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# 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 *Saccharomyces 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. 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 deletions strains were in common in the longest-lived decile (2.80%) and thirteen were in common in the shortest-lived decile (4.05%) of 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-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

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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.



## 1. 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 et al., 2007).

There are two types of aging commonly studied in budding yeast (Longo et al., 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 et al., 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 hematopoetic 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 et al., 2011; Delaney et al., 2013; Mirisola and Longo, 2012; Polymenis and Kennedy, 2012; Smith et al., 2008). Similarly, nutrient signaling pathways and dietary interventions exhibit analogous effects on RLS, CLS, and aging of multicellular organisms (Fabrizio et al., 2001; Jiang et al., 2000; Kaeberlein et al., 2005).

For technical reasons, CLS has been more amenable to high throughput studies, as a genome-wide RLS study was only recently completed (McCormick et al., 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 et al., 2010; Matecic et al., 2010; Powers et al., 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 (Fig. 1).

Two different techniques for high-throughput CLS estimation include: (1) outgrowth in liquid media in a 96-well format (Murakami and Kaeberlein, 2009; Powers et al., 2006) and (2) outgrowth of pooled cultures on agar followed by DNA extraction and relative hybridization to microarray chips (Fabrizio et al., 2010; Matecic et al., 2010). Each gene deletion cassette has been designed with unique flanking oligonucleotides that anneal to complementary oligonucleotides synthesized on hybridization chips (Giaever et al., 2002; Winzeler et al., 1999), which are used to estimate changes in relative abundance of particular deletion strains over time (Irizarry et al., 2003; Ooi et al., 2003; Yuan et al., 2005). There have been two genome-wide CLS screens performed by the pooled library approach (Fabrizio et al., 2010; Matecic et al., 2010) and one by the individual cultures grown in micro-well plates (Powers et al., 2006). The main focus of this article is on the comparison of these threes 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, Figs. 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 *Caenorhabditis elegans*, have also resulted in low concordance, which could also be explained by nutritional differences (Curran and Ruvkun, 2007; Hamilton et al., 2005; Hansen et al., 2005; Kennedy, 2008; Smith et al., 2007). Consistent with this possibility, nutrient interventions alone such as methionine restriction exert influence on aging across multiple species (Johnson and Johnson, 2014; Orentreich et al., 1993; Troen et al., 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 et al., 1995; Harrison et al., 2014; Strong et al., 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-nutrient interaction) in the three genome-wide CLS screens published to date.

## 2. Results and discussion

### 2.1. 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 et al., 2010; Matecic et al., 2010; Powers et al.,

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