SILAC (stable isotope labeling by amino acids in cell culture) that allows for the quantification of the incorporation rate of stable-isotope-labeled amino acids in each protein in the proteome. This approach has been used successfully in complex organisms (Doherty et al., 2005, 2009; Pratt et al., 2002; Schwanhäusser et al., 2009). To apply this method in C. elegans, we took advantage of an adaptation of the SILAC protocol for C. elegans that feeds the worms with bacteria grown on heavy isotope Lys. Worms were harvested at different time points after switching to heavy label. The heavy-to-light ratio of each protein at every time point was determined by quantitative mass spectrometry, after which these ratios were further processed for mass spectrometry analysis. Incorporation of heavy-labeled Lys-containing peptides over time is quantified by liquid chromatography-tandem mass spectrometry. The ratio of heavy Lys over light Lys is calculated for each protein by MaxQuant software. From these ratios, the fraction heavy Lys is calculated. Interpolation of the log-transformed fraction heavy label yields the estimated turnover rate expressed as THPLH.

RESULTS

Workflow for Measuring Protein Turnover in C. elegans

To measure whole proteome protein turnover in worms, a method based on stable isotope labeling was set up. A summary of the experimental method is shown in Figure 1. In short, worms were synchronized and, from the desired stage (developing L4 or day 5 adult) on, fed with heavy-labeled bacteria to incorporate this label into the proteome of the worm. Animals were harvested at different time points after switching to heavy label. The heavy-to-light ratio of each protein at every time point was determined by quantitative mass spectrometry, after which these ratios were used to calculate the time it takes until half of each protein is labeled heavy (THPLH).

As an example, the fraction heavy label at each time point is plotted for 2,441 individual proteins for which THPLH was calculated in the developing wild-type N2 strain (Figure S1). From Figure S1, it is clear that most proteins incorporate heavy-labeled Lys at a more or less constant rate over the duration of the experiment. An important step when comparing different worm strains is to assure developmental synchronization, especially because some of the used strains are known to display differences in developmental growth rate. The volume of the worms more or less doubles between L4 and adulthood; hence, small variations in synchronization have a large impact on estimated THPLH. Because of this volume-doubling, net protein synthesis will be higher than net protein degradation. This is why we use THPLH rather than protein half-lives, because the latter would likely be underestimated under these conditions. THPLH can still be compared among strains provided that within the same experiment, these strains were well synchronized. In addition to visual analysis, the quantitative mass spectrometry data can be used to assess the degree of developmental synchronization. Somatic cell division and sperm production are largely completed when the worms reach the L4 stage (the start of the experiments in developing worms) (Sulston and Horvitz, 1977); hence, protein...
the extent of developmental synchronization in our experiments. The label-free quantification intensities for vitellogenins and two oscillating gene clusters as described in Kim et al. (2013) plotted versus time were used to see which strains were synchronized properly in each experiment (Figure S2). From this assessment, we concluded that we had experiments in which strains were well synchronized to compare developing N2, daf-16(mu86) (CF1038), daf-2(e1370) (CB1370), and daf-2(e1370);daf-16(mu86) (DR1209) to one another and the strains expressing α-synuclein-YFP (OW450) to those expressing α-synuclein-YFP (OW40). The synchronization in day 5 adult worms was assessed in the same way and showed that these strains were synchronized (Figure S3). When making comparisons among samples, we include only those proteins for which ratios were measured in at least three time points and for which the slope of the log-transformed fraction heavy label versus time was calculated with a $p < 0.05$ in the compared strains. From here on, this will be referred to as our comparison criteria.

**Protein Turnover in Developing Short-Lived and Long-Lived Insulin Signaling Mutant Worms**

The long lifespan of the insulin signaling mutant daf-2(e1370) has been shown to be fully dependent on the daf-16 gene (Kenyon et al., 1993); hence, the daf-2(e1370);daf-16(mu86) double mutant has a normal (or even slightly decreased) lifespan compared to wild-type worms. As mentioned, earlier studies have shown that protein translation in daf-2(e1370) is decreased compared to that in daf-2(e1370);daf-16(mu86). We therefore compared protein turnover in these strains. When comparing daf-2(e1370) (3,565 proteins) with daf-2(e1370);daf-16(mu86) (2,966 proteins), the combined average fraction heavy label of all proteins shows that in general, the heavy label is incorporated at a slower rate in the developing daf-2(e1370) strain (Figure 2A). Figure 2B shows the distribution of THPLH in these strains, which was, on average, 19 hr in the daf-2(e1370) versus 15 hr in the daf-2(e1370);daf-16(mu86) mutant. In Figure 2C, THPLH of proteins measured in both strains (1,940 proteins) are plotted against each other. See Table S1 for an overview of calculated THPLH per strain and statistics for the comparisons. These figures show that THPLH between proteins varies during development from L4 to young adult but that in general, the whole proteome is shifted toward shorter THPLH in the daf-2(e1370);daf-16(mu86) mutant, which suggests that DAF-16 dictates global rather than individual changes in protein homeostasis. Average THPLH found for these strains and, more importantly, their relative difference are highly similar compared to those found in a replicate experiment (Figure S4, 23 hr for daf-2(e1370) versus 16 hr for daf-2(e1370);daf-16(mu86)). Protein turnover was also assessed in daf-16(mu86), and as expected from the phenotype of daf-16(mu86) being largely identical to that of daf-2(e1370);daf-16(mu86), average THPLH is similar for these strains, as well as for the wild-type N2 strain (Figure 2A; Figure S5; Table S1). Altogether, THPLH in developing daf-2(e1370) worms is about one-third longer compared to daf-2(e1370);daf-16(mu86), daf-16(mu86), and N2 worms and the changes are more or less equal over the entire proteome.
worms, the amount of light Lys rapidly dropped over time, with a mean change) compared to the average change in THPLH in either the THPLH are due to changes in clearance of light-labeled Lys in the young adult stage. To this end, we used a strain that expresses human α-synuclein-YFP in the body-wall muscle cells (OW40). These worms develop α-synuclein-YFP-containing aggregates that increase with age (van Ham et al., 2008). Overexpression of α-synuclein-YFP has a dramatic effect on THPLH compared to a strain only over-expressing YFP (OW450) under the same body-wall muscle-specific promoter. Henceforth, we refer to OW40 as α-synuclein-YFP and to OW450 as YFP. These data come from a different experiment from that one that produced the preceding data for the insulin signaling mutants. The average fraction heavy label versus time of 2,333 proteins in the α-synuclein-YFP strain and 2,239 proteins in the YFP strain are plotted in Figure 3A. The distribution of calculated THPLH represented in Figures 3B and 3C shows the comparison of 1,512 proteins that met our comparison criteria and that were identified in both the α-synuclein-YFP and the YFP strains. Again, THPLH of most of the worm proteome is lengthened (48 ± 31 versus 26 ± 31 hr; see Table S1 for details), even though the transgene is only expressed in the body-wall muscle, and this experiment was performed before the appearance of immobile aggregates (van Ham et al., 2008). Both strains had similar feeding rates (Figure S6A). To exclude that the observed large differences in measured THPLH are due to changes in recycling of the light label, we again assessed miscleaved peptides as described earlier (Figure S6B). Although light Lys levels drop slightly more slowly in the α-synuclein-YFP strain compared to the YFP strain, these differences do not account for the large change in THPLH. For instance, after 4 hr on heavy-labeled bacteria, newly synthesized proteins contain about 88% heavy Lys in the YFP strain versus about 85% heavy Lys in the α-synuclein-YFP strain. In addition, we compared the incorporation of heavy label in a developmental protein in these strains (C50F2.3) (Spencer et al., 2011). We chose C50F2.3 because it had reliable peptide counts in both strains. This protein was not yet detectably expressed at the start of the experiment but was highly expressed later in the experiment. If substantial recycling of the light Lys were to occur, we would expect to find this in the newly synthesized protein. However, incorporation of light label is very low and comparable for the α-synuclein-YFP and YFP strains. The amount of remaining light Lys found in this protein after the worms were grown for 24–32 hr on heavy-labeled bacteria was comparable to what was calculated using the miscleaved peptide method. We therefore conclude that recycling is not a major confounding factor in the estimation of THPLH in the developing Parkinson’s model strain (Figures S7B and S7C).
Protein Turnover Rates in Aging Worms

We next asked how proteome-wide protein turnover would change in adult worms of the preceding tested strains. We chose to investigate protein turnover at day 5 of adulthood because at this time point, egg laying has largely stopped but there is no major age-related death yet. To circumvent contamination of day 5 adult worms with eggs and progeny, they were washed with S Basal buffer over a 40 μm pore size nylon cell strainer every day from day 1 to day 5 of adulthood. Visual inspection confirmed the effectiveness of this approach. For determination of THPLH by pulsed SILAC, the adult worms have the benefit that small differences in synchronization have less effect than they do in developing worms and that the total protein concentration per worm stays largely the same over the course of the experiment. However, total label incorporation is lower (Vukoti et al., 2015), which makes estimation of THPLH less reliable. Worms were pulse labeled for 6, 24, and 48 hr and harvested. As is clear from Figure S3, all strains were well synchronized over the course of the experiment.

As expected, the calculated THPLH are much longer in day 5 adult worms compared to developing worms. This might be partially explained by THPLH being affected by the high net protein synthesis in the latter due to considerable net growth in this phase of life. Nevertheless, average calculated THPLH in N2 in day 5 adult worms is considerably longer, taking 198 ± 102 hr versus 15 ± 4.4 hr during development. The daf-16((mu86)) and daf-2(e1370);daf-16((mu86)) strains have shorter but highly similar THPLH compared to N2: 142 ± 59 and 148 ± 65 hr, respectively. Whereas in developing worms the daf-2(e1370) strain had longer proteome-wide THPLH compared to N2, daf-2(e1370);daf-16((mu86)), and daf-16((mu86)), in day 5 adult worms this strain has the shortest average THPLH of 77 ± 53 hr (Figure 4).

When only the 316 proteins for which reliable THPLH could be calculated in all six strains are taken into account, these numbers remain largely the same (see Table S1 for an overview of calculated THPLH per strain and statistics for the comparisons). Compared to the YFP strain, with an average THPLH of 104 ± 57 hr, the α-synuclein-YFP-expressing strain had a dramatically increased average THPLH of 277 ± 155 hr (Figure 5).

Similar to what was observed in developing worms, it appeared that in all cases, the protein turnover rates of the entire proteome were affected more or less equally and there was no clear shift of a certain group of proteins. This will be discussed further.

The fraction of remaining or recycled light Lys used for protein synthesis at each time point was again calculated from the miscleaved peptides containing two Lys, as previously described. In day 5 adult worms, more light Lys was available for protein synthesis than was available in developing worms (Figure S6C). However, levels were similar when comparing daf-2(e1370);daf-16((mu86)) and daf-2(e1370) mutants, suggesting that they have similar rates of amino acid recycling. In the Parkinson’s model and YFP control strains, the amount of light Lys was equal after 6 hr (~50%) but then remained higher in the Parkinson’s model compared to the control strain (Figure S6D). Nevertheless, if one assumes that the maximum fraction heavy label that can be achieved over a given period is the same as the fraction heavy Lys calculated from the miscleaved peptides, the α-synuclein-YFP strain still has a much longer THPLH compared to the YFP control.

THPLH Is Largely Proportionally Affected over the Proteome of Different Strains

We hypothesized that proteins associated with different biological processes might also display different turnover and that in the tested strains, average THPLH of certain biological processes could be changed more than that of others. If this were the
high correlations suggest that, at least in developing worms, there is a strong hierarchy in the relative rates at which specific proteins are being turned over.

In day 5 adult worms, the same analysis showed that in general, the Spearman’s rank correlations for proteins sorted by THPLH were lower when worms with different phenotypes were compared. The phenotypically similar daf-16(mu86) and daf-2(e1370);daf-16(mu86) had a Spearman’s rank correlation of 0.907, and these strains both had high correlations with N2 wild-type worms (0.859 for daf-16(mu86) and 0.918 for daf-2(e1370);daf-16(mu86)). Far less correlation was observed when these strains were compared to the long-lived daf-2(e1370) worms: 0.380 for daf-2(e1370) versus daf-16(mu86) and 0.422 for daf-2(e1370) versus daf-2(e1370);daf-16(mu86).

Because the rank order of THPLH in daf-2(e1370) changed dramatically in the day 5 adult worms, we wondered whether certain biological processes were affected more than others. To test this, 316 proteins for which THPLH was determined for the day 5 adult worms in all six strains were ranked from long to short, and this list was subsequently divided into quartiles. Using ranking rather than absolute THPLH allows one to determine whether there is a different distribution of THPLH for certain biological processes in a strain and makes it possible to compare strains with large changes in overall THPLH (Figure 6).

Per quartile, the overrepresented KEGG pathways (Figure 6A), biological processes (Figure 6B), and cellular components (Figure 6C) were determined through enrichment analysis. To make the comparison among strains easier, the order of the depicted terms is based on the ranking they have in the wild-type strain when sorted from long to short THPLH. Furthermore, only terms found to be significantly enriched in at least one of the four quartiles in all strains are shown. In Data S1, S2, and S3, the terms, the significance of the enrichment and the proteins, and the number of proteins included in those terms can be found. In general, the data show that proteins involved in certain biological processes are replaced at a faster rate than others. The enrichment analysis by rank order of THPLH shows that, despite large differences in average THPLH that were observed between the tested strains, the rank order of turnover rates of biological processes, KEGG pathways, or subcellular components is generally similar in most strains. However, many biological processes and components in the daf-2(e1370) strain are enriched in quartiles that represent shorter THPLH compared to other strains, suggesting that in this strain, there could be a shift in allocation of resources. In both the KEGG pathways (Figure 6B) and the cellular components (Figure 6C), the terms associated with the ribosome are enriched.
in a lower quartile in the *daf-2(e1370)* worms. This could perhaps explain why average protein turnover is faster in the day 5 adult worms in this strain. Although average THPLH of the α-synuclein-YFP versus YFP is dramatically longer, the rank order of THPLH of the biological processes and components stays largely identical, again suggesting that in the α-synuclein-YFP strain protein turnover is affected equally over the entire proteome.

The preceding Gene Ontology Consortium (GO)-term analysis compares 316 proteins for which THPLH was calculated in all six tested strains in day 5 adult worms. We next sought to investigate whether specific groups of proteins deviated more (*p* < 0.05) in average change in THPLH when directly comparing *daf-2(e1370)* to *daf-2(e1370);daf-16(mu86)* and YFP to α-synuclein-YFP in both developing and day 5 adult worms. These proteins are shaded red in Figures 2C, 3C, 4C, and 5C. GO-term analysis was performed to identify whether pathways or processes were significantly enriched among these proteins, using all proteins in these plots as a background (see Table 1 for the results of GO-term analysis). No significant enrichment was found for the proteins with longer or shorter than average THPLH when comparing YFP to α-synuclein-YFP both in developing and adult worms. In developing *daf-2(e1370)* worms, several proteins involved in the uridine 5'-diphospho (UDP)-glucuronosyl/UDP-glucosyltransferase pathway had a significantly longer THPLH compared to the average change in THPLH observed in *daf-2(e1370)* versus *daf-2(e1370);daf-16(mu86)*. This pathway has been implicated in so-called Phase II of detoxification and

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**Figure 6. GO-Term Analysis in Day 5 Adult Worms**

(A–C) Proteins were ranked by calculated THPLH and divided into quartiles from longest (red) to shortest THPLH (blue) for each strain. Per-quartile GO-term analysis was performed to identify groups of proteins for which the ranked THPLH was enriched in a certain quartile. GO terms that are related to one another appear in the same color code. Only pathways found in all strain are depicted. GO-term analysis was performed based on (A) KEGG pathways, (B) biological processes, and (C) cellular components.
has been shown to be transcriptionally downregulated in daf-2(e1370) mutants (Patel et al., 2008). No GO terms were enriched in developing daf-2(e1370);daf-16(mu86) worms; however, a shorter than average change in THPLH was observed in developing day 5 adult daf-2(e1370) compared to daf-2(e1370);daf-16(mu86). Several vitellogenins were enriched among proteins with a shorter than average change in THPLH. Vitellogenins have been shown to be transcriptionally downregulated in daf-2(e1370) worms and have been implicated in their longevity phenotype (Murphy et al., 2003). However, the protein levels of vitellogenins in daf-2(e1370) were also shown to be controlled by post-translational regulation (DePina et al., 2011).

Another set of proteins with a reduced THPLH in day 5 adult daf-2(e1370) versus daf-2(e1370);daf-16(mu86) worms is linked to the GO terms P granule and germ plasm. These proteins are expressed in the germline, and it has been suggested that some of these contribute to the long lifespan of daf-2(e1370) (Curran et al., 2009; Stout et al., 2013). P granules are involved in the post-translational regulation of RNA, which could be linked to the observed altered THPLH (Parker and Sheth, 2007).

**DISCUSSION**

In past decades, several studies have suggested a mechanistic link between protein homeostasis and aging. This has been based on experiments that determined changes in protein translation rates or proteome remodeling over time in C. elegans models of aging and longevity (Depuydt et al., 2013, 2016; Hansen et al., 2007; Kirstein-Miles et al., 2013;...
Pan et al., 2007; Stout et al., 2013). These studies showed that changes in the proteome go hand in hand with the aging process. However, these studies did not address how long each protein is present from synthesis to degradation or whether the observed proteome changes are due to an altered combined rate of synthesis and degradation of a subset of the proteome. Nevertheless, several hypotheses have been formulated in which damaged proteins need to be replaced rapidly to defer the aging process. Furthermore, it has been suggested that protein homeostasis is lost or changed during the aging process (Walther et al., 2015).

Given that changes in translation could be balanced, in principle, by changes in degradation, this would allow for rapid turnover of more abundant proteins and their continuous replacement with undamaged protein. It is therefore of added value to measure relative protein turnover rates instead of or in combination with protein abundance. In addition, accumulation followed by aggregation of proteins could be due to a change in protein turnover dynamics of only a subset of proteins. Furthermore, loss of protein turnover implies that an imbalance in the proteome develops over time, meaning that certain proteins will accumulate and that the equilibrium between certain biological processes will therefore be lost. THPLH, as measured by the pulsed stable isotope labeling and quantitative mass-spectrometry-based method presented here, gives insight into the changes in the protein turnover of each protein. This allowed us to measure how rapidly proteins involved in certain biological processes are turned over and whether and how this is changed in C. elegans’s model strains of longevity and age-related protein aggregation disease. This led to a number of surprising observations in this study.

First, large changes in average THPLH were observed when comparing different worm strains, and these differences were larger in day 5 adult worms than in worms developing from L4 to young adult. In developing worms, wild-type, daf-2(e1370);daf-16(mu86), and daf-16(mu86) animals have a more or less equal protein turnover profile: on average, 50% of all proteins are heavy Lys labeled about 15 hr after the start of the pulse in these strains. The long-lived daf-2(e1370) worms have a slower protein turnover during development, and it takes about 19 hr to reach the same amount of heavy label after the start of the pulse. The α-synuclein-YFP-expressing Parkinson’s model worms need more than twice as much time before 50% of the proteome has incorporated the heavy label compared to the strain expressing YFP only, and developmental time, feeding rate, and recycling of light-labeled Lys do not explain the observed difference in this strain. Hence, during development from L4 to young adult, before any aging phenotype is apparent, the deletion or overexpression of a single gene can have dramatic effects on protein turnover of the entire proteome. We conclude that a slower protein turnover during development occurs both in a model of longevity and a model of aging and hence does not necessarily predict eventual lifespan. Our observations regarding proteome-wide protein turnover in developing insulin signaling mutant worms are similar to those recently obtained by the Braeckman group (Dhondt et al., 2016). This study had a comparable approach but used heavy nitrogen labeling and day 2 adult worms in a sterile glp-4 background. Like us, the authors came to the conclusion that protein turnover is slowed in the daf-2(e1370) versus daf-2(e1370);daf-16(mu86) strains. The same study confirmed that protein turnover rate measured by pulsed labeling and quantitative mass spectrometry nicely correlates with classical 35S-Met-based methods. Furthermore, this study used time-lapse imaging of a photoconvertible Dendra2 fusion protein to corroborate the observations on differences in THPLH in the daf-2(e1370) versus daf-2(e1370); daf-16(mu86) background.

In day 5 adult worms, average THPLH is much longer, which is in line with observations by Vukoti et al. (2015). This study used a pulsed SILAC-based approach similar to ours to study protein turnover in wild-type worms during adulthood and found that protein turnover drastically dropped after day 5. No comparison was made with strains with longevity or aging phenotypes in this study. We found that at this stage of life, the long-lived daf-2(e1370) worms had protein turnover rates that more closely resembled those observed in developing worms compared to the other strains. Unexpectedly, the daf-16(mu86) and daf-2(e1370); daf-16(mu86) strains, both of which have a slightly reduced lifespan, had faster protein turnover rates compared to wild-type worms, albeit still much slower than daf-2(e1370). A study by the Braeckman group (Depuydt et al., 2016) describes 35S-Met pulse-chase experiments and showed that in daf-2(e1370); daf-16(mu86), worm protein translation and protein degradation rates are initially higher than those in daf-2(e1370) but that they drop drastically between day 2 and day 5 of adulthood. In daf-2(e1370) worms, rates of both processes remain largely the same over this period. These observations could potentially explain our observations that in day 5 adult worms, daf-2(e1370) animals have a lower THPLH than do daf-2(e1370); daf-16(mu86) animals, whereas the Braeckman group (Dhondt et al., 2016) finds the opposite in day 2 adults; the latter is similar to what we find in developing L4 worms. The day 5 adult α-synuclein-YFP strain had by far the slowest average THPLH, although part of this might be explained by the higher levels of remaining light label, which could, for instance, come from increased protein recycling. At first, this may seem to contradict earlier (Depuydt et al., 2013) and recent (Walther et al., 2015) studies that find a link between lifespan and protein abundance, but again, protein abundance and protein turnover rates do not necessarily correlate.

Second, our data indicate that the protein homeostasis network has a remarkable plasticity. Although large differences in protein turnover were found in the analyzed strains at day 5 of adulthood, the THPLH rank order in these strains remains largely unaffected, except for in the daf-2(e1370) worms. The latter could be in accordance with the idea that long-lived animals allocate energy toward maintenance of the soma rather than to production of offspring (Kirkwood, 2005). We found that ribosomal proteins had a relatively faster turnover rate in this strain, which could mean that the synthesis of new ribosomes is important for maintenance of the remainder of the proteome. Proteins involved in the biological processes of aging and determination of adult lifespan are both significantly and oppositely enriched in a quartile with shorter THPLH for daf-2(e1370) and longer THPLH for α-synuclein-YFP (Figure 6B). Groups of proteins previously implicated in daf-16-dependent longevity in daf-2 mutants (vitellogenins,
proteins involved in the UDP-glucuronosyl/UDP-glucosyltransferase pathway, and P granule proteins) were also found to have changed protein turnover rates.

Third, we conclude that protein turnover is regulated in a non-cell autonomous fashion, at the level of the whole organism. The best example presented in this study that illustrates this finding is the Parkinson’s model that expresses human α-synuclein-YFP exclusively in the body-wall muscles, which make up less than 10% of the worm’s 959 somatic cells (Altun and Hall, 2009). THPLH in this worm is more than twice as long for all proteins in the organism in both developing and day 5 adult worms, meaning that the muscle-specific expression of the transgene affects protein homeostasis networks throughout the organism. Non-cell autonomous regulation of protein homeostasis has been described previously: Van Oosten-Hawle et al. (2013) found that disturbed protein homeostasis led to changes in chaperone expression in other tissues. In future experiments, it will be interesting to see whether expression of aggregation-prone proteins in different tissue types can also exert this whole-body response in altered protein turnover. Another mechanistic explanation for the observed global changes in protein turnover is a difference in the rates of feeding or heavy label absorption by the intestine (although we did not measure differences in food intake in the developing worms). The used daf-2(e1370) strain has been shown to have a lowered metabolism (Depuydt et al., 2014), but it is not clear whether this would be a rate-limiting factor for heavy label incorporation in the proteome. The described differences in metabolism would imply a slower rather than a faster protein turnover like we found here. Finally, even if the observed differences in protein turnover could be explained by lowered food intake, it does not mean that these differences are not important: food intake and absorption are likely part of the intricate protein homeostasis network.

Collectively, our data suggest that changes in proteome-wide protein turnover occur over the life of the organism but that there is no simple link between protein turnover rates and eventual adult lifespan. Measured average THPLH does not fully correlate with the eventual lifespan of the tested strains. However, in day 5 adult worms, which are at the start of post-reproductive aging, the fastest protein turnover is seen in the strain with the longest lifespan, whereas a slow protein turnover is observed in a model of age-related disease. In accordance with the observed hierarchy in THPLH, we propose that overall protein turnover must be tightly regulated to achieve protein homeostasis. One could imagine that in the case of slower protein turnover, damaged proteins are not cleared rapidly enough, leading to accumulation of dysfunctional proteins. However, when protein turnover is faster, it means that both synthesis and degradation pathways are working at high speed. This could potentially make the protein homeostasis network vulnerable, because if in this situation one of the two pathways were slightly delayed, it would cause rapid proteome imbalance. The method described in this study may be used to further elucidate the interplay between protein turnover and aging.

**EXPERIMENTAL PROCEDURES**

The experimental procedures are summarized in Figure 1. See Supplemental Information for full details.

**ACCESSION NUMBERS**

The accession number for the raw mass spectrometry proteomics data reported in this paper is PRIDE: PXD004561.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, seven figures, two tables, and three data files and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.08.025.

**AUTHOR CONTRIBUTIONS**


**ACKNOWLEDGMENTS**

Several C. elegans strains were purchased from the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). T.B.D. and M.V. are supported by grants from The Dutch Cancer Society (KWF Kankercultuurd), H.R.V. and R.M.v.E. are supported by the ProteinsWork initiative of the Netherlands Proteomics Centre. We thank all members of the T.B.D. and B.M.T.B. labs and Dr. Geert Depuydt for discussion.

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**REFERENCES**


Understanding the odd science of aging.


Supplemental Information

Proteome-wide Changes in Protein Turnover Rates in C. elegans Models of Longevity and Age-Related Disease

Marieke Visscher, Sasha De Henau, Mattheus H.E. Wildschut, Robert M. van Es, Ineke Dhondt, Helen Michels, Patrick Kemmeren, Ellen A. Nollen, Bart P. Braeckman, Boudewijn M.T. Burgering, Harmjan R. Vos, and Tobias B. Dansen
Figure S1. Related to Figure 1. Typical output of pulsed SILAC experiment. Fractions Heavy of all 2441 proteins detected in wild type worms. Whisker Box plots shows the 25th and 75th percentile as a box, and the 2.5th and 97.5th percentiles as whiskers. The median is indicated by the divider in the box. Note that the majority of proteins has a more or less constant rate of incorporation over the duration of the experiment.
Figure S2, related to Figures 1, 2 and 3, Visscher et al.
Figure S3, related to Figures 1, 4 and 5, Visscher et al.
Figure S4, related to Figure 2, Visscher et al.
Figure S5, related to Figure 2, Visscher et al.
Suppl. Figure S6, related to Figures 2, 3, 4 and 5 Visscher et al.
Figure S7, related to Figures 2 and 3

A

Feeding rate

-OD600/hr

- Δ

N2, daf-2, daf-16, daf-2;daf-16, OW40, OW450

B

protein C50F2.3

Total Intensity

0 5 10 15 20 25 30

0 50000000 100000000 150000000 200000000 250000000 300000000

Hours

ow40 ow450 ow40 ow450 ow40 ow450 ow40 ow450 ow40 ow450 ow40 ow450

4 6 8 13 24 28 32

Light

Heavy

C

Fraction Light

0.00 0.03 0.06 0.09 0.12 0.15

0.00 0.03 0.06 0.09 0.12 0.15

4 6 8 13 24 28 32

Time on Heavy (hrs)

a-syn-YFP (OW40)

YFP (OW450)
## Supplementary Table 1. Calculated THPLH for the used strains and statistical analysis

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### Comparisons between strains

#### Developing worms

- half-life determined in both strains
- half-life determined in all 4 strains

#### 5 day adult worms

- half-life determined in both strains
- halflife determined in all 6 strains

This table is continued on the next page
Table 1 continued

Statistics

results Mann-Whitney test

daf-2 vs daf-2;daf-16

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YFP vs a-syn-YFP

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OW40 OW450

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Suppemental Table 2

Spearman's Rank Correlation
based on proteins for which Time until Half the Protein is Labeled Heavy was determined for all strains.

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Developing worms

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Supplemental data:

Data used to prepare figures 2B, 2C, 3B, 3C, 4B, 4C, 5B, 5C and S5 can be found in the supplemental Excel sheet entitled:
\textbf{supplworkbookDataforFigs.xlsx}

Lists of proteins used for the GO term analysis presented in Table 1 can be found in the supplemental Excel sheet entitled:
\textbf{Supplemental information GO Table 1.xlsx}

Output of the GO term analysis presented in Figure 6 can be found in the supplemental Excel sheet entitled:
\textbf{Supplemental excel sheet old worms GO terms.xlsx}
Legends to Supplemental Figures

Figure S1. related to Figure 1. Typical output of a pulsed SILAC experiment

Fractions Heavy of all 2441 proteins detected in wild type worms. Whisker Box plots shows the 25th and 75th percentile as a box, and the 2.5th 97.5th percentiles as whiskers. The divider in the box indicates the median. Note that the majority of proteins have a more or less constant rate of incorporation over the duration of the experiment.

Figure S2. related to Figures 1, 2 and 3. Synchronization of developing worms as measured by the expression patterns of vitellogenins and gene clusters.

A) LFQ (Label free quantification) intensities of vitellogenins identified in developing wild type, daf-16(mu86), daf-2(e1370);daf-16(mu86), daf-2(e1370). Somatic cell division as well as sperm production is largely complete at L4 stage. Vitellogenins are expressed only in the adult worm intestinal compartment and hence mark the onset of adulthood in this experiment.

B) LFQ intensities of expression of ‘cluster 1’ genes(Kim et al., 2013) identified in developing wild type, daf-16(mu86), daf-2(e1370);daf-16(mu86), daf-2(e1370).

C) LFQ intensities of expression of ‘cluster 8’ genes(Kim et al., 2013) identified in developing wild type, daf-16(mu86), daf-2(e1370);daf-16(mu86), daf-2(e1370).

D) LFQ (Label free quantification) intensities of vitellogenins identified in developing α-synuclein-YFP and YFP strains.

E) LFQ intensities of expression of ‘cluster 1’ genes(Kim et al., 2013) identified in developing α-synuclein-YFP and YFP strains.

F) LFQ intensities of expression of ‘cluster 8’ genes(Kim et al., 2013) identified in developing α-synuclein-YFP and YFP strains.

Figure S3. related to Figures 1, 4 and 5. Synchronization of day 5 adult worms as measured by the expression patterns of vitellogenins and gene clusters.
A) LFQ intensities of vitellogenins identified in day 5 adult wild type, daf-16(mu86), daf-2(e1370);daf-16(mu86), daf-2(e1370) as well as in α-synuclein-YFP and YFP strains.

B) LFQ intensities of expression of ‘cluster 1’ genes (Kim et al., 2013) identified in day 5 adult wild type, daf-16(mu86), daf-2(e1370);daf-16(mu86), daf-2(e1370) as well as in α-synuclein-YFP and YFP strains.

C) LFQ intensities of expression of ‘cluster 8’ genes (Kim et al., 2013) identified in day 5 adult wild type, daf-16(mu86), daf-2(e1370);daf-16(mu86), daf-2(e1370) as well as in α-synuclein-YFP and YFP strains.

Figure S4. related to Figure 2. Protein turnover in developing insulin/IGF signaling pathway mutant worms: duplicate experiment.

A) Average of the fractions heavy label of all proteins measured in the long-lived daf-2(e1370) strain (2424 proteins) and in the short-lived daf-2(e1370);daf-16(mu86) strain (2209 proteins). Note that the daf-2(e1370) strain incorporates heavy Lysine at a much slower rate in its proteome.

B) Binned THPLH in hours for proteins for which in at least 3 of 7 time points a ratio heavy over light was detected and for which the slope of a linear regression line, fitted through the log-transformed fraction heavy versus time, could be estimated with a significance of p<0.05. 1605 proteins met these criteria for the daf-2(e1370);daf-16(mu86) mutant and 1892 for the daf-2(e1370) mutant strain.

C) THPLH in hours as in (B). The THPLH of each protein in daf-2(e1370);daf-16(mu86) is plotted against the THPLH of the same protein in the daf-2(e1370) mutant (1518 proteins). Note that the entire cloud shifts towards longer protein half-lives in daf-2(e1370);daf-16(mu86)

Figure S5. related to figure 2. Comparison of Protein half-lives in developing insulin/IGF signaling pathway mutant worms.

A) The THPLH of each protein in daf-2(e1370) worms is plotted against the THPLH of the same protein in wild type worms.
B) The THPLH of each protein in *daf-16(mu86)* worms is plotted against the THPLH of the same protein in wild type worms.

C) The THPLH of each protein in *daf-2(e1370);daf-16(mu86)* worms is plotted against the THPLH of the same protein in wild type worms.

D) The THPLH of each protein in *daf-2(e1370)* worms is plotted against the THPLH of the same protein in *daf-16(mu86)* worms.

E) The THPLH of each protein in *daf-2(e1370);daf-16(mu86)* worms is plotted against the THPLH of the same protein in *daf-16(mu86)* worms.

Figure S6. related to Figures 2, 3, 4 and 5. Assessment of Light label availability from miscleaved peptides.

The relative pools of Heavy and Light Lysine was estimated in each time-point by searching for miscleaved tryptic peptides of newly synthesized proteins (recognized by the presence of Heavy Lysine) that contain two instead of one Lysine. The fraction Light Lysine at the time of synthesis of the identified miscleaved peptide can be estimated from the ratio of the intensities of pairs of miscleaved peptide containing two Heavy \(I_{LYS\text{Lys}}\) or one Heavy and one Light Lysine \(I_{LYS\text{Lys}}\) as described in the Methods section. Bar graphs represent the middle two quartiles of the Fraction Light calculated from all miscleaved peptide pairs as well as the median fraction Light Lysine in each time point. The number of identified miscleaved peptides used for the analysis is indicated below each bar. A) Fraction Light Lysine over time for developing IIS signaling mutants. B) Fraction Light Lysine over time for the developing Parkinson’s model and YFP control strain. C) Fraction Light Lysine over time for day5 adult IIS signaling mutants. D) Fraction Light Lysine over time for the day 5 adult Parkinson’s model and YFP control strain.

Figure S7. related to Figures 2 and 3. Feeding rates and amino acid recycling in developing worms.

A) Feeding rates as assessed by the decrease in turbidity at OD600 (inversed values). Error bars represent standard deviations of two biological replicates. The used strains had comparable feeding rates during development.
B) Amino acid recycling was assessed by determining the amount of Light Lysine containing peptides in the protein C50F2.3 that is expressed late during development from L4 to young adult. Because the protein is not present in early time-point, any light Lysine should come from recycling. There is very little light Lysine present, which means that recycling is not a major factor in both YFP and α-synuclein-YFP strains.

C) Fraction Light Lysine as calculated from the data presented in B

Full Experimental Procedures

C. elegans strains and maintenance

C. elegans strains were maintained at 15°C on standard NGM plates seeded with E. coli OP50 (Stiernagle, 2006). In this study the following strains were used: Bristol N2 (wild type), CB1370 (daf-2(e1370)III), CF1038 (daf-16(mu86)I), DR1209 (daf-16(mu86)I;daf-2(e1370)III), OW40 (zglIs15[P(unc-54)::α-synuclein::YFP]IV), OW450 (rmIs126[P(unc-54)Q0::YFP]V).

Stable isotope labeling of E. coli

The HT115Δlys E. coli strain (F-, Δlys, mcrA, mcrB, IN(rrnD-rrnE)I, rnc14::Tn10(DE3 lysogen: lacUV5 promoter -T7 polymerase) was created using bacteriophage P1 transduction to introduce an in-frame deletion in lysA from a BW25113 strain that was rendered auxotrophic for Lysine (Datsenko and Wanner, 2000). For heavy labeling, bacteria were cultured in M9 minimal medium as described by Larance et al. (Larance et al., 2011). This medium was supplemented with 15N2-13C6-L-Lysine (Silantes) and 0.2 g/L Lysine amino acid dropout mix (US Biologicals). Bacteria were grown until an optical density at 600 nm (OD600) of about 1.0 was reached. Bacteria were concentrated 50x and diluted 6x in S Medium, made
according to standard protocol (Stiernagle, 2006). Heavy isotope incorporation was checked using trypsin digestion and LC-MS analysis and in all experiments exceeded 95%.

**Pulse labeling of *C. elegans***

*C. elegans* strains were synchronized by bleaching gravid adults and overnight hatching of the remaining eggs. The *daf-2(e1370)* strain develops slightly slower and was bleached and allowed to hatch one day earlier than the other strains to optimize synchronization. For each strain, synchronized L1 stage worms were placed on 10 standard 10 cm NGM plates seeded with *E. coli* grown on normal, light Lysine at 15°C. At L4 stage (checked visually by microscopy), worms were rinsed off the plates and washed with M9 four times. Subsequently each worm strain was dispensed into 7 wells of a 24 wells plate containing 1 ml heavy labeled HT115Δlys bacteria in S Medium and the worms were grown at 20°C under continuous rocking until harvest at different time points (4, 6, 8, 13, 24, 28 and 32 hours or 4, 7, 9 and 24 hours depending on the experiment). The timespan of the experiment, covering the late development phase, was chosen to prevent interference of progeny with the measurement. For the experiments using adult *C. elegans*, worms were synchronized as above, switched from 15°C to 20°C at L4 stage and allowed to grow until day 5 of adulthood. Adult worms were separated from eggs and larvae every day until egg laying had stopped by washing them with S-basal buffer over a 40 micron nylon Cell Strainer (Corning). At day 5 of adulthood worms were transferred to into 3 wells of a 24 wells plate containing 1 ml heavy labeled HT115Δlys bacteria in S Medium and the worms were grown at 20°C under continuous rocking until harvest at different time points (6, 24 and 48 hours). For harvesting, worms were washed and pelleted through centrifugation, snap frozen in liquid nitrogen and stored at -80°C.

**Preparation of mass spectrometry samples from *C. elegans* material**

Worms were lysed in 150 µl 8M urea, 100 mM Tris pH 7.5 supplemented with protease inhibitors (Roche complete EDTA-free) by interval sonication (10x 30’’ at high) with a bioruptor sonication device and water bath (Diagenode). After sonication, samples were
centrifuged and 40 µl of the supernatant was separated from other contaminating worm parts on a 10% SDS-PAGE gel (electrophoresed for ±1 cm). With help of SimplyBlue staining the samples could be visualized and excised from the gel and further sliced into pieces of ±1 mm³. A mixture of H₂O and acetonitrile (ACN) (1:1) was used for destaining, followed by 20’ incubation with 6.5 mM dithiothreitol (DTT) and 10’ with 55 mM iodoacetamide for reduction and alkylation. Between all steps the gel pieces were shrunk with 100% ACN to allow the next solution to reach the sample completely. Next the samples were washed with 50 mM ammonium bicarbonate in milli-Q. 100 ng trypsin + Lys-C (Promega) was added on ice for 30’, after which another 100 ng was added and proteins were digested overnight at 37° C.

The next day C18-stagetips were used for filtering and loading of the protein digest. The stage tips were activated first by washing with methanol, followed by washing with buffer B (0.5% formic acid in 80% ACN) and buffer A (0.5% formic acid). After loading of the digested sample, stage tips were washed with buffer A and peptides were eluted with buffer B. Buffer B was removed by evaporation in a SpeedVac, followed by dissolving of the samples in buffer A.

**Mass spectrometry**

Peptide samples were separated on a 30 cm column (75 µm ID fused silica capillary with emitter tip (New Objective)) packed with 3 µm aquapur gold C-18 material (dr. Maisch) using a 140 minute gradient (7% to 80% ACN 0.1% FA), delivered by an easy-nLC 1000 (Thermo). Peptides were electro-sprayed directly into a Thermo Scientific Orbitrap Fusion Tribrid Mass Spectrometer and analyzed in Top Speed data dependent mode with the resolution of the full scan set at 240000 and an intensity threshold of 5000 ions. Most intense ions were isolated by the quadrupole and fragmented with a HCD collision energy of 30%. The maximum injection time of the iontrap was set to 35 milliseconds.

**Data analysis**
Raw files were analyzed with the Maxquant software version 1.5.1.0 (Cox and Mann, 2008) with deamidation of glutamine and asparagine as well as oxidation of methionine set as variable modifications, and cysteine alkylation with iodoacetamide set as fixed modification. For identification, the *C. elegans* wormpep208 database from wormbase.org was searched with both the peptide as well as the protein false discovery rate set to 1%. The SILAC quantification algorithm was used in combination with the ‘match between runs’ tool (option set at two minutes), the IBAQ and the LFQ algorithm, all of which are integral parts of the Maxquant software (Luber et al., 2010; Schwanhausser et al., 2011). Proteins identified with two or more unique peptides were filtered for reverse hits, decoy hits and standard contaminants.

The median heavy (H)/light (L) ratios were further analyzed using Microsoft Excel. First, for each protein, the fraction heavy labeled protein was determined from the H/L ratio by the formula ‘(H/L ratio)/(1 + (H/L ratio))’. To linearize heavy label incorporation over time, these fractions were log-transformed by ‘-LN(1-Fraction H)’. The slopes of the log-transformed fraction H vs time were determined for all proteins for which a H/L-ratio was determined for at least three different time points using the macro ‘LinEstGap’ (https://newtonexcelbach.wordpress.com/downloads download Linest-poly.xls). LinEstGap uses the "least squares" method to calculate a regression line, along with the respective *p*-values of the fit of the data to this regression line. An advantage over the normal LinEst function is that it allows gaps in data series. Next to the measured time points, time point zero with a fraction H of zero was included in the calculations because there is no heavy labeling at the start of the experiment, as the natural occurrence of $^{15}$N$_2$-$^{13}$C$_6$-L-Lysine is next to zero. Furthermore, for the same reason, the regression lines were forced through zero. Another advantage of forcing through zero is the elimination of possible developmental differences by withdrawing proteins out of the analysis that are only synthesized during early or late development. In further analyses only proteins were selected with at least 3 measured H/L ratios that after log-transformation returned a value for their slopes with *p*-values smaller than 0.05, thereby selecting for the most reliably quantified proteins. Using the formula ‘Slope/(−
LN(0.5)), the Time until Half the Protein is Labeled Heavy (THPLH) was interpolated for every single protein.

To visualize fractions of each protein in a sample, MaxQuant Perseus was used ((http://www.maxquant.org/links.htm).

The density plots of the distribution of THPLH were generated using the density function in R.

**Statistics**

Statistical significance of changes in THPLH between strains was analyzed using Mann-Whitney tests. For comparisons of calculated THPLH of all proteins between two strains, regardless of whether a THLPH was calculated for these proteins in both strains unpaired tests were performed. For comparisons of all proteins for which THPLH was calculated in both strains paired tests were performed. In cases where more than two strains were compared the Mann-Whitney test was corrected according to Benjamini and Hochberg.

**Estimation of available Light and Heavy Lysine pools.**

Incorporation of Heavy Lysine depends on 1) its availability but also on 2) clearance of Light Lysine that remains from before switching the worms to Heavy Lysine labeled bacteria and 3) on the rate of recycling of Light Lysine from proteins. The relative pools of Heavy and Light Lysine were estimated in each time-point by searching for miscleaved tryptic peptides of newly synthesized proteins (recognized by the presence of Heavy Lysine) that contain two instead of one Lysine. The fraction Light Lysine can be estimated from the ratio of the intensities of pairs of miscleaved peptide containing two Heavy ($I_{LysBlysB}$) or one Heavy and one Light Lysine ($I_{LysBLysO}$) according to the formula:

\[
\frac{1}{2 \cdot \left( \frac{I_{LysBlysB}}{I_{LysBLysO}} + 1 \right)} = (\text{Fraction Lys0})
\]
Note that \( I_{LYS1,LYS2} \) is the combined intensity of peptides with either only the first or only the second Lysine Light labeled. Tens to hundreds of miscleaved peptide pairs were identified per sample and the median values and 2nd and 3rd quartiles of the fraction Light Lysine were calculated.

**GO-term analysis**

The online database STRING (Search Tool for the Retrieval of Interacting Genes) (http://string-db.org/) was used (Jensen et al., 2009) for the GO term enrichment analysis. For the strain to strain comparison, proteins with a significantly longer THPLH (p<0.05 assuming normal distribution around the mean change) than the average change in THPLH when comparing two strains were searched against all proteins for which a THPLH was calculated in both strains (these proteins are shaded in red in Figure 2C, 3C, 4C and 5C and lists of these can be found in the Supplemental information). Enrichment was subsequently determined by significant, Bonferroni corrected \( p \)-values. For the GO-term analysis used to determine whether certain biological processes were affected more than others in the used *C. elegans* strains (Figure 6) the identified proteins were divided into four groups based on their THPLH. Per quartile proteins were compared against a background of all identified proteins in this mass spec experiment.

**Determination of feeding rates**

Worms were synchronized as described above, counted and dispensed in baffled Erlenmeyer flasks at equal concentrations of worms in S Basal buffer with HT115Δlys bacteria as food source. The consumption of food was measured by a decrease in turbidity (OD600) at 1, 2, 4, 6, 8, 10, 24 and 28 hours after addition of worms. Bacteria without worms were used as a control for spontaneous lysis.
Supplemental References


