Published in final edited form as:

Exp Gerontol. 2016 December 15; 86: 113–123. doi:10.1016/j.exger.2016.04.012.

Gene-Nutrient Interaction Markedly Influences Yeast Chronological Lifespan

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Abstract

Purpose—Research into the genetic mechanisms of aging has expanded rapidly over the past two decades. This has in part been the result of the use of model organisms (particularly yeast, worms and flies) and high-throughput technologies, combined with a growing interest in aging research. Despite this progress, widespread consensus regarding the pathways that are fundamental to the modulation of cellular aging and lifespan for all organisms has been limited due to discrepancies between different studies. We have compared results from published genome-wide, chronological lifespan (CLS) screens of individual gene deletion strains in *S. cerevisiae* in order to identify gene deletion strains with consistent influences on longevity as possible indicators of fundamental aging processes from this single-celled, eukaryotic model organism.

Methods—Three previous reports have described genetic modifiers of chronological aging in the budding yeast (*S. cerevisiae*) using the yeast gene deletion strain collection. We performed a comparison among the data sets using correlation and decile distribution analysis to describe concordance between screens and identify strains that consistently increased or decreased CLS.

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Disclosures

John L. Hartman IV has a financial interest in Spectrum PhenomX, LLC, which aims to commercialize Q-HTCP technology. All other authors declare no competing interests.

We used gene enrichment analysis in an effort to understand the biology underlying genes identified in multiple studies. We attempted to replicate the different experimental conditions employed by the screens to identify potential sources of variability in CLS worth further investigating.

Results—Among 3209 strains present in all three screens, nine (2.80%) deletions strains were in common in the longest-lived decile and thirteen (4.05%) were in common in the shortest-lived decile for all three screens. Similarly, pairwise overlap between screens was low. When the same comparison was extended to three deciles to include more mutants studied in common between the three screens, enrichment of cellular processes based on gene ontology analysis in the long-lived strains remained very limited. To test the hypothesis that different parental strain auxotrophic requirements or media formulations employed by the respective genome-wide screens might contribute to the lack of concordance, different CLS assay conditions were assessed in combination with strains having different ploidy and auxotrophic requirements (all relevant to differences in the way the three genome-wide CLS screens were performed). This limited but systematic analysis of CLS with respect to auxotrophy, ploidy, and media revealed several instances of gene $\times$ nutrient interaction.

Conclusions—There is surprisingly little overlap between the results of three independently performed genome-wide screens of CLS in *S. cerevisiae*. However, differences in strain genetic background (ploidy and specific auxotrophic requirements) were present, as well as different media and experimental conditions (e.g., aeration and pooled vs. individual culturing), which, along with stochastic effects such as genetic drift or selection of secondary mutations that suppress the loss of function from gene deletion, could in theory account for some of the lack of consensus between results. Considering the lack of overlap in CLS phenotypes among the set of genes reported by all three screens, and the results of a CLS experiment that systematically tested (incorporating extensive controls) for interactions between variables existing between the screens, we propose that discrepancies can be reconciled through deeper understanding of the influence of cell intrinsic factors such as auxotrophic requirements ploidy status, extrinsic factors such as media composition and aeration, as well as interactions that may occur between them, for example as a result of different pooling vs. individually aging cultures. Such factors may have a more significant impact on CLS outcomes than previously realized. Future studies that systematically account for these contextual factors, and can thus clarify the interactions between genetic and nutrient factors that alter CLS phenotypes, should aid more complete understanding of the underlying biology so that genetic principles of CLS in yeast can be extrapolated to differential cellular aging observed in animal models.

Keywords

yeast gene deletion strain library; *S. cerevisiae*; aging; chronological lifespan (CLS); genome-wide screen; longevity; quantitative high throughput cell array phenotyping; gene-nutrient interaction

Introduction

Knowledge about aging-related cellular mechanisms has advanced rapidly in recent decades, yet an integrated understanding of age-related deterioration lacks consensus. While aging is multi-factorial and organism-specific in some respects, genetic and nutritional interventions

can influence the onset and rate of age-related decline similarly across different species, indicating fundamental processes of cellular aging are evolutionarily conserved. Thus leveraging the powerful genetic analysis of the single-celled organism, *Saccharomyces cerevisiae*, to dissect the complexity of gene-nutrient interaction effects on cellular aging could aid derivation of biological principles that apply to higher eukaryotes (Curran and Ruvkun 2007; Kennedy 2008; Smith and others 2007).

There are two types of aging commonly studied in budding yeast (Longo and others 2012). Replicative lifespan (RLS) is the number of times a mother cell produces daughters prior to senescence (mothers and daughters are easily distinguished due to asymmetric budding). RLS is seen as a useful model of cellular aging for mitotically active cells, such as stem cells. The second model for aging in budding yeast, which is the topic of this article, is chronological lifespan (CLS), referring to the maintenance of viability of non-mitotic cells. The percentage of colony forming units versus time is considered to be the gold standard measure of CLS. Assuming a cessation of cell division in 'stationary' culture and thus a constant number of total cells, CLS reflects the duration of cell survival after transitioning from a dividing to non-dividing state, which is quantified by shifting cells back to fresh media after increasing intervals of time in stationary phase. In contrast to animal cells yeast do not enter a post-mitotic state independently of nutrient depletion, however the nutrient milieu during logarithmic growth (non-starvation state) ultimately affects yeast CLS, which could therefore be indicative of factors generally affecting eukaryotic cellular health and post-mitotic survival (Fabrizio and Wei 2011; Ocampo and others 2012). In summary, the yeast CLS model offers advantages for large-scale genetic analysis, yet much remains to be discovered regarding its relevance, alongside other experimental models, for understanding human healthspan.

RLS and CLS are considered to have complementary relevance for understanding aging in multi-cellular organisms; RLS being relevant to mitotic tissues with regenerative reserve capacity such as epithelial linings and hematopoietic tissue, whereas CLS has greater relevance to cells comprising non-dividing tissues such as brain and cardiac or skeletal muscle. Although the biological relationship between yeast CLS and RLS and its relevance to aging across the broader eukaryotic kingdom remains to be further demonstrated, overlapping mechanisms have been suggested (Burtner and others 2011; Delaney and others 2013; Mirisola and Longo 2012; Polymenis and Kennedy 2012; Smith and others 2008). Similarly, nutrient signaling pathways and dietary interventions exhibit analogous effects on RLS, CLS, and aging of multicellular organisms (Fabrizio and others 2001; Jiang and others 2000; Kaeberlein and others 2005).

For technical reasons, CLS has been more amenable to high throughput studies, as a genome-wide RLS study was only recently completed (McCormick and others 2015). Though CLS (plating and colony counting) is higher throughput than RLS (dissection and counting of daughter cells), neither accommodate the throughput for routine genome-wide assessment of the entire YKO/KD collection. Other approaches have been developed to estimate CLS, (Fabrizio and others 2010; Matecic and others 2010; Powers and others 2006), which we discuss below. High throughput CLS techniques sample large cell populations in stationary culture over days to weeks to estimate the decline in viable cells vs.

time, based on the percentage of cells re-entering proliferative growth when exposed to fresh media (Figure 1).

Two different techniques for high-throughput CLS estimation include: (1) outgrowth in liquid media in a 96-well format (Murakami and Kaerberlein 2009; Powers and others 2006) and (2) outgrowth of pooled cultures on agar followed by DNA extraction and relative hybridization to microarray chips (Fabrizio and others 2010; Matecic and others 2010). Each gene deletion cassette has been designed with unique flanking oligonucleotides that anneal to complementary oligonucleotides synthesized on hybridization chips (Giaever and others 2002; Winzeler and others 1999), which are used to estimate changes in relative abundance of particular deletion strains over time (Irizarry and others 2003; Ooi and others 2003; Yuan and others 2005). There have been two genome-wide CLS screens performed by the pooled library approach (Fabrizio and others 2010; Matecic and others 2010) and one by the individual cultures grown in micro-well plates (Powers and others 2006). The main focus of this article is on the comparison of these three screens (Table 1), including whether genes and biological processes known to influence cellular aging are revealed by their overlap, and consideration of possible reasons for any lack of consensus (Table 2, Figures 2 and 3).

We found remarkable lack of agreement between the three genome-wide screens and hypothesize that gene-environment interaction is a major cause of discordance because different media were used (Table 2). It is notable that large-scale aging studies performed in other organisms, in particular *C. elegans*, have also resulted in low concordance, which could also be explained by nutritional differences (Curran and Ruvkun 2007; Hamilton and others 2005; Hansen and others 2005; Kennedy 2008; Smith and others 2007). Consistent with this possibility, nutrient interventions alone such as methionine restriction exert influence on aging across multiple species (Johnson and Johnson 2014; Orentreich and others 1993; Troen and others 2007). Furthermore, in mouse or rat models with otherwise tightly controlled experimental conditions, variable longevity results obtained for interventions performed at different sites (e.g. National Institute on Aging's Interventions Testing Program) or at the same site across multiple years appear to be in part due to unexpected effects of different rodent chow impacting lifespan (Ghirardi and others 1995; Harrison and others 2014; Strong and others 2008). Although there are other plausible explanations for the discordant CLS results observed for the yeast gene deletion strain collection, we conclude that yeast genetic analysis of CLS (and RLS) provides a powerful platform to investigate gene-environment and gene-gene interaction from a comprehensive perspective to gain additional clarity regarding their effects on cellular health and aging. To investigate gene-nutrient interaction with respect to CLS, we performed a controlled experiment using quantitative high throughput cell array phenotyping to systematically compare the effect of different media on CLS, as well as the possible interaction of media with auxotrophic requirements and/or ploidy. The results indicate broad dependency of the CLS phenotype on media, auxotrophy, and ploidy (i.e., gene $\times$ nutrient interaction) in the three genome-wide CLS screens published to date.

Results and Discussion

Comparison of three independent, genome-wide CLS screens

CLS data were obtained from supplemental data files and compared by correlation and decile distribution analysis to describe concordance between genome-wide screens performed by three independent laboratories (Fabrizio and others 2010; Matecic and others 2010; Powers and others 2006). Differences between the screen methodologies are illustrated in Figure 1, and summarized in Table 1. Differences in media composition are summarized in Table 2.

The total number of Yeast Gene Deletion Strains (YGDS) reported on by each of the three screens was variable, and less than the total number of strains in the respective collections, due to lost detection of strains in the respective screens (Table 1, Supplemental Table 1). The Matecic et al study reported 3209 unique strains identified by the “UP” tags, and 2538 by the “DOWN” tags, thus the more inclusive UP tag set was used for comparisons with the other two screens. The three studies were compared pair-wise by Pearson correlation, revealing little agreement between them considering the studies shared essentially the same objective (Figure 2). In contrast, a within-study analysis of Matecic *et al.* showed reproducibility of viability estimates by comparing barcode abundance of the UP tags and DOWN tags for each ORF deletion construct (Matecic and others 2010) (Table 1, Supplemental Table 1). The correlation coefficient (R) for the up and down tags from Matecic et al was 0.79 (Figure 2A), while that for Matecic et al (UP tags) with Powers et al was $R=0.11$ (Figure 2B). The correlation coefficient for Matecic et al and Fabrizio et al was $R=0.09$ (Figure 2C), while the lowest correlation was between Powers et al and Fabrizio et al, with $R=0.018$ (Figure 2D).

We compared decile distributions to look with finer detail for possible correlation in extension or shortening of CLS between the different screens (Figure 3, Supplemental Tables 1–5). In the within-study comparison of Matecic et al (UP and DOWN tags), there was smooth overlap in the distribution across all CLS deciles (Figure 3A, Supplemental Figure 1A). Among the across study comparisons, there was discernable correlation for short-lived and long-lived deletion strains, albeit very limited (Figure 3B, 3C, 3D and Supplemental Figure 1B, 1C, 1D). There appeared to be slightly greater agreement between the Matecic et al and Fabrizio et al screens (Figure 3C and Supplemental Figure 1C), particularly for short-lived strains, however the correlation was low for every between-screen comparison.

Unlike other genetic models where screens are non-saturating or differences in genetic backgrounds exist, thus providing potential explanations for discrepancies among studies, the results with the YGDS suggest factors in addition to gene deletions of interest are contributing to the longevity effects. Discrepancy may be best explained by differences in experimental conditions used such as media recipe and/or subtle differences in the auxotrophic requirements or ploidy of the YGDS employed in the respective studies.

Overlap in genes found by different screens to influence CLS

We next investigated whether the overlap in genes identified between screens could be related to common aging mechanisms, as each published report implicated specific classes of genes in longevity regulation, such as nutrient sensing pathways including TOR1 or SCH9, purine biosynthesis, and autophagy (Fabrizio and others 2001; Kennedy and others 2005; Matecic and others 2010; Powers and others 2006). Consistent with the low correlation of results overall, the small overlapping set of CLS extending mutants, common to all three screens, did not over represent any particular pathway (Table 3).

Although there was no statistically significant enrichment based on gene ontology assessments, it was notable that deletion strains related to autophagy (e.g. *ATG14*, *VPS27*) and nutrient sensing/stationary phase transition (*RIM8*, *RIM15*, *RIM101*) were identified among the overlapping short-lived deletion strains (Table 4). Expanding the analysis to include overlap in the top two deciles from all three screens revealed 52/642 long-lived and 102/642 short-lived strains in common between the three screens (Figure 4, Supplemental Tables 2–5), yet when compared to the background of genes (Supplemental Table 1) provided no enrichment for biological processes, molecular functions or cellular components associated with long-lived or short-lived phenotypes. However, expansion of the analysis to inclusion of the top three deciles (Figure 4) revealed functional enrichment for chromatin accessibility complex among long-lived mutants and enrichment of autophagy-related genes among short-lived mutants (Supplemental Table 6).

Although there are relatively few overlapping genes between the screens, and the genes that do overlap for longevity extension show little significant enrichment in pathways or functions known to regulate aging, they may nevertheless suggest clues to consensus CLS pathways. For example, the identification of long-lived deletion strains with connections to gene expression regulation (*ITC1*), mRNA turnover (*PUF3*) and transcriptional regulation (*SSN2*) could emphasize the importance of a coordinated response for entry into and maintenance of stationary phase for successful cell survival (Table 3). Similarly, the handful of overlapping short-lived deletion strains might suggest the proper establishment of stationary phase transcriptional and metabolism state (*RIM15*, *RIM8*, *RIM101*, *UBP8*); maintenance of redox balance through glutathione metabolism (*GCV3*, *DUG2*); protein production, stability and turnover (*VPS27*, *MON2*, *ATG14*); and possibly DNA integrity exemplified by telomere maintenance (*MTC7*, *NMD2*, *ECM29*) are important for the long-term survival of cells in non-dividing states (Table 4). Perhaps, more importantly, the consistency between the technical replicates within the Matecic et al study suggests that reproducibility is possible (Figures 2 and 3) and thus experimental factors such as media and genetic background (Tables 1 and 2) may explain differences in CLS results obtained between the three YGDS screens. Genetic background differences can occur due to the presence of different auxotrophic markers, mating type, and ploidy of the respective deletion strain collections as well as from genetic drift and/or selection of secondary mutations resulting from suppression of the engineered gene deletions (known to occur in some deletion strains). From this perspective, the network of genes that regulates aging could exhibit plasticity in response to variations in dietary, nutritional, and/or environmental context. Although variable CLS poses a challenge, analogous challenges exist for

understanding aging in multi-cellular organisms, and thus genome-wide CLS in the YGDS provides a relatively tractable genetic model to address the complexity of aging resulting from genes interacting with the environment and other genes.

Experimental variables that might cause disparate CLS screen results

Below we consider experimental variables that could potentially explain disparate results between the three previous genome-wide screens (Tables 1 and 2):

Media Composition—Cellular health and longevity depend in part on the availability and sensing of nutrients available for growth, reproduction and maintenance, and coordinating cellular activities accordingly. For yeast, nutrients can be freely manipulated via liquid or agar-based media, allowing highly controlled experimentation about nutrient effects. The most common media used in yeast research consist of “rich” media, “YPD”, comprised of three ingredients: 1) Yeast Extract, 2) Peptone and 3) Dextrose (glucose). Yeast extract is derived from cultures of yeast, while peptone is generally derived from enzymatic digestion of animal protein, which are ‘undefined’ due to variability between proportions of molecular species between preparations. In contrast, ‘defined’ media has been commonly used in genome-wide CLS assays, which is interchangeably called “SC” (Synthetic Complete) or “SD” (Synthetic Defined) media. The specific makeup of synthetic media differs between laboratories, as was the case for the three CLS studies (Table 2). Most laboratories use a component of defined media, called yeast nitrogen base (YNB), for which the standard formula derives from the work of Wickerham (Wickerham 1946). Ammonium sulfate is often added in a standard amount (5 gm/L) as a nitrogen source, and amino acids are added separately in varying combinations and amounts (e.g., Table 2). With YPD, only the carbon source is typically manipulated, whereas the amino acids and additional nitrogen sources can also be individually adjusted in composition or amount with defined media. Media constitution likely has significant effects on CLS, yet media differences have received little attention in the study design relative to genetic differences. It is also worth considering that genetic factors and media components can interact, for example auxotrophic markers and corresponding nutrient requirements. Leucine, histidine, uracil, methionine and lysine are frequently used auxotrophic markers in the genetic background of the YGDS library, and thus the amino acid complements are provided in excess, but often in varying amounts (Table 2). Notably, amino acid levels (e.g., leucine and glutamine) can impact nutrient signaling pathways shown to influence cellular aging, such as TOR (target of rapamycin) (Ocampo and others 2012; Pan and others 2011). These and related considerations are further complicated with screens employing pooled cultures, where strain-specific metabolite production, secretion and response are not readily tracked, but co-occur and potentially interact. CLS in isolated monocultures provides greater control in experimental design with regard to detection of main effects of auxotrophy and media composition as well as gene-gene and gene-media interactions, but in principle reduces throughput for genome wide CLS analysis.

As shown in Table 2, the three studies all used 2% dextrose as carbon source and 5 gm/L ammonium sulfate for excess nitrogen. *Matecic et al.* also screened with media containing 0.5% dextrose, but it was the only study to do so and thus the 2% condition was used for

comparative analysis. The amino acids component of the recipes varied considerably. The Matecic *et al.* and Longo *et al.* studies used the *MATa* haploid genetic background, which has the *his3*, *leu2*, *ura3*, and *met17* auxotrophies, while the Powers *et al.* study used the homozygous diploid background with complete auxotrophy for *his3*, *leu2*, and *ura3* and heterozygous for *met17/MET17* and *lys2/LYS2*. Additionally, the Longo media contains a phosphate buffer (Fabrizio and others 2010; Wei and others 2011), and their protocol calls for removal of the media and assay of CLS in water. Whether media differences account for the low concordance between different genome-wide CLS screens remains to be formally tested.

Aeration effects on oxygenation, respiration, and media acidification—Yeast transition between metabolic states in response to available nutrients and oxygen, engaging to variable extents in fermentative and respiration-based processes. Yeast repress respiration in a high glucose, nutrient rich environment, undergoing glycolysis and fermentation until glucose or other nutrient limitation de-represses respiration, leading to further extraction of energy by conversion of ethanol to organic acids, in particular acetic acid, which reduces CLS. Respiration increases with reduction of glucose concentration to 0.5% or lower (“calorie-restricted”) from 2% (“non-restricted”) (Ocampo and others 2012; Pan and others 2011), but the significance of this metabolic change with regard to the many genetic effects on the CLS phenotype is not well understood, for example, there is less organic acid production by cells growing in media with lower glucose (Burtner and others 2009a). Thus, aerobic glycolysis and fermentation of glucose in the log phase of growth, organic acid production and secretion into the media, and its consequent effects on CLS represent an important mechanism of CLS, which can be greatly affected by media conditions and aeration status (Burtner and others 2009a; Murakami and others 2011; Ocampo and others 2012; Wierman and others 2015). Buffering the media (e.g., in the Longo lab media), or washing away the acetic acid in conditioned media, and aging in water can also potentially increase CLS. However, there may also be CLS-extending factors that are altered by these same interventions, and the production of either CLS- extending or shortening factors may themselves depend upon nutrient factors, genetic background, and gene interaction. The “acetic acid mechanism” appears to be fairly complex, but it may further be only one of many related or independent mechanisms of CLS. The number of factors that influence CLS is only beginning to be characterized (Matecic and others 2010; Ocampo and others 2012; Wierman and others 2015).

Unidentified gene interaction—The *S. cerevisiae* YGDS has contributed greatly to understanding that gene interaction is ubiquitous; i.e., the knowledge that networks of genes contribute to every phenotype is a direct result of widespread use of the YGDS library (Costanzo and others 2010; Giaever and Nislow 2014; Hartman and others 2015). Gene interaction in the YGDS is usually reported in terms of surprising changes in cell proliferation of deletions mutants relative to reference strain, in response to genetic (e.g., double mutants or expression of heterologous gene) or chemical perturbation (Mani and others 2008). One way of quantifying gene interaction involves a dose response to perturbations such as concentrations of a drug, levels of gene expression, or both (Hartman IV 2007; Hartman IV and Tippery 2004; Louie and others 2012), which can be seen as

analogous to CLS, where the perturbation is time (Figure 5). Gene interaction effects are observed extensively with respect to different yeast media (Hartman and others 2015), which is likely to also be true for CLS - a more complicated phenotype that scores cell proliferation as a function of age. Thus media recipes appear to have a greater effect on CLS than the simple logarithmic growth phenotype (Burtner and others 2009a; Murakami and Kaerberlein 2009; Murakami and others 2008). In summary, the complexities of high throughput growth phenotyping are amplified in CLS by serial assay of aging cultures. Based on these observations, it is plausible that lack of consensus between different laboratories could be explained by differences in strains, media and related biology rather than failed replication or stochastic effects. Carefully controlled experiments, for example involving paired analysis of the *MAT α /met17/LYS2*, *MAT α /MET17/lys2* and or homozygous diploid libraries (also controlling for media components) would help to further explore gene $\times$ nutrient interaction in CLS.

Differences in CLS phenotyping methodologies—Another potential source of discrepancy between large-scale CLS studies involves specific phenotypic screening methodologies employed, which can influence outcome and interpretation. The gold standard for measuring of CLS is to plate cells from an aerated stationary phase liquid culture to fresh agar media to determine the percentage change in CFU vs time (Figure 1). The high throughput screens used different strategies to estimate CLS; alternatively, outgrowth measured by re-inoculation in liquid media and measurement of growth curves by OD, or plating of pooled YGDS cultures (aged together in one stationary phase culture) onto agar followed by bulk harvest of colonies, genomic extraction of DNA and fluorometric labeling and microarray hybridization. Each methodology can provide accurate estimates, however contributions to total cell proliferation other than the number of colony forming cells potentially exist, such as doubling time (exponential growth rate) or carrying capacity (final density in nutrient-exhausted media), which in principle should not impact CLS by the gold standard CFU-estimating assays. Additionally, cell-extrinsic factors are secreted differentially by YGDS, which could affect CLS (Matecic and others 2010; Wierman and others 2015). The production of such factors and the dependence on genetic context of their CLS influence (i.e., interaction between gene deletion, CLS-factor production, and CLS-factor effect) could be detected differently by CLS of pooled vs. individually cultured YGDS. Finally, the aeration status influences CLS as evidenced by the different durations of analysis between the non-pooled (lower aeration of cultures in individual wells of 96 micro-well plates) approach and the pooled/barcode strategy (higher aeration in flask cultures). Delineation of these different contributors to the CLS phenotype could also provide greater ability to reconcile discordant results between different genome-wide screens for CLS (Fig. 5).

A controlled experiment suggests gene $\times$ media interaction markedly affects CLS

To clarify the potential for media and genetic factors to influence CLS, we investigated the different experimental conditions employed by the three previous genome-wide CLS screens using a single platform for measuring CLS (Louie and others 2012; Rodgers and others 2014; Shah and others 2007). Quantitative high-throughput cell array phenotyping (Q-HTCP) was used to measure CLS in six different media conditions and three strains with

differing ploidy and auxotrophic requirements. Replicate cultures (n=192 for BY4741 and n=122 for BY4743) were assayed in the two different YGDS parental backgrounds (Brachmann and others 1998) used for the prior genome-wide CLS screens. Additionally, we analyzed BY4742 (n=96), the haploid strain that yields BY4743 if mated to BY4741. Many replicates were used in order to assess the variance in CLS under the different defined conditions. The media analyzed included the three used in prior genome-wide screens (Table 2). Additionally, low-aeration was assessed for the Powers et al media, since that screen was carried out with reduced aeration. In our experiment, 384 well plates were inverted to achieve aeration by cells settling at the air-liquid interface (surface tension holds the media in place); the non-aerated condition being obtained by housing plates in the standard upright position. A recently described ‘human-like’ (HL) media (Supplemental Table 7) was tested with and without 0.1M MES (2-(N-morpholino)ethanesulfonic acid) to buffer extracellular pH, which has been shown to extend CLS (Burtner and others 2009b; Burtner and others 2011; Fabrizio and Wei 2011; Mirisola and Longo 2012). CLS was estimated approximately once per week, assaying the aging, 384-culture arrays by Q-HTCP, consisting of time series imaging of agar cell arrays, image analysis, growth curve fitting and use of resulting parameters to estimate CLS (Figure 5 and Supplemental Figure 2). Some cultures appeared to lose viability completely (no growth curve detected). Occasional cultures appeared to regain viable cells, a phenomenon previously described as ‘gaspings’ (Fabrizio and others 2004). Gaspings occurred at different ages and to different extents across the strains and media conditions (Figure 5). Several observations from this experiment indicate that genetic factors that distinguish the BY reference strain backgrounds (BY4741, BY4742, or BY4743), media factors and interactions between them can exert influence on CLS. CLS differences among strains could result from different mating type, ploidy or auxotrophic requirements (Table 1), while the possible explanations for media effects on CLS are more numerous (Table 2). Examples of the genotype- and media-specific differences in CLS observed include (Figure 5): (1) In the Powers et al media, aeration shortens lifespan to greater extent for BY4742, than BY4741 or BY4743; (2) In the Powers et al media, we found CLS to be longer for BY4743 than either haploid strain, and observed BY4741 to have longer CLS than BY4742 between days 10 and 24; (3) In the HL media buffered with 0.1M MES, as expected, all strains exhibit increased CLS. The effect of media buffering on increasing CLS was particularly notable for the BY4742 strain between days 31 and 46; (4) The pattern of CLS for the three strains aged in buffered HL media most resembled that of the Longo media, having BY4742 with the longest CLS, followed by BY4743, and with BY4741 having the shortest CLS. This is likely due to the Fabrizio et al media also employing pH buffering (see Table 2); however, we note our assay omitted a step in the Longo CLS protocol of washing the cells with water after 3 days (growth saturation) to perform the CLS assessment in water. (5) In the Matecic et al media, CLS was much shorter for BY4743. (6) In the Powers et al media, reducing the aeration increased CLS to different extents for the three parental strains, but the relative CLS (BY4743 >> BY4742 >BY4741) was similar.

In summary, these results support the hypothesis that interactions between genetic factors such as auxotrophy, mating type and ploidy in addition to media markedly influence CLS and thus could potentially explain some discrepancies between the results of the different

genome-wide CLS screens. Toward the goal of extrapolating observations about yeast chronological aging to human cells, and given the potential influence of media on CLS, it is conceivable that the use of yeast media that more closely resembles human tissue culture media could help to reduce gene $\times$ media interaction as a confounding factor thwarting the validation of yeast CLS findings for human cells (Hartman and others 2015).

Other possible influences on CLS that contribute to lack of consensus

Though beyond the intended scope of this review, other contributors to variability in YGDS phenotypes exist and probably affect CLS. Replacing a genomic sequence with a constitutively expressed, selectable marker can affect expression of its neighboring genes such that the resulting phenotype of the deletion strain is incorrectly attributed to the gene deletion (Giaever and Nislow 2014; Winzeler and others 1999). Mechanisms of neighboring gene effects (NGEs) include alteration of local chromatin structure and transcription factor binding. The importance of NGEs for interpretation of YGDS phenotypes has been described (Ben-Shitrit and others 2012), and should also be considered in the CLS phenotype.

Another phenomenon that potentially clouds the interpretation of CLS is gasping, which is the regrowth of viable cells after a stationary phase culture begins to lose viability (Fabrizio and others 2004). Gasping appears to be stochastic, which can make it difficult to detect, and depending on its timing with respect to the CLS assay points, gasping can contribute to differences between studies by masquerading as extended CLS. It is also possible that genetic or nutrient perturbations could influence gasping (Figure 5).

As mentioned above, gene deletion strain cultures can accumulate secondary mutations due to drift or to selection for suppression of growth defects resulting from the original deletion. Secondary mutations, like neighboring gene effects of gene deletion, can confound YGDS phenotypes leading to either false positive (phenotype independent of the gene deletion) or false negative (secondary mutation masks the phenotype of gene deletion) results (Hughes and others 2000). Assuming that secondary mutations are not fixed in the population, replicating phenotypic analysis on sub-clones of YGDS cultures can be used to assess the likelihood of stochastic or indirect/secondary effects. The confounding effects of secondary mutations can be reduced by minimizing passaging of the library and assessed by inclusion of replicate cultures and defining phenotypic variance in the parental strain (Figure 5).

In summary, neighboring gene effects, gasping, and secondary mutations are examples of phenomena that can cloud the genome-wide study of CLS in the YGDS. These confounders compound the complexity of gene-media interaction. Though challenging, systematically investigating gene $\times$ nutrient interaction in CLS requires high throughput phenotyping to achieve adequate replication for characterization of variance. Addressing these issues should provide clarity regarding the core set of genes directly responsible for aging phenotypes of interest. Advancing systems level modeling of yeast aging will improve our ability to more accurately model more complex aging processes in multi-cellular organisms, including extending human health and longevity.

Conclusion/Summary

It appears that multiple genetic and environmental interactions influence CLS in yeast, and thus there is a need for consistent, standardized, and well-documented procedures when performing CLS assays. We expect genetic and environmental interaction is also important for understanding CLS in multi-cellular organisms such as worms, flies, mice, monkeys. Factors affecting CLS have also been shown to influence RLS (Delaney and others 2013; Mirisola and Longo 2012; Murakami and others 2012; Polymenis and Kennedy 2012), perhaps broadening the relevance of gene-nutrient interaction to the RLS model of aging. Moreover, genetic $\times$ environmental interactions are likely to differentially affect cellular aging in different cell types, harnessing the power of simple model systems like yeast to derive applicable principles of gene-nutrient interaction and aging should generate hypotheses useful for investigating the cellular complexities of human aging.

Methods

Comparisons of relative CLS from three genome-wide screens

Data were obtained from the supplemental files provided with the three publications reporting genome-wide analysis of CLS in the yeast gene deletion strain collection (Fabrizio and others 2010; Matecic and others 2010; Powers and others 2006). The time points used from comparison (see Table 1) were 21 days (Matecic et al), 20 days (Fabrizio et al), and 7 weeks (Powers et al). Pearson Product-Moment Correlations were calculated using SAS Software (v.9.3). Screen hit analyses were performed using YeastMine, provided through a collaboration of the Saccharomyces Genome Database and the InterMine project (Cherry and others 2012). Lists of overlapping genes were obtained for the indicated comparisons (Tables 3, 4, Supplemental Tables 2–5) by YeastMine Intersection analysis of the corresponding gene lists (Supplemental Table 1). The Widget for Gene Ontology Enrichment analysis was used to assess significant enrichment (using the Holm-Bonferonni Test Correction, and $p=0.05$ significance cut off) for biological process, cellular component or molecular function, setting the background population for comparison to the total overlapping ORF set for all genes shared among the three CLS screens (Supplemental Table 1, Matecic upstream tag only).

Quantitative high throughput cell array phenotyping (Q-HTCP)

Q-HTCP was performed as previously described (Hartman IV and Tippery 2004; Rodgers and others 2014; Shah and others 2007). Briefly, a 384-well array containing the 3 reference strains was grown in YPD for 2 days and used to inoculate the six different media (see Fig. 5, Table 2, and Supplemental Table 7). The aging arrays were covered with adhesive gas permeable membranes and placed with well opening side down at 30 °C in a humidified incubator. At the indicated times, the aging arrays were printed to fresh HL agar media (Supplemental Table 7, and Supplemental Figure 2). At each time point, a custom robotic system was used to collect cell array images every two hours (Supplemental Figure 2), and custom image analysis software used to quantify the images, fit each numerical time series to a logistic growth function, and calculate the area under the growth curve (Figure 5) at 96 hrs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by grants to JLH (Research Scholar Grant 10-066-01-TBE American Cancer Society, Physician-Scientist Early Career Award 57005927 Howard Hughes Medical Institute, and NIH grant R01 AG043076) and by T32 DK062710 and P30 DK056336. The opinions expressed herein are those of the authors and not necessarily those of the NIH or any other organization with which the authors are affiliated.

References

- Ben-Shitrit T, Yosef N, Shemesh K, Sharan R, Ruppin E, Kupiec M. Systematic identification of gene annotation errors in the widely used yeast mutation collections. *Nat Methods*. 2012; 9:373–378. [PubMed: 22306811]
- Brachmann CB, Davies A, Cost GJ, Caputo E, Li J, Hieter P, Boeke JD. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast*. 1998; 14:115–132. [PubMed: 9483801]
- Burtner CR, Murakami CJ, Kaeberlein M. A genomic approach to yeast chronological aging. *Methods Mol Biol*. 2009a; 548:101–114. [PubMed: 19521821]
- Burtner CR, Murakami CJ, Kennedy BK, Kaeberlein M. A molecular mechanism of chronological aging in yeast. *Cell Cycle*. 2009b; 8:1256–1270. [PubMed: 19305133]
- Burtner CR, Murakami CJ, Olsen B, Kennedy BK, Kaeberlein M. A genomic analysis of chronological longevity factors in budding yeast. *Cell Cycle*. 2011; 10:1385–1396. [PubMed: 21447998]
- Cherry JM, Hong EL, Amundsen C, Balakrishnan R, Binkley G, Chan ET, Christie KR, Costanzo MC, Dwight SS, Engel SR, Fisk DG, Hirschman JE, Hitz BC, Karra K, Krieger CJ, Miyasato SR, Nash RS, Park J, Skrzypek MS, Simison M, Weng S, Wong ED. *Saccharomyces* Genome Database: the genomics resource of budding yeast. *Nucleic Acids Res*. 2012; 40:D700–705. [PubMed: 22110037]
- Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, Ding H, Koh JL, Toufighi K, Mostafavi S, Prinz J, St Onge RP, VanderSluis B, Makhnevych T, Vizeacoumar FJ, Alizadeh S, Bahr S, Brost RL, Chen Y, Cokol M, Deshpande R, Li Z, Lin ZY, Liang W, Marback M, Paw J, San Luis BJ, Shuteriqi E, Tong AH, van Dyk N, Wallace IM, Whitney JA, Weirauch MT, Zhong G, Zhu H, Houry WA, Brudno M, Ragibizadeh S, Papp B, Pal C, Roth FP, Giaever G, Nislow C, Troyanskaya OG, Bussey H, Bader GD, Gingras AC, Morris QD, Kim PM, Kaiser CA, Myers CL, Andrews BJ, Boone C. The genetic landscape of a cell. *Science*. 2010; 327:425–431. [PubMed: 20093466]
- Curran SP, Ruvkun G. Lifespan regulation by evolutionarily conserved genes essential for viability. *PLoS Genet*. 2007; 3:e56. [PubMed: 17411345]
- Delaney JR, Murakami C, Chou A, Carr D, Schleit J, Sutphin GL, An EH, Castanza AS, Fletcher M, Goswami S, Higgins S, Holmberg M, Hui J, Jelic M, Jeong KS, Kim JR, Klum S, Liao E, Lin MS, Lo W, Miller H, Moller R, Peng ZJ, Pollard T, Pradeep P, Pruett D, Rai D, Ros V, Schuster A, Singh M, Spector BL, Wende HV, Wang AM, Wasko BM, Olsen B, Kaeberlein M. Dietary restriction and mitochondrial function link replicative and chronological aging in *Saccharomyces cerevisiae*. *Exp Gerontol*. 2013; 48:1006–1013. [PubMed: 23235143]
- Fabrizio P, Battistella L, Vardavas R, Gattazzo C, Liou LL, Diaspro A, Dossen JW, Gralla EB, Longo VD. Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*. *J Cell Biol*. 2004; 166:1055–1067. [PubMed: 15452146]
- Fabrizio P, Hoon S, Shamalnasab M, Galbani A, Wei M, Giaever G, Nislow C, Longo VD. Genome-wide screen in *Saccharomyces cerevisiae* identifies vacuolar protein sorting, autophagy, biosynthetic, and tRNA methylation genes involved in life span regulation. *PLoS Genet*. 2010; 6:e1001024. [PubMed: 20657825]
- Fabrizio P, Pozza F, Pletcher SD, Gendron CM, Longo VD. Regulation of longevity and stress resistance by Sch9 in yeast. *Science*. 2001; 292:288–290. [PubMed: 11292860]

- Fabrizio P, Wei M. Conserved role of medium acidification in chronological senescence of yeast and mammalian cells. *Aging* (Albany NY). 2011; 3:1127–1129. [PubMed: 22184281]
- Ghirardi O, Cozzolino R, Guaraldi D, Giuliani A. Within- and between-strain variability in longevity of inbred and outbred rats under the same environmental conditions. *Exp Gerontol*. 1995; 30:485–494. [PubMed: 8557096]
- Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, Arkin AP, Astromoff A, El-Bakkoury M, Bangham R, Benito R, Brachat S, Campanaro S, Curtiss M, Davis K, Deutschbauer A, Entian KD, Flaherty P, Foury F, Garfinkel DJ, Gerstein M, Gotte D, Guldener U, Hegemann JH, Hempel S, Herman Z, Jaramillo DF, Kelly DE, Kelly SL, Kotter P, LaBonte D, Lamb DC, Lan N, Liang H, Liao H, Liu L, Luo C, Lussier M, Mao R, Menard P, Ooi SL, Revuelta JL, Roberts CJ, Rose M, Ross-Macdonald P, Scherens B, Schimmack G, Shafer B, Shoemaker DD, Sookhai-Mahadeo S, Storms RK, Strathern JN, Valle G, Voet M, Volckaert G, Wang CY, Ward TR, Wilhelmy J, Winzeler EA, Yang Y, Yen G, Youngman E, Yu K, Bussey H, Boeke JD, Snyder M, Philippsen P, Davis RW, Johnston M. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature*. 2002; 418:387–391. [PubMed: 12140549]
- Giaever G, Nislow C. The Yeast Deletion Collection: A Decade of Functional Genomics. *Genetics*. 2014; 197:451–465. [PubMed: 24939991]
- Hamilton B, Dong Y, Shindo M, Liu W, Odell I, Ruvkun G, Lee SS. A systematic RNAi screen for longevity genes in *C. elegans*. *Genes Dev*. 2005; 19:1544–1555. [PubMed: 15998808]
- Hansen M, Hsu AL, Dillin A, Kenyon C. New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a *Caenorhabditis elegans* genomic RNAi screen. *PLoS Genet*. 2005; 1:119–128. [PubMed: 16103914]
- Harrison DE, Strong R, Allison DB, Ames BN, Astle CM, Atamna H, Fernandez E, Flurkey K, Javors MA, Nadon NL, Nelson JF, Pletcher S, Simpkins JW, Smith D, Wilkinson JE, Miller RA. Acarbose, 17-alpha-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males. *Aging Cell*. 2014; 13:273–282. [PubMed: 24245565]
- Hartman JL IV. Buffering of deoxyribonucleotide pool homeostasis by threonine metabolism. *Proc Natl Acad Sci U S A*. 2007; 104:11700–11705. [PubMed: 17606896]
- Hartman JL IV, Tippery NP. Systematic quantification of gene interactions by phenotypic array analysis. *Genome Biol*. 2004; 5:R49. [PubMed: 15239834]
- Hartman JL IV, Stisher C, Outlaw DA, Guo J, Shah NA, Tian D, Santos SM, Rodgers JW, White RA. Yeast Phenomics: An Experimental Approach for Modeling Gene Interaction Networks that Buffer Disease. *Genes*. 2015; 6:24–45. [PubMed: 25668739]
- Hughes TR, Roberts CJ, Dai H, Jones AR, Meyer MR, Slade D, Burchard J, Dow S, Ward TR, Kidd MJ, Friend SH, Marton MJ. Widespread aneuploidy revealed by DNA microarray expression profiling. *Nat Genet*. 2000; 25:333–337. [PubMed: 10888885]
- Irizarry RA, Ooi SL, Wu Z, Boeke JD. Use of mixture models in a microarray-based screening procedure for detecting differentially represented yeast mutants. *Stat Appl Genet Mol Biol*. 2003; 2 Article1.
- Jiang JC, Jaruga E, Repnevskaya MV, Jazwinski SM. An intervention resembling caloric restriction prolongs life span and retards aging in yeast. *FASEB J*. 2000; 14:2135–2137. [PubMed: 11024000]
- Johnson JE, Johnson FB. Methionine restriction activates the retrograde response and confers both stress tolerance and lifespan extension to yeast, mouse and human cells. *PLoS One*. 2014; 9:e97729. [PubMed: 24830393]
- Kaeberlein M, Powers RW 3rd, Steffen KK, Westman EA, Hu D, Dang N, Kerr EO, Kirkland KT, Fields S, Kennedy BK. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science*. 2005; 310:1193–1196. [PubMed: 16293764]
- Kennedy BK. The genetics of ageing: insight from genome-wide approaches in invertebrate model organisms. *J Intern Med*. 2008; 263:142–152. [PubMed: 18226092]
- Kennedy BK, Smith ED, Kaeberlein M. The enigmatic role of Sir2 in aging. *Cell*. 2005; 123:548–550. [PubMed: 16286003]
- Longo VD, Shadel GS, Kaeberlein M, Kennedy B. Replicative and chronological aging in *Saccharomyces cerevisiae*. *Cell Metab*. 2012; 16:18–31. [PubMed: 22768836]

- Louie RJ, Guo J, Rodgers JW, White R, Shah N, Pagant S, Kim P, Livstone M, Dolinski K, McKinney BA, Hong J, Sorscher EJ, Bryan J, Miller EA, Hartman JLA IV. yeast phenomic model for the gene interaction network modulating CFTR- F508 protein biogenesis. *Genome Med.* 4(103): 2012.
- Mani R, St Onge RP, Hartman JL IV, Giaever G, Roth FP. Defining genetic interaction. *Proc Natl Acad Sci U S A.* 2008; 105:3461–3466. [PubMed: 18305163]
- Matecic M, Smith DL, Pan X, Maqani N, Bekiranov S, Boeke JD, Smith JS. A microarray-based genetic screen for yeast chronological aging factors. *PLoS Genet.* 2010; 6:e1000921. [PubMed: 20421943]
- McCormick MA, Delaney JR, Tsuchiya M, Tsuchiyama S, Shemorry A, Sim S, Chou AC, Ahmed U, Carr D, Murakami CJ, Schleit J, Sutphin GL, Wasko BM, Bennett CF, Wang AM, Olsen B, Beyer RP, Bammler TK, Prunkard D, Johnson SC, Pennypacker JK, An E, Anies A, Castanza AS, Choi E, Dang N, Enerio S, Fletcher M, Fox L, Goswami S, Higgins SA, Holmberg MA, Hu D, Hui J, Jelic M, Jeong KS, Johnston E, Kerr EO, Kim J, Kim D, Kirkland K, Klum S, Kotireddy S, Liao E, Lim M, Lin MS, Lo WC, Lockshon D, Miller HA, Moller RM, Muller B, Oakes J, Pak DN, Peng ZJ, Pham KM, Pollard TG, Pradeep P, Pruett D, Rai D, Robison B, Rodriguez AA, Ros B, Sage M, Singh MK, Smith ED, Snead K, Solanky A, Spector BL, Steffen KK, Tchao BN, Ting MK, Vander Wende H, Wang D, Welton KL, Westman EA, Brem RB, Liu XG, Suh Y, Zhou Z, Kaeberlein M, Kennedy BK. A Comprehensive Analysis of Replicative Lifespan in 4,698 Single-Gene Deletion Strains Uncovers Conserved Mechanisms of Aging. *Cell Metab.* 2015; 22:895–906. [PubMed: 26456335]
- Mirisola MG, Longo VD. Acetic acid and acidification accelerate chronological and replicative aging in yeast. *Cell Cycle.* 2012; 11:3532–3533. [PubMed: 22951542]
- Murakami C, Delaney JR, Chou A, Carr D, Schleit J, Sutphin GL, An EH, Castanza AS, Fletcher M, Goswami S, Higgins S, Holmberg M, Hui J, Jelic M, Jeong KS, Kim JR, Klum S, Liao E, Lin MS, Lo W, Miller H, Moller R, Peng ZJ, Pollard T, Pradeep P, Pruett D, Rai D, Ros V, Schuster A, Singh M, Spector BL, Vander Wende H, Wang AM, Wasko BM, Olsen B, Kaeberlein M. pH neutralization protects against reduction in replicative lifespan following chronological aging in yeast. *Cell Cycle.* 2012; 11:3087–3096. [PubMed: 22871733]
- Murakami C, Kaeberlein M. Quantifying yeast chronological life span by outgrowth of aged cells. *J Vis Exp.* 2009
- Murakami CJ, Burtner CR, Kennedy BK, Kaeberlein M. A method for high-throughput quantitative analysis of yeast chronological life span. *J Gerontol A Biol Sci Med Sci.* 2008; 63:113–121. [PubMed: 18314444]
- Murakami CJ, Wall V, Basisty N, Kaeberlein M. Composition and acidification of the culture medium influences chronological aging similarly in vineyard and laboratory yeast. *PLoS One.* 2011; 6:e24530. [PubMed: 21949725]
- Ocampo A, Liu J, Schroeder EA, Shadel GS, Barrientos A. Mitochondrial respiratory thresholds regulate yeast chronological life span and its extension by caloric restriction. *Cell metabolism.* 2012; 16:55–67. [PubMed: 22768839]
- Ooi SL, Shoemaker DD, Boeke JD. DNA helicase gene interaction network defined using synthetic lethality analyzed by microarray. *Nat Genet.* 2003; 35:277–286. [PubMed: 14566339]
- Orentreich N, Matias JR, DeFelice A, Zimmerman JA. Low methionine ingestion by rats extends life span. *J Nutr.* 1993; 123:269–274. [PubMed: 8429371]
- Pan Y, Schroeder EA, Ocampo A, Barrientos A, Shadel GS. Regulation of yeast chronological life span by TORC1 via adaptive mitochondrial ROS signaling. *Cell metabolism.* 2011; 13:668–678. [PubMed: 21641548]
- Polymenis M, Kennedy BK. Chronological and replicative lifespan in yeast: do they meet in the middle? *Cell Cycle.* 2012; 11:3531–3532. [PubMed: 22951539]
- Powers RW 3rd, Kaeberlein M, Caldwell SD, Kennedy BK, Fields S. Extension of chronological life span in yeast by decreased TOR pathway signaling. *Genes Dev.* 2006; 20:174–184. [PubMed: 16418483]
- Rodgers J, Guo J, Hartman JL IV. Phenomic assessment of genetic buffering by kinetic analysis of cell arrays. *Methods Mol Biol.* 2014; 1205:187–208. [PubMed: 25213246]

- Shah NA, Laws RJ, Wardman B, Zhao LP, Hartman JL IV. Accurate, precise modeling of cell proliferation kinetics from time-lapse imaging and automated image analysis of agar yeast culture arrays. *BMC Syst Biol.* 1(3):2007.
- Smith ED, Kennedy BK, Kaerberlein M. Genome-wide identification of conserved longevity genes in yeast and worms. *Mech Ageing Dev.* 2007; 128:106–111. [PubMed: 17126379]
- Smith ED, Tsuchiya M, Fox LA, Dang N, Hu D, Kerr EO, Johnston ED, Tchao BN, Pak DN, Welton KL, Promislow DE, Thomas JH, Kaerberlein M, Kennedy BK. Quantitative evidence for conserved longevity pathways between divergent eukaryotic species. *Genome Res.* 2008; 18:564–570. [PubMed: 18340043]
- Strong R, Miller RA, Astle CM, Floyd RA, Flurkey K, Hensley KL, Javors MA, Leeuwenburgh C, Nelson JF, Ongini E, Nadon NL, Warner HR, Harrison DE. NORDIHYDROGUAIARETIC ACID AND ASPIRIN INCREASE LIFESPAN OF GENETICALLY HETEROGENEOUS MALE MICE. *Aging Cell.* 2008; 7:641–650. [PubMed: 18631321]
- Troen AM, French EE, Roberts JF, Selhub J, Ordovas JM, Parnell LD, Lai CQ. Lifespan modification by glucose and methionine in *Drosophila melanogaster* fed a chemically defined diet. *Age (Dordr.)* 2007; 29:29–39. [PubMed: 19424828]
- Wei M, Madia F, Longo VD. Studying age-dependent genomic instability using the *S. cerevisiae* chronological lifespan model. *J Vis Exp.* 2011
- Wickerham LJ. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. *J Bacteriol.* 1946; 52:293–301.
- Wierman MB, Matecic M, Valsakumar V, Li M, Smith DL Jr, Bekiranov S, Smith JS. Functional genomic analysis reveals overlapping and distinct features of chronologically long-lived yeast populations. *Aging (Albany NY).* 2015; 7:177–194. [PubMed: 25769345]
- Winzler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H, Chu AM, Connelly C, Davis K, Dietrich F, Dow SW, El Bakkoury M, Foury F, Friend SH, Gentalen E, Giaever G, Hegemann JH, Jones T, Laub M, Liao H, Davis RW, et al. Functional characterization of the *S cerevisiae* genome by gene deletion and parallel analysis. *Science.* 1999; 285:901–906. [PubMed: 10436161]
- Yuan DS, Pan X, Ooi SL, Peyser BD, Spencer FA, Irizarry RA, Boeke JD. Improved microarray methods for profiling the Yeast Knockout strain collection. *Nucleic Acids Res.* 2005; 33:e103. [PubMed: 15994458]

Highlights

- *S. cerevisiae* provides a unique platform to assess genes and gene interactions that influence cellular aging.
- A remarkable lack of consensus exists between screens of the yeast gene deletion strain collection for increased CLS.
- Deletions in mitochondrial or autophagy-related genes consistently shorten CLS.
- CLS appears to be markedly influenced by media, which could account for lack of concordance between screens.
- Auxotrophy, ploidy, and media effects on CLS suggest gene $\times$ nutrient interaction could account for discordance between prior genome-wide screens.

Figure 1. Pictorial view of traditional and high-throughput methods of measuring and estimating yeast chronological lifespan (CLS)

(A) Yeast CLS is defined by the rate of temporal decline in the percentage of cells that can form colonies when plated to fresh media. The gold standard method to quantify CLS is to grow cultures to stationary phase (~3 days) in glass tubes, aerated on a roller. Periodically, an equal volume of the culture is removed, diluted, and plated onto fresh agar media, and colonies are counted after period of growth (3–4 days). (B) The Powers *et al* genome screen for CLS cultured the entire yeast gene deletion strain collection in 96 well plates and periodically removed a small volume of the aging culture for inoculation of fresh liquid media. CLS estimation of the relative abundance of colony forming cells vs time was based on regrowth of the culture, as assayed by OD readings of the inoculated microwell plates. (C) The Matecic *et al* and Fabrizio *et al* screens estimated differences between the yeast gene deletion strains by growing them together in a pooled liquid culture. Periodically, pooled cultures was sampled and plated to fresh agar and allowed to grow into a lawn. The genomic DNA was harvested from the freshly grown lawns of cells and the classic method of two-color competitive hybridization to oligonucleotide microarrays was used to estimate relative changes in the relative viability of each yeast gene deletion strain, possible because each gene deletion is flanked by unique 20 nucleotide sequences designed to specifically anneal to the oligonucleotide micro-arrays.

Figure 2. Correlation of CLS phenotypes between previous genome wide screens of the yeast gene deletion strains

(A) The Matecic et al study estimated CLS values by relative abundance of the upstream (UP) or downstream (DOWN) 20-nucleotide sequences flanking each gene deletion in the yeast gene deletion collection, which were detected by pcr amplification for 2-color competitive hybridization to custom-designed oligonucleotide microarrays. Each genomic DNA prep, obtained from outgrowth of aging cultures, was assayed for both sets of tags, and the correlation between the CLS estimates for the UP and DOWN tags (at 21 days) is shown. (B) The relative CLS estimates from the upstream tags of *Matecic et al* were plotted against the CLS estimates for the same strains from the *Powers et al* publication. Negative values reported by *Powers et al* (presumed to be short-lived strains) were removed since they could not be plotted on a logarithmic scale. (C) Similar to panel B, the relative CLS estimates from the upstream tags in *Matecic et al* were plotted with all corresponding estimates published by *Fabrizio et al*. (D) Similar to panels B and C, the relative CLS estimates for all yeast gene deletion strains reported by both *Fabrizio et al* and *Powers et al* are plotted together.

Figure 3. Correlation of relative CLS estimates between different genome-wide screens of the yeast gene deletion strain collection

The genes that were reported in common by the *Matecic et al* (upstream and downstream tags), *Powers et al*, and *Fabrizio et al* publications were separately ranked according to the relative CLS reported by each screen. The corresponding rankings were divided into deciles and each decile was assessed for overlap as a percentage of the total number of genes assigned to the decile. The shortest-lived strains are assigned to decile 1 and strains with the greatest CLS are assigned decile 10. (A) The decile overlaps in *Matecic et al* relative CLS values from comparing the upstream and downstream tags. (B) The decile overlaps comparing *Matecic et al* with *Powers et al*. (C) The decile overlaps comparing *Matecic et al* with *Fabrizio et al*. (D) The decile overlaps comparing *Powers et al* with *Fabrizio et al*. In panels A–D, the heat maps are colored to reflect the relative amount of overlap between comparisons of the indicated deciles, with dark blue indicating high overlap, white indicating lack of overlap, and medium intensity blue indicating lack of a trend (random).

amounts of overlap). The heat maps are represented in the form of histograms in Supplemental Figure 1.

Figure 4. Overlap in rank-ordered relative CLS between the three genome-wide screens
 The 3209 genes reported by all three screens were rank-ordered, and the overlaps in the three shortest-lived and longest-lived deciles are shown. The gene lists used to perform the analysis are provided in Supplemental Table 1, and for the resulting gene lists are in Tables 3 and 4 and Supplemental Tables 2–5.

Figure 5. CLS is affected by interactions between genetic and nutrient factors

Each row contains data for a different media condition (indicated at far left). In the left-most column, box plots represent the area under the growth curve (AUGC) vs. chronological age after reaching stationary phase (0, 3, 10, 17, 24, 31, 46, 60, 79 days) for replicate cultures of BY4741 (red), BY4742 (green), and BY4743 (blue). The plots of AUGC vs time are shown for all individual cultures in the second (BY4741), third (BY4742), and fourth (BY4743) columns. Thus, each box plot summarizes the three graphs shown to its right, revealing how the strain background and media can interact to affect CLS. Each colored box represents the middle 75% of data at each indicated time point for each strain (BY4741 is red, BY4742 is green, BY4743 is blue). The black bars are the average value, and the whiskers represent the range. For representative images, see Supplemental Figure 2. The number of cultures that do not generate a growth curve at a particular time point are indicated at the top of each box plot (total number of replicate cultures: BY4741 = 192; BY4742 = 96; BY4743 = 122).

Overview/comparison of published genome-wide chronological lifespan screens in the YGDS

Table 1

Author	Strain	Conditions	YGDS Reported	Viability Assessment	CLS measures*
Powers et al (2006)	BY4743 ¹	96 well plates, 150ul SC media, monocultures, non-aerated, 30°C	4,759	Monocultures sampled for outgrowth by OD ₆₀₀ , 24 h at 25°C (in triplicate)	wks 1, 2, 5 and 7 (7 weeks)
Mateic et al (2010)	BY4741 ²	10 ml glass test tube cultures (20 tubes 2% 20 tubes 0.5% glucose), pooled YGDS collection, aerated, 30°C	DOWN Tags 2,698 (n=2538 unique); UP tags 3,478 (n=3209 unique)	Pooled samples for outgrowth, DNA extraction and PCR Barcode tags, competitive hybridization	Every 3 days (21 days)
Fabrizio et al (2010)	BY4741 ²	Volume not reported, pooled YGDS collection, aerated, 30°C	4,130	Pooled samples for outgrowth, DNA extraction and PCR Barcode tags, competitive hybridization	Every 4–7 days (20 days)

¹ BY4743 – homozygous deletion diploids (*MataM Ta his3 1 leu2 0/leu2 0 ura3 0 met17 0MET17 lys2 0LYS2*).

² BY4741 – haploid deletions (*MATa his3 1 leu2 0 met17 0 ura3 0*)

* bolding indicates time points used for analyses performed in this manuscript

Table 2

Different media Compositions used for three genome-wide screens.

<u>Ingredient</u>	Matecic^a [mg/L]	Powers^b [mg/L]	Fabrizio^c [mg/L]
Adenine	29.6	40	80
L-Alanine	105	0	0
L-Arginine	105	20	40
L-Asparagine	105	0	0
L-Aspartic Acid	105	100	100
L-Cysteine	105	0	0
L-Glutamine	105	0	0
L-Glutamic Acid	105	100	100
L-Glycine	105	0	0
L-Histidine	62.7	20*	80
Inositol	105	0	0
L-Isoleucine	105	0	60
L-Leucine	217.5	60*	120
L-Lysine	105	30	60
L-Methionine	105	20	80
para-Aminobenzoic Acid	105	0	0
L-Phenylalanine	105	50	60
L-Proline	105	0	0
L-Serine	105	375	400
L-Threonine	105	200	200
L-Tryptophan	160	40	80
L-Tyrosine	105	30	40
Uracil	22.4	20*	80
L-Valine	105	150	150
	<u>[g/L]</u>	<u>[g/L]</u>	<u>[g/L]</u>
Wickerham yeast nitrogen base	1.7 [§]	1.7 [§]	1.7 [§]
Ammonium Sulfate	5	5	5
NaH ₂ PO ₄	0	0	1.4 [‡]
Glucose	20	20	20

Table 3

Gene deletions that extend lifespan (overlap in longest-lived decile of genes reported by all three studies).

ORF	Gene Name	Name meaning
YDR443C	SSN2	Suppressor of SNfl
YGL133W	ITC1	Imitation switch Two Complex
YGL139W	FLC3	FLavin Carrier
YGL197W	MDS3	Mck1 Dosage Suppressor
YJL134W	LCB3	Long-Chain Base
YKL046C	DCW1	Defective Cell Wall
YLL013C	PUF3	PUmilio-homology domain Family
YLR280C		
YML029W	USA1	U1-Snpl Associating

Table 4

Gene deletions that shorten lifespan (overlap in shortest-lived decile of genes reported by all three studies).

ORF	Gene Name	Name meaning
YAL044C	GCV3	GlyCine cleavage
YAL056W	GPB2	G protein beta
YBR041W	FAT1	FATty acid transporter
YBR128C	ATG14	AuTophagy related
YBR281C	DUG2	Deficient in Utilization of Glutathione
YCR087W		
YDR512C	EMU	Early Meiotic Induction
YEL033W	MTC7	Maintenance of Telomere Capping
YER005W	YND1	Yeast Nucleoside Diphosphatase
YFL033C	RIM 15	Regulator of IME2
YGL045W	RIM8	Regulator of IME2
YHL027W	RIM 101	Regulator of IME2
YHL030W	ECM29	Extracellular Mutant
YHR077C	NMD2	Nonsense-mediated MRNA Decay Ribosomal Small subunit of
YIL093C	RSM25	Mitochondria
YIL125W	KGD1	alpha-KetoGlutarate Dehydrogenase
YLR087C	CSF1	Cold Sensitive for Fermentation
YMR158W-B		
YMR223W	UBP8	UBiquitin-specific processing Protease
YNL297C	MON2	MONensin sensitivity
YNR006W	VPS27	Vacuolar Protein Sorting
YOR002W	ALG6	Asparagine-Linked Glycosylation