Systematic analysis of asymmetric partitioning of yeast proteome between mother and daughter cells reveals "aging factors" and mechanism of lifespan asymmetry

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Budding yeast divides asymmetrically, giving rise to a mother cell that progressively ages and a daughter cell with full lifespan. It is generally assumed that mother cells retain damaged, lifespan limiting materials ("aging factors") through asymmetric division. However, the identity of these aging factors and the mechanisms through which they limit lifespan remain poorly understood. Using a flow cytometry-based, high-throughput approach, we quantified the asymmetric partitioning of the yeast proteome between mother and daughter cells during cell division, discovering 74 mother-enriched and 60 daughter-enriched proteins. While daughter-enriched proteins are biased toward those needed for bud construction and genome maintenance, mother-enriched proteins are biased towards those localized in the plasma membrane and vacuole. Deletion of 23 of the 74 mother-enriched proteins leads to lifespan extension, a fraction that is about six times that of the genes picked randomly from the genome. Among these lifespan-extending genes, three are involved in endosomal sorting/endosome to vacuole transport, and three are nitrogen source transporters. Tracking the dynamic expression of specific mother-enriched proteins revealed that their concentration steadily increases in the mother cells as they age, but is kept relatively low in the daughter cells via asymmetric distribution. Our results suggest that some mother-enriched proteins may increase to a concentration that becomes deleterious and lifespan-limiting in aged cells, possibly by upsetting homeostasis or leading to aberrant signaling. Our study provides a comprehensive resource for analyzing asymmetric cell division and aging in yeast, which should also be valuable for understanding similar phenomena in other organisms.

aging | asymmetric cell division | proteome

Cellular aging and asymmetric cell division are intimately linked. In budding yeast, asymmetric cell division yields a mother cell and a daughter cell that are easily distinguishable under the microscope. Tracking the fate of the mother lineage led to the discovery that individual mother cells have a finite replicative lifespan, defined by the number of daughters a mother cell produced before senescence (1). It is known that although the mother cell ages with each division, their daughters retain the same full lifespan independent of the age of the mother at least until the last few mother cell divisions (2, 3). Thus, the asymmetry in cell division leads to asymmetry of aging.

Even in single-celled organisms in which cell division is seemingly morphologically symmetric, such as fission yeast or *Escherichia coli*, asymmetric partitioning of cellular contents can still occur and have a differential impact on the aging/death fate of the two offspring (4–8). Asymmetric cell division is also a general phenomenon in mammalian cells (e.g., during development or in mitotically active tissues), where cell division typically leads to two cells with distinct fates, often with different replicative potential. It has been argued on theoretical grounds that asymmetric cell division may be favored by natural evolution (9–11); when the accumulation of lifespan-limiting damage outpaces the dilution by symmetric cell division, keeping the damage to one of the two offspring via asymmetric partitioning is a general strategy to avoid population senescence.

It is generally assumed that budding yeast mother cells retain damaged/lifespan-limiting materials (referred to as "aging factors" hereafter), allowing their daughter cells to reset the clock. Indeed, a number of potential aging factors have been reported to accumulate preferentially in mother cells through asymmetric partitioning. One example is extrachromosomal rDNA circles, which are known to be a limiting factor for lifespan (12, 13) and are retained in mother cells (14–16). Protein aggregates, carbonylated proteins, and reactive oxygen species have also been reported to distribute asymmetrically between old mothers and their daughters (17–22). Preferential retention of membrane transporters in the mother cells has also been associated with lifespan asymmetry (23, 24). These observations support the general notion that mother cells retain aging factors to themselves, enabling their daughters to rejuvenate. However, a global view of the identities of asymmetrically partitioned aging factors and the mechanism through which they influence lifespan is still lacking.

Significance

In this work, we took a proteome-centric view to analyze the cell division and lifespan asymmetry between mother and daughter cells in budding yeast. Using a flow cytometry-based, high-throughput approach, we quantified the partitioning of the proteome and identified 74 mother-enriched and 60 daughter-enriched proteins. Functional analysis of these proteins suggests mechanisms of asymmetric partitioning at an organelle/suborganelle level. We found that mother-enriched proteins are much more likely to becoming aging factors than those proteins chosen at random. The proposed mechanism, as supported by our single-cell observations, is that these proteins accumulate in old mother cells to a high level that becomes lifespan-limiting. Our work sheds new light on the mechanisms of asymmetric cell division and aging.

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SYSTEMS BIOLOGY

In this work, we took a proteome-centric approach to explore cell division asymmetry and its connection to lifespan asymmetry in budding yeast. Using high-throughput proteomics based on a green fluorescent protein (GFP) library and mother cell labeling, we quantified the asymmetric partitioning of the proteome between mother and daughter cells and systematically identified mother-enriched and daughter-enriched proteins. Functional analyses of these proteins suggest that macrostructures are one basis for asymmetric partition. We found that mother-enriched proteins tend to accumulate in mother cells over time and that the deletion mutants are much more likely to extend lifespan than genes chosen at random, arguing that it is the high concentration that limits lifespan. These observations provide a consistent picture of how asymmetric partitioning of the proteome influences lifespan asymmetry, and serve as a starting point for generating new hypotheses on the mechanism of asymmetry and aging.

Results

Identification of Mother- and Daughter-Enriched Proteins by Quantifying the Asymmetric Partitioning of the Yeast Proteome. To systematically identify proteins that are asymmetrically segregated, we have developed a high-throughput approach to measure quantitatively the partition of the whole proteome between mother and daughter cells during cell division. To measure the partition of a GFP-tagged protein from the yeast GFP tagging library, we labeled mother cells with Cy5 and let the population grow for about one generation. The resulting cell population was then subjected to flow cytometry analysis for both the Cy5 and GFP signals (Fig. 1*A*). Because the daughter cells synthesize a new cell membrane and cell wall, they do not inherit the surface dye, and thus can be distinguished from the Cy5-positive mothers (Fig. 1*B*). Measuring the GFP signal normalized by the cell size (using the side-scatter data) then gives the intensity of the protein in the mother and daughter cell populations. We systematically screened the GFP-tagging library (25) in 96-well plates using a BD Biosciences LSR II flow cytometer with a high-throughput sampler (HTS). Fig. 1*C* shows the average intensity in mother vs. daughter cells for all ∼4,000 GFP-tagged strains. The majority of the proteins fall on a straight line on the diagonal, with the abundance spanning three orders of magnitude. This result indicates that most of the proteins are evenly (or symmetrically) distributed, with the intensity in the mother cells the same as the intensity in the daughter cells; therefore the total amount of protein is proportional to the average cell volume of the two populations.

However, there is a small subset of proteins that deviate from the diagonal; they are either enriched in mother cells (below the diagonal) or enriched in the daughter cells (above the diagonal), and therefore asymmetrically distributed. We quantified the deviation from the diagonal by a z-score, defined as the vertical distance from the diagonal normalized by the standard deviation calculated from all of the strains (*Methods*). Mother-enriched proteins have a negative z-score, whereas daughter-enriched proteins have a positive z-score (Fig. 1*C*).

We verified some of these asymmetrically partitioned proteins directly by microscopy and found that there is a clearly visible difference between mother and daughter cells for high z-score proteins (Fig. S1 and images of ref. 25). However, it is difficult to identify asymmetrically partitioned proteins with moderate z-scores from fluorescent images. Our method based on mother cell labeling and flow cytometry is much more sensitive and allows better quantification because a large number of cells (10^3 to 10^4) are measured for each GFP-tagged strain.

To follow the dynamics of proteome partitioning, we performed experiments at three different time points after the original cell population was labeled with Cy5: 130 min, 170 min, and overnight (about 16 h). The first two time points compare mother and daughter cells with different degrees of maturation (the unlabeled daughter population becomes obvious around 130 min), whereas the third time point gives a comparison between labeled middle-aged mother cells (∼10 generations old after overnight growth) and an unlabeled population that is a mixture of daughter cells and mother cells at different ages. A negative z-score for the first two time points indicates a protein enriched in mother cells, whereas a negative z-score for the third time point indicates that the protein is enriched in the middle-aged mother cells (∼10 generations old) compared with the general mixed-cell population. In general, the magnitude of the z-scores (thus, the asymmetry) decreases over time as the unlabeled cell population develops into more mature daughter cells and eventually becomes mixed with both mother and daughter cells (Fig. S2 and Dataset S1).

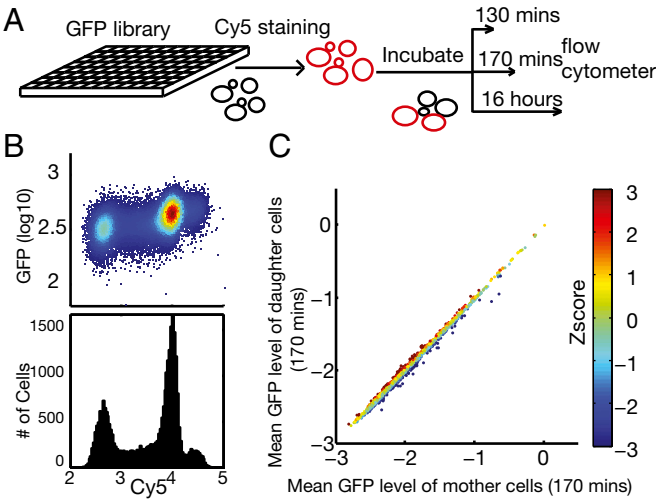

Fig. 1. High-throughput screening for proteins asymmetrically distributed between mother and daughter cells. (*A*) Schematic of the experimental procedure. *S. cerevisiae* strains from the GFP tagging library were grown in 96-well plates to exponential phase and then stained with Cy5 dye (cells in red). Newly budded daughter cells after the initial staining carry little Cy5 dye (cells in black) because they do not inherit the cell wall from their mothers. (*B*) Example of the flow cytometry data collected from one well at 170 min. Mother and daughter populations are clearly visible from the 2D density plot of Cy5 vs. GFP signals (*Upper*) and the histogram of Cy5 signal (*Lower*). (*C*) Identification of mother- or daughter-enriched proteins. The $\log_{10}$-transformed mean GFP intensity (normalized by cell volume) of the mother population is plotted against the $\log_{10}$-transformed mean GFP intensity of the daughter population (170-min data), with each dot representing one tagged strain. The straight line is the linear fit (slope = 0.985, intercept = −0.034). The deviation of the GFP intensity of the daughter population from the fitted line was converted to a z-score (indicted by the color scale) to measure the asymmetry, where negative or positive z-scores indicate enrichment in mother or daughter cells, respectively.

Functional Bias of Mother- and Daughter-Enriched Proteins Suggests Specific Mechanisms for Asymmetry. To analyze the biological function of asymmetrically partitioned proteins, we first performed gene ontology (GO) analysis. For each time point, we ranked genes by their z-scores from the most negative to the most positive and performed rank sum test for genes in each GO category associated with a specific molecular function, biological process, or cellular component. GO categories that ranked at the top tend to have more negative z-scores and are preferentially retained in mother cells, whereas GO categories ranked at the bottom are enriched in daughter cells. A number of GO categories are significantly enriched in either the mother or daughter cells (Fig. 2). With a few exceptions, the statistical significance of these categories is generally highest at the 130-min time point, decreases at 170 min, and diminishes overnight, indicating that the asymmetry is highest between mother cells and newborn daughter cells.

Several clear functional themes emerge from the GO analysis. For daughter-enriched proteins, there is a strong bias toward

certain cellular components, such as the cell cortex, cytoskeleton, site of polarized growth, and biological processes like endocytosis (Fig. 2*A*). Many of these proteins are known to be preferentially distributed to the bud neck, inside the bud, or at the bud tip (Dataset S2 provides a list of the daughter-enriched proteins discussed below). For example, the nuclear protein encoded by the *LOC1* gene is required for the daughter-specific localization of the *ASH1* mRNA, and *CRN1* encodes a cortical actin cytoskeletal component that regulates actin patch assembly. This analysis indicates that proteins needed for the emergence, construction, and scission of the bud are enriched in daughter cells, possibility through active transport processes. Another theme for daughter-enriched proteins is reflected by those proteins localized at chromosomes or in the nucleus that function in chromatin organization. Examples are *HST2*, which encodes a cytoplasmic NAD(+)-dependent protein deacetylase and a member of the *SIR2* family, and *HMO1*, a chromatin-associated high-mobility group family member involved in genome maintenance. This functional theme suggests that maintenance of chromatin in a silenced and repressive state at certain loci is important for the newborn daughter cells.

Examination of GO categories enriched in mother cells revealed an interesting pattern suggestive of organelle-based asymmetry. Among highly significant GO categories are the vacuole and plasma membrane (cellular components) and transmembrane transporters (molecular function), many of them involved in ion and amino acid transport (Fig. 2*B*). Because newly budded daughter cells do not inherit the plasma membrane from their mothers, it is expected that many proteins in the plasma membrane of the daughter cells need to be synthesized anew, and thus will appear depleted in the young daughter cells relative to the mother cells (or, equivalently, mother-enriched). Somewhat unexpectedly, the most significant GO category is the vacuole, because a large number of proteins localized to the vacuole or vacuolar membranes are enriched in mother cells (rank sum test, $P < 10^{-17}$; Dataset S3 provides a list of the mother-enriched proteins discussed below). This enrichment suggests that, quantitatively, the vacuole is unevenly distributed, although it is known that a daughter does inherit the vacuole from its mother in an active process (26, 27).

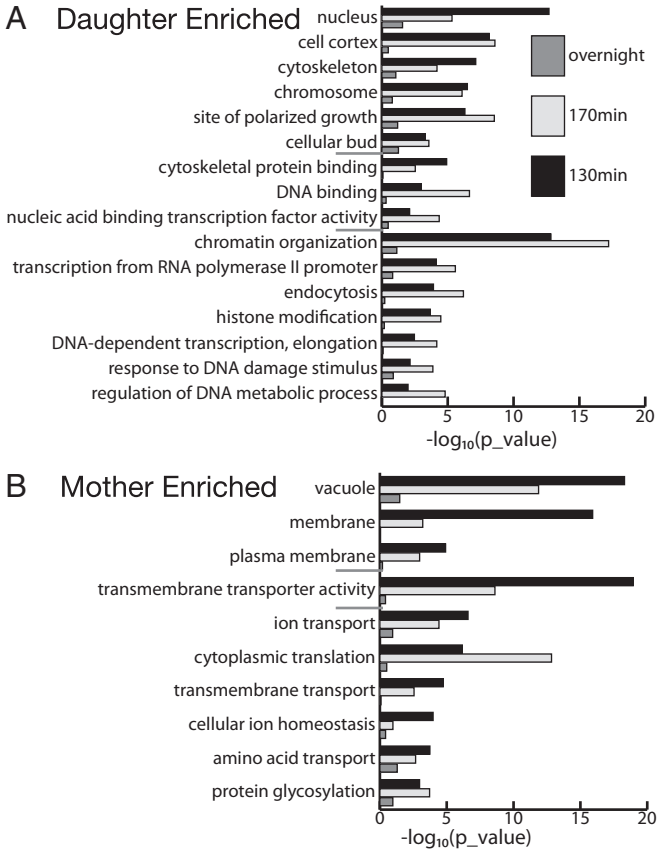

Fig. 2. GO analysis of mother/daughter-enriched proteins. At each of the three time points, proteins are rank-ordered by their z-scores, and a rank sum test was performed for each of the GO-slim categories. GO categories with proteins ranked toward positive z-scores are daughter-enriched (*A*), whereas GO categories with proteins ranked toward negative z-scores are mother-enriched (*B*). *P* values for the significant GO categories associated with a specific cellular component, molecular function, or biological process are shown in the upper, middle, and lower portions of the graphs (separated by short gray lines).

To analyze specific asymmetrically partitioned proteins, we defined a set of mother-enriched and daughter-enriched proteins based on their z-scores at the three time points. For mother-enriched proteins, we picked those proteins with strong negative z-scores at any of the three time points ($z < -3.0$) or with robust negative z-scores across time points ($z < -1.0$ for all three time points). Seventy-four genes are mother-enriched by these criteria. Similarly, 60 proteins are found to be daughter-enriched, defined by $z > 3.0$ at any time point or $z > 1.0$ across all three time points. These criteria ensure that the probability of including a random gene in either one of the lists is less than 0.008, assuming that the z-scores are independent and normally distributed.

GO enrichment analysis of the mother-enriched and daughter-enriched proteins gave similar results to the rank sum test. For example, the membrane and vacuole are the two most significantly enriched categories for the mother-enriched proteins, with 37 of 74 proteins and 18 of 74 proteins annotated as localized to the membrane and vacuole, respectively ($P = 2.2 * 10^{-11}$ and $P = 2 * 10^{-8}$, respectively). For daughter-enriched proteins, the cell periphery (17 of 60 proteins; $P = 3 * 10^{-4}$) and site of polarized growth (10 of 60 proteins; $P = 2 * 10^{-3}$) are the two most significant categories.

In addition to the plasma membrane and vacuole, mother-enriched proteins are found in other organelles. However, these organelles did not score as significantly in the GO analysis because most of the proteins localized in these organelles are symmetrically distributed. This observation suggests that proteins localized in symmetrically partitioned organelles can still be preferentially retained in the mother cell. For example, although most of the mitochondrially localized proteins are symmetrically distributed, several are found enriched in the mother cells, including the cytochrome C protein Cyc1, pyruvate dehydrogenase protein Pda1, the TCA cycle enzymes Idh2 and Kgd1, and the ATPase components Atp1 and Atp2. Interestingly, three of the six proteins (Pda1, Kgd1, and Atp1) are known to be associated with the mitochondrial nucleoid (28). It has been shown that the actively replicating mitochondrial nucleoid is associated with the endoplasmic reticulum (ER)/mitochondria junction (29). It is plausible that the part of the mitochondria tethered to the ER may not be partitioned symmetrically. Thus, asymmetry can be caused by asymmetric partitioning of a substructure of an organelle that is symmetrically partitioned overall.

Mother-Enriched Proteins Are More Likely to be Lifespan-Limiting. To analyze the relationship between cell division asymmetry and lifespan asymmetry, we focused on mother-enriched proteins. We reasoned that proteins preferentially retained in mother cells are more likely to be aging factors because their accumulation in the mother cells may be deleterious, eventually becoming lifespan-limiting. To test this hypothesis, we first examined the overlap between this set of genes and 228 long-lived mutants from a previous genomics screen (30) and found 13 that were in common [enrichment: $P < 8.6 \times 10^{-6}$]. We then measured the lifespan of the deletion mutants of all 74 mother-enriched proteins. Strikingly, we found that deletion of 23 of the 74 genes extends lifespan (Table 1); this fraction is approximately sixfold

the frequency of lifespan extension observed in viable genome-wide gene deletions.

There are clear functional themes among these newly discovered lifespan-extending genes. Besides two genes involved in mitochondrial energy generation and several localized to the vacuole, we found three genes involved in endosomal sorting and endosome-to-vacuole transport, including *VPS24*, encoding one of the four subunits of the endosomal sorting complex required for transport III (ESCRT-III) complex; *DID2*, encoding a class E protein of the vacuolar protein-sorting (Vps) pathway that binds Vps4p and directs it to dissociate from the ESCRT-III complex; and *VPS60*, encoding a protein involved in late endosome-to-vacuole transport (Fig. 3 *A*–*C*). Interestingly, another component of the ESCRT-III complex, *SNF7*, is also mother-enriched. However, deletion of *SNF7* leads to shortened lifespan. Snf7 is a core component of the ESCRT-III complex, suggesting that reduced but not abolished activity of the ESCRT-III complex extends lifespan. To our knowledge, our study represents the first time that genes involved in endosomal sorting/endosome-to-vacuole transport have been implicated in lifespan regulation, although it was known previously that ESCRT complexes are tightly linked to metabolic regulation and target of rapamycin (TOR) activity (31).

As another functional theme, we found that deletion of three genes that encode various transporters for nitrogen sources extends lifespan. These genes are *MEP2*, encoding an ammonia transporter; *CAN1*, encoding an arginine transporter; and *TPO3*, encoding a polyamine transporter (Fig. 3 *D*–*F*). The strong enrichment of transporters for nitrogen sources suggests there might be a common mechanism. It is possible that the unregulated uptake of nitrogen sources may lead to the overproduction of metabolic intermediates that become lifespan-limiting in aged cells.

Mother-Enriched and Lifespan-Limiting Proteins Tend to Accumulate over Time in Aged Mother Cells. To connect the asymmetric division in young cells to the aging phenotype in old cells, we tracked how these proteins change over time in single mother cells throughout their lifespan, using a recently developed microfluidic device (32, 33). We analyzed Did2p and Tpo3p, which represent a protein involved in endosomal sorting/endosome-to-vacuole transport and a nitrogen source transporter, respectively.

We followed the intensity of the proteins in single mother cells and their daughter cells as a function of the mother cell age (Fig. 4). A common trend is that the protein level in the mother cells increases steadily as they age. As the protein level increases in the mother cell, the daughter cells also inherit more from the mother. However, there is a clear asymmetry between mother and daughter cells; this asymmetry keeps the protein level in the daughter cells low even when they are born from old mother cells. For Did2p, daughters born from old mother cells aged around 20 generations have a protein level comparable to middle-aged mothers of about 10 generations. For Tpo3p, daughter cells (regardless of the age of their mothers) always have a level lower than the level of the young mother cells. It is known that the lifespan of daughter cells is independent of the age of their mother cells, except for the daughters produced in the last few cell divisions of the mother (3). A possible scenario is that these proteins become lifespan-limiting only when they reach the same level as in the old mother cells.

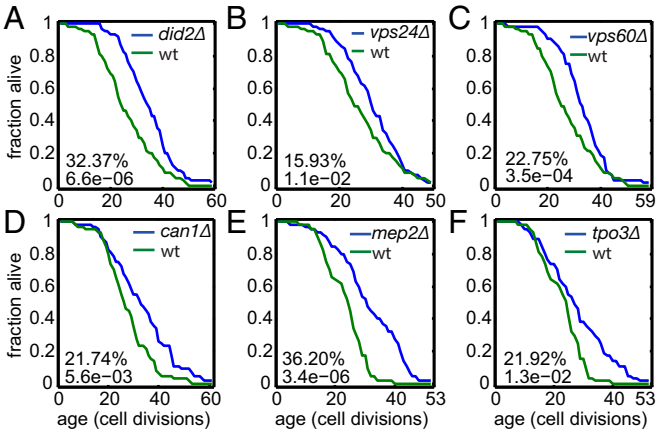

Fig. 3. Examples of mother-enriched proteins, the deletion of which extends lifespan. Shown are lifespan curves for the deletion strains of three genes involved in endosomal sorting/endosome-to-vacuole transport (*A*–*C*) and three nitrogen source transporters (*D*–*F*). The percent lifespan increase relative to the wild-type (wt) control and the *P* value based on a rank sum test are indicated.

Table 1. Mother-enriched lifespan-limiting proteins

Name	Mean lifespan	WT lifespan	Percent increase
Did2 (1)	31.6 (125)	27.2 (125)	16.2 (0.0006)
Vps24 (1)	29.7 (125)	27.4 (125)	8.4 (0.0348)
Vps60 (1)	32.2 (125)	27.5 (125)	17.1 (0.0002)
Can1 (2)	35.6 (165)	25 (225)	42.4 (<0.0001)
Mep2 (2)	30.6 (125)	25 (125)	22.4 (<0.0001)
Tpo3 (2)	28.8 (125)	24.8 (125)	16.1 (0.0013)
Itr1 (2)	29.6 (125)	27.3 (125)	8.4 (0.0257)
Fet3 (2)	27.9 (50)	24.2 (50)	15.3 (0.0325)
Yro2 (2)	32.3 (145)	27.2 (185)	18.8 (<0.0001)
Fcy2 (2)	28.9 (125)	25.6 (165)	12.9 (0.0015)
Scw4 (2)	27.8 (45)	23 (45)	20.9 (0.0142)
Cyc1 (3)	29.3 (125)	25.6 (125)	14.5 (0.0015)
Idh2 (3)	30.8 (735)	26.8 (904)	14.9 (<0.0001)
Fmp42 (3)	31.5 (45)	24.2 (45)	30.2 (0.0022)
Pml39 (4)	29.2 (45)	25.2 (45)	15.9 (0.0234)
Rai1 (4)	35.3 (45)	23.5 (45)	50.2 (<0.0001)
Pgm2 (5)	31.3 (125)	27.2 (125)	15.1 (0.0011)
Ypt6 (5)	30.7 (205)	27 (245)	13.7 (0.007)
Rpl7a (5)	32.1 (172)	26 (208)	23.5 (<0.0001)
Gsy1 (5)	30.7 (125)	25.7 (125)	19.5 (<0.0001)
Yeh1 (5)	30.4 (45)	26.8 (65)	13.4 (0.0143)
YEL020C (5)	29 (45)	25.3 (45)	14.6 (0.0468)
YER128W (5)	30 (45)	25 (45)	20 (0.0036)

Column 1 shows the protein name (grouped based on related function or similar subcellular localization). 1, ESCRT; 2, transporters/cell periphery; 3, mitochondrial; 4, nuclear; 5, others. Columns 2 and 3 show the mean lifespan (number of cells dissected) for the deletion mutant and the WT control. Column 4 shows the percent increase in mean lifespan (*P* values).

Discussion

One major mechanism for lifespan asymmetry between yeast mother and daughter cells is asymmetric division of cellular contents. Using a novel high-throughput approach, we have quantified the asymmetric partitioning of the yeast proteome between mother and daughter cells and identified proteins that are enriched in mother or daughter cells. These proteins are interesting candidates for the future investigation of the mechanisms of asymmetric cell division and aging.

How can a protein asymmetrically partition between mother and daughter cells? For a typically sized protein (~400 amino acids) freely floating in cytosol, the time it takes to diffuse throughout the cell (a few microns in size) is a few seconds; thus, the concentration can quickly equilibrate between the mother and the bud, and it is difficult to develop asymmetry. Consistent with this notion, we observed that the majority of proteins are symmetrically distributed, with equal concentrations in mother and daughter cells. However, we did observe a subset of proteins that are preferentially distributed to one of the two cell types. Interestingly, mother-specific proteins are not more likely to form complexes [based on

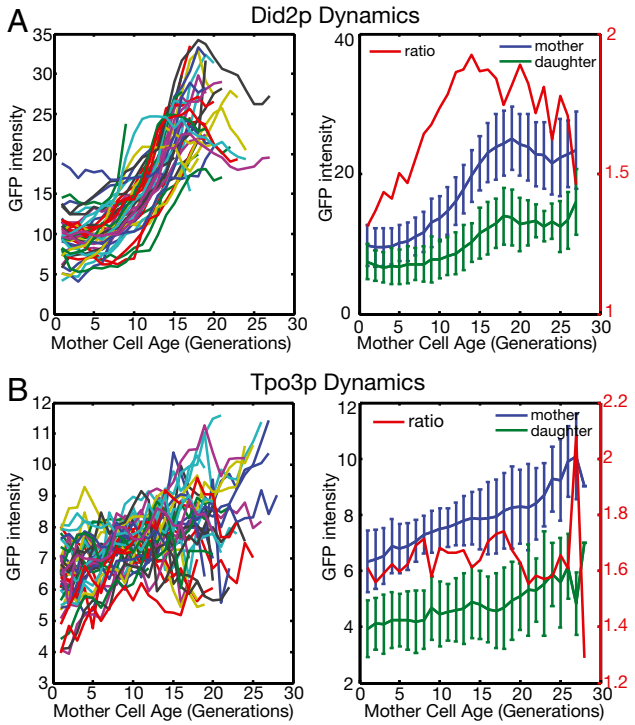

Fig. 4. Dynamic expression of mother-enriched proteins in single cells as a function of mother cell age. Mother cells were tracked using a microfluidic device and a time-lapsed microscope. GFP intensities of C-terminal–tagged Did2p (*A*) and Tpo3p (*B*) were measured in individual mother cells as well as in their daughters. (*Left*) GFP intensity in individual mother cells, where each colored line represents one cell. (*Right*) Mean and SD of the GFP intensity in mother cells and in daughter cells produced by mother cells at a given age. The red line is the ratio of the means of the mother and daughter cells.

the protein complex data from the Saccharomyces Genome Database (SGD), downloads.yeastgenome.org/curation/literature/interaction_data.tab], further contraindicating diffusion constraints as a major mechanism of asymmetry.

Instead, analysis of the mother- and daughter-enriched proteins suggested two scenarios for their asymmetric partitioning: (*i*) active transport and (*ii*) confinement within an organelle or a substructure of an organelle that is not symmetrically distributed. Proteins preferentially distributed to daughter cells are highly enriched for those proteins needed for the construction of the bud, many of which are delivered to bud neck and bud through active transport processes. Mother-specific proteins are highly enriched for those proteins localized to membranes or in the vacuole, suggesting that the vacuole is preferentially retained in the mother cell. This result is consistent with the observation that the vacuole in the mother cell keeps increasing in size as the cell ages, and eventually reaches a high-volume fraction of the cell. Quantitative analysis also revealed a faster than linear scaling of the vacuole size with the cell volume, consistent with a rapid increase of vacuole size as mother cells become older and bigger (27).

We have observed preferential retention of a number of mitochondrial proteins (e.g., Kgd1, Pda1, Atp1) in the mother cells, even though the majority of mitochondrial proteins are symmetrically distributed. It is known that the mitochondria-to-cell volume ratio of daughter cells is very close to, and even slightly higher than, the mitochondria-to-cell volume ratio of their mother cells (34). Therefore, the preferential retention of these mitochondrial proteins cannot be explained by the preferential distribution of mitochondria in mother cells. Kgd1, Pda1, and Atp1 are physically associated with the mitochondrial nucleoid and are likely bifunctional enzymes involved in both energy metabolism and mitochondrial genome maintenance (28). Previous studies have shown that the actively replicating mitochondrial nucleoid is associated with the mitochondria/ER junction (29). We thus conjecture that a substructure of mitochondria that is tethered to the ER is not equally transmitted to the daughter cell. Consistent with this conjecture is a recent observation showing that aggregated proteins in the ER are captured by mitochondria and preferentially retained in the mother cells (18).

How does asymmetric protein partitioning connect with lifespan asymmetry? Our data show that mother-enriched proteins are more likely to have a lifespan extension phenotype when deleted, suggesting that they are more likely to become lifespan-limiting in old cells. Dynamic tracking of these proteins in single mother cells showed a steady increase to a high level in the old cells, whereas the daughter cells keep a relatively low level through asymmetry, arguing that the high concentration may be deleterious in old cells.

Thayer et al. (35) recently used isotope labeling and total proteome MS to identify long-lived asymmetrically retained proteins (LARPs). They identified several full-length proteins (Mrh1, Pma1, Sur7, Thr1, and Hsp26) and many protein fragments. We found that Pma1 is significantly enriched in mother cells (z-score of −4.5 for the 170 min), but is filtered out due to a small number of cells (*Methods*). Mrh1 is also in our list of mother-enriched proteins, but the deletion mutant is short-lived. Unlike the LARPs, which are long-lived proteins, the mother-enriched proteins we identified have a normal half-life (Fig. S3), and hence normal turnover. This observation suggests that the lifespan-limiting effect is not due to old and nonfunctional proteins; rather, it is the high concentration in mothers that interferes with cell homeostasis and/or leads to aberrant cell signaling, limiting lifespan.

Are there fitness benefits of the preferential retention of proteins in the mother cell? Theoretical studies suggest that asymmetric segregation may evolve as a general strategy to cope with cellular damage (9–11), conferring a fitness benefit at the population level by producing rejuvenated offspring at the expense of an aging parent. Such asymmetric segregation may operate at the level of the organelle, where the daughter cell synthesizes an organelle de novo or grows it from a seed inherited from the mother. Mother-specific retention may also confer a fitness benefit by ensuring that certain proteins (e.g., those proteins not needed for, or even inhibitory to, the early development of the bud) are expressed just in time in the daughter cell, and that their level of expression does not become too high in the daughter/young mother cells. Interestingly, we found that none of 74 mother-enriched proteins are essential, which is a significant depletion from the genome-wide average of about 18%.

The observed asymmetric distribution of many vacuolar proteins and the rapid increase in vacuole size as the mother cell ages suggest that vacuoles in old mother cells might be a major source of aging factors limiting lifespan. Interestingly, it was observed that the spores do not inherit the vacuole from the mother during meiosis (36), and sporulation was found to rejuvenate the gametes generated from old cells (37). Three of the 23 proteins we identified (Did2, Vps24, and Vps60) are involved in endosomal sorting and endosome-to-vacuole transport via the multivesicular body (MVB) pathway. It is known that the MVB pathway is strongly linked to the regulation of cellular metabolism, and mutants of ESCRT complexes exhibit decreased TOR activity (31, 38). Interestingly, chemical-genetic profiling in yeast revealed that *DID2* and *VPS60* deletion mutants show strong sensitivity to rapamycin treatment (39), adding to the evidence that these genes positively influence TOR activity. Thus, one possible scenario is that accumulated proteins involved in endosomal sorting and endosome-to-vacuole transport eventually lead to aberrant TOR signaling that limits the lifespan. Consistent with this scenario, our dynamic tracking of the protein Did2 showed

that a decrease of the concentration later in life in a subset of cells correlates with a longer lifespan (Fig. 4*A*).

In this study, we have focused mainly on mother-enriched proteins. However, our genome-wide screening also produced a comprehensive set of daughter-enriched proteins. These proteins may be important for bud development, or may act as rejuvenating factors necessary for the reset of the lifespan. Data from our genome-wide screening indicate that the deletion mutants are enriched for those mutants with an increased lifespan (Dataset S4). For example, deletion of *CRN1* and *LOC1*, involved in construction of the bud- and daughter-specific mRNA localization, significantly extends lifespan; the latter is consistent with a previous finding (40). The enrichment of lifespan-extending mutants is consistent with the antagonistic pleiotropy hypothesis that genes beneficial to early development may become detrimental later in life (41). This hypothesis has been explored in other species (e.g., in worms), where a strong overlap between longevity genes and genes important for development was observed (42). For potential rejuvenating factors, we expect that the deletion will shorten lifespan. We did found a number of mutants with a significantly shortened lifespan, among them *SRS2* and *HMO1*, which are involved in DNA repair and genome maintenance.

In summary, we have used a high-throughput approach to identify asymmetrically distributed proteins between mother and daughter cells during division. Proteins enriched in mother cells also tend to be lifespan-limiting, suggesting that their accumulation during mother cell aging is restrictive to continued cell division. We also identified a set of daughter-enriched proteins with lifespan phenotypes. These findings set the stage for a comprehensive understanding of cell division asymmetry and replicative aging in yeast, and point to a number of new mechanisms that may modulate aging in more complex eukaryotes.

Methods

All strains of *Saccharomyces cerevisiae* used in high-throughput flow cytometry screening were taken from the GFP tagging library (25). Deletion strains used for lifespan analysis were derived from BY4741 (*MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and BY4742 (*MAT*α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*). Yeast cultures were grown in selective minimal media containing 2% (mass/vol) glucose.

Replicative lifespan analysis and single-cell tracking using the microfluidic device were performed as previously described (33, 43, 44).

Protocols for cell staining and flow cytometry experiments and FACS data analysis and z-score calculation are described in *SI Methods*.

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1. Mortimer RK, Johnston JR (1959) Life span of individual yeast cells. *Nature* 183(4677): 1751–1752.
2. Jazwinski SM (1990) An experimental system for the molecular analysis of the aging process: The budding yeast Saccharomyces cerevisiae. *J Gerontol* 45(3):B68–B74.
3. Kennedy BK, Austriaco NR, Jr, Guarente L (1994) Daughter cells of Saccharomyces cerevisiae from old mothers display a reduced life span. *J Cell Biol* 127(6 Pt 2): 1985–1993.
4. Coelho M, et al. (2013) Fission yeast does not age under favorable conditions, but does so after stress. *Curr Biol* 23(19):1844–1852.
5. Barker MG, Walmsley RM (1999) Replicative ageing in the fission yeast Schizosaccharomyces pombe. *Yeast* 15(14):1511–1518.
6. Stewart EJ, Madden R, Paul G, Taddei F (2005) Aging and death in an organism that reproduces by morphologically symmetric division. *PLoS Biol* 3(2):e45.
7. Lindner AB, Madden R, Demarez A, Stewart EJ, Taddei F (2008) Asymmetric segregation of protein aggregates is associated with cellular aging and rejuvenation. *Proc Natl Acad Sci USA* 105(8):3076–3081.
8. Wang P, et al. (2010) Robust growth of Escherichia coli. *Curr Biol* 20(12):1099–1103.
9. Ackermann M, Chao L, Bergstrom CT, Doebeli M (2007) On the evolutionary origin of aging. *Aging Cell* 6(2):235–244.
10. Evans SN, Steinsaltz D (2007) Damage segregation at fissioning may increase growth rates: A superprocess model. *Theor Popul Biol* 71(4):473–490.
11. Watve M, Parab S, Jogdand P, Keni S (2006) Aging may be a conditional strategic choice and not an inevitable outcome for bacteria. *Proc Natl Acad Sci USA* 103(40): 14831–14835.
12. Sinclair DA, Guarente L (1997) Extrachromosomal rDNA circles—A cause of aging in yeast. *Cell* 91(7):1033–1042.
13. Defossez PA, et al. (1999) Elimination of replication block protein Fob1 extends the life span of yeast mother cells. *Mol Cell* 3(4):447–455.
14. Denoth-Lippuner A, Krzyzanowski MK, Stober C, Barral Y (2014) Role of SAGA in the asymmetric segregation of DNA circles during yeast ageing. *eLife* 3:3.
15. Shcheprova Z, Baldi S, Frei SB, Gonnet G, Barral Y (2008) A mechanism for asymmetric segregation of age during yeast budding. *Nature* 454(7205):728–734.
16. Gehlen LR, et al. (2011) Nuclear geometry and rapid mitosis ensure asymmetric episome segregation in yeast. *Curr Biol* 21(1):25–33.
17. McFaline-Figueroa JR, et al. (2011) Mitochondrial quality control during inheritance is associated with lifespan and mother-daughter age asymmetry in budding yeast. *Aging Cell* 10(5):885–895.
18. Zhou C, et al. (2014) Organelle-based aggregation and retention of damaged proteins in asymmetrically dividing cells. *Cell* 159(3):530–542.
19. Zhou C, et al. (2011) Motility and segregation of Hsp104-associated protein aggregates in budding yeast. *Cell* 147(5):1186–1196.
20. Liu B, et al. (2010) The polarisome is required for segregation and retrograde transport of protein aggregates. *Cell* 140(2):257–267.
21. Erjavec N, Larsson L, Grantham J, Nyström T (2007) Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p. *Genes Dev* 21(19):2410–2421.
22. Aguilaniu H, Gustafsson L, Rigoulet M, Nyström T (2003) Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* 299(5613):1751–1753.
23. Eldakak A, et al. (2010) Asymmetrically inherited multidrug resistance transporters are recessive determinants in cellular replicative ageing. *Nat Cell Biol* 12(8):799–805.
24. Henderson KA, Hughes AL, Gottschling DE (2014) Mother-daughter asymmetry of pH underlies aging and rejuvenation in yeast. *eLife* 3:e03504.
25. Huh WK, et al. (2003) Global analysis of protein localization in budding yeast. *Nature* 425(6959):686–691.
26. Weisman LS (2003) Yeast vacuole inheritance and dynamics. *Annu Rev Genet* 37: 435–460.
27. Chan YH, Marshall WF (2014) Organelle size scaling of the budding yeast vacuole is tuned by membrane trafficking rates. *Biophys J* 106(9):1986–1996.
28. Chen XJ, Butow RA (2005) The organization and inheritance of the mitochondrial genome. *Nat Rev Genet* 6(11):815–825.
29. Meeusen S, Nunnari J (2003) Evidence for a two membrane-spanning autonomous mitochondrial DNA replisome. *J Cell Biol* 163(3):503–510.
30. McCormick MA, et al. (2015) A comprehensive analysis of replicative lifespan in 4,698 single-gene deletion strains uncovers conserved mechanisms of aging. *Cell Metabolism*, in press.
31. Jones CB, et al. (2012) Regulation of membrane protein degradation by starvation-response pathways. *Traffic* 13(3):468–482.
32. Xie Z, et al. (2012) Molecular phenotyping of aging in single yeast cells using a novel microfluidic device. *Aging Cell* 11(4):599–606.
33. Zhang Y, et al. (2012) Single cell analysis of yeast replicative aging using a new generation of microfluidic device. *PLoS One* 7(11):e48275.
34. Rafelski SM, et al. (2012) Mitochondrial network size scaling in budding yeast. *Science* 338(6108):822–824.
35. Thayer NH, et al. (2014) Identification of long-lived proteins retained in cells undergoing repeated asymmetric divisions. *Proc Natl Acad Sci USA* 111(39): 14019–14026.
36. Roeder AD, Shaw JM (1996) Vacuole partitioning during meiotic division in yeast. *Genetics* 144(2):445–458.
37. Unal E, Kinde B, Amon A (2011) Gametogenesis eliminates age-induced cellular damage and resets life span in yeast. *Science* 332(6037):1554–1557.
38. Babst M, Odorizzi G (2013) The balance of protein expression and degradation: An ESCRTs point of view. *Curr Opin Cell Biol* 25(4):489–494.
39. Hillenmeyer ME, et al. (2008) The chemical genomic portrait of yeast: Uncovering a phenotype for all genes. *Science* 320(5874):362–365.
40. Long RM, et al. (2001) An exclusively nuclear RNA-binding protein affects asymmetric localization of ASH1 mRNA and Ash1p in yeast. *J Cell Biol* 153(2):307–318.
41. Williams G (1957) Pleiotropy, natural selection, and evolution of senescence. *Evolution* 11:398–411.
42. Chen D, Pan KZ, Palter JE, Kapahi P (2007) Longevity determined by developmental arrest genes in Caenorhabditis elegans. *Aging Cell* 6(4):525–533.
43. Kaeberlein M, Kirkland KT, Fields S, Kennedy BK (2004) Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol* 2(9):E296.
44. Steffen KK, Kennedy BK, Kaeberlein M (2009) Measuring replicative life span in the budding yeast. *J Vis Exp* 25(28):e1209.
45. Wilcoxin F (1947) Probability tables for individual comparisons by ranking methods. *Biometrics* 3(3):119–122.
46. Wilcoxon F (1946) Individual comparisons of grouped data by ranking methods. *J Econ Entomol* 39:269.