Yeast as a model to understand the interaction between genotype and the response to calorie restriction

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Abstract
Calorie restriction is reported to enhance survival and delay the onset of age-related decline in many different species. Several proteins have been proposed to play a role in mediating the response to calorie restriction, including the target of rapamycin kinase, sirtuins, and AMP kinase. An enhanced mechanistic understanding of calorie restriction has popularized the concept of “calorie restriction mimetics”, drugs that mimic the beneficial effects of calorie restriction without requiring a reduction in nutrient intake. In theory, such drugs should delay the onset and progression of multiple age-related diseases, similar to calorie restriction in mammals. Despite the potential benefits of such calorie restriction mimetics, however, relatively little is known about the interaction between genetic variation and individual response to calorie restriction. Limited evidence from model systems indicates that genotype plays a large role in determining both the magnitude and direction of effect that calorie restriction has on longevity. Here we present an overview of these data from the perspective of using yeast as a model to study aging and describe an approach we are taking to further characterize the molecular mechanisms underlying genotype-dependent responses to calorie restriction.

1. Introduction
Aging is a highly complex biological process associated with myriad physiological changes. As an organism matures, many of the initial changes, such as increased strength and improved motor skills, are beneficial for reproductive success. However, later in life, aging related processes tend to be detrimental. In humans, advanced age is associated with increased incidence of many pathological processes such as heart disease, cancer, and neurodegenerative diseases. Therefore enhancing our understanding of the molecular basis of aging may provide insight into the mechanisms of age-related pathologies, and interventions that delay aging may also prevent or delay multiple age-related diseases.

A number of genetic and environmental interventions have been shown to increase life span in laboratory model organisms; the best characterized of these is known as calorie restriction (CR, also referred to as dietary restriction or DR). First reported in 1935, CR is defined as a reduction in caloric intake without malnutrition [1]. CR has since been shown to increase life span in multiple organisms including yeast, flies, nematodes, mice, rats, and monkeys [2–6]. In addition to the life span extending effects of CR, experimental cohorts displayed reductions in age-related disease [6]. In one study of non-human primates, for example, animals subjected to CR maintained a more youthful appearance and showed reduced incidences of cancer, cardiovascular disease, and diabetes compared to age-matched controls fed ad libitum [7]. Given the promising results from numerous species, significant interest has developed in using CR in humans as a means of increasing life span and health span [8].

A key question in the field is whether CR will have similar effects on aging and age-related diseases in humans as has been observed in laboratory animals. Although the answer to this question remains unknown, controlled clinical trials have begun to assess the feasibility and metabolic effects of short-term CR in patients [9]. Studies of individuals who have been self-practicing CR have also been reported, with evidence for similar metabolic and physiological changes as those observed in laboratory animals, including decreased levels of circulating IGF-1 and testosterone, decreased blood pressure, reduced risk of cardiovascular disease and diabetes, and reduced inflammation [10,11].

Genetic and molecular studies in model organisms have led to a large body of literature on the potential mechanisms by which CR slows aging, and several proteins have been implicated in mediating the beneficial health effects of CR in different organisms, including the target of rapamycin (TOR) kinase, the sirtuin family of protein deacetylases and ADP ribosyltransferases, AMP-activated protein

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kinase (AMPK) and FOXO-family transcription factors, among others (see Refs. [12–16] for reviews of this topic). One outcome of advances in understanding the mechanistic underpinnings of CR has been popularization of the idea that pharmacological intervention in pathways mediating CR could lead to similar positive effects on aging and age-related diseases [17,18]. Sirtuin activators, TOR inhibitors, and AMPK activators have all been proposed as CR mimetics – pharmacological agents that mimic the effects of CR on longevity and health without requiring reduced nutrient intake [19–23]. Since it is generally recognized that getting a majority of people to comply with a CR lifestyle is not feasible, the development of bona fide CR mimetics could allow a majority of individuals to receive the health benefits of CR without requiring them to drastically reduce their calorie consumption.

A careful analysis of the literature suggests, however, that in certain situations CR can have a negligible or even detrimental effect on survival [24]. The individual response to CR will, of course, be determined by a combination of environmental and genetic factors. Relative to most laboratory studies, humans represent a genetically and environmentally heterogeneous population. Therefore, before CR mimetics become widely used, it will be important to gain a better understanding of the interaction between nutrient availability, other environmental parameters, and genotype.

2. Evidence for genotype-dependent responses to CR in model organisms

Experiments performed in model organisms have yielded several observations which demonstrate the important role that genotype plays in the response to CR. Studies in invertebrate species aimed at defining the genetic pathways involved in the response to CR, for example, have identified numerous mutations that blunt, abrogate, or enhance life span extension under CR conditions. For example, in the nematode Caenorhabditis elegans, mutation or RNAi knockdown of two different transcription factors, the forkhead transcription factor PHA-4 or the heat shock transcription factor HSF-1, completely prevents life span extension from CR [25,26]. In contrast, RNAi knockdown of the mitochondrial prohibitin complex causes CR to extend life span to a much greater extent than in wild type nematodes [27]. These specific cases, among others, demonstrate that single-gene mutations can have a profound impact on the effect of identical CR regimens on lifespan.

The importance of genetic variation has also been demonstrated in mice, through studies that examined the life spans of ILS/ISS recombinant inbred mouse strains. The ILS/ISS strains were originally derived from crosses between eight inbred mouse strains [28,29]. Progeny from these crosses were then subjected to selective breeding for alcohol sensitivity. The resulting strains, therefore, share a common origin but are genetically distinct. For these reasons the ILS/ISS panel has proven to be a useful tool for studying multigenic traits and complex phenotypes. Liao et al. [30] examined the life spans of 41 ILS/ISS strains fed ad libitum or subjected to a CR diet representing 60% of ad libitum consumption. The ILS/ISS strains showed remarkable variation in life span under both feeding conditions, with differences ranging from an increase in life span exceeding 400% to a decrease of more than 90% in males from two different strains (Fig. 1). Unexpectedly, the majority of ILS/ISS strains did not experience a statistically significant increase in life span on CR. Only 5% of male strains and 21% of female strains had their life span significantly extended by CR [30]. These findings are consistent with other studies that have failed to observe life span extension in wild-derived and some inbred mouse strains subjected to CR [24,31,32].

The mechanisms underlying the differential responses to CR in the ILS/ISS strains are currently not understood. Liao et al. [30] observed no correlations between the response to CR and gender, female fertility, or life span under ad libitum feeding. Subsequent studies in the ILS/ISS strains have since suggested a correlation between the response to CR and the ability of mice to increase or maintain fat mass while on a CR regimen [33]. ILS/ISS strains that responded positively to CR maintained their fat mass better under CR conditions than strains that responded poorly. There is evidence to suggest, however, that fat mass itself is not the primary determinant of CR longevity, since ob/ob mice, which are unable to produce leptin and are genetically predisposed to obesity, have life spans similar to wild-type mice when subjected to CR [34]. Therefore the response to CR in mice is most likely not mediated by the presence or absence of fat, but perhaps is controlled by underlying metabolic processes that influence fat metabolism and storage under conditions of limited nutrient availability.

As is the case for nearly all of the studies of CR in mice, the ILS/ISS strains were derived from inbred lab strains, and it remains unclear whether the allelic diversity in inbred populations can serve as a suitable model for genetic diversity in natural populations or in humans. Thus, it may be that the effect of genotype on the response to CR in the ILS/ISS mice may overestimate or underestimate the true impact of genetic variation on the response to CR in individual people. Nonetheless, these studies clearly demonstrate that genetic variation in mice can strongly influence the effect of CR on longevity in a polygenic and complex manner and illustrate the need for additional studies to define the molecular mechanisms accounting for these differential responses.

3. Modeling aging and calorie restriction in yeast

The budding yeast Saccharomyces cerevisiae provides a useful model for studying aging at the cellular level, due to its relatively short life span and ease of genetic and environmental manipulation [35]. Two types of aging have been described in yeast: replicative and chronological. Replicative aging refers to the aging of mitotically active cells, with replicative life span defined as the number of daughter cells a mother cell can produce before irreversible mitotic arrest [36]. Replicative life span is most commonly measured by following individual mother cells maintained on rich growth medium (YEpd, 1% yeast extract, 2% bacto peptone, 2% glucose) and manually removing and counting daughter cells by micromanipulation [38]. Chronological aging refers to the aging of cells in a quiescence-like state, with chronological life span defined as the length of time that a non-dividing yeast cells can maintain replicative potential [37]. Chronological aging is most often measured by culturing cells in synthetic defined medium with glucose as the carbon source, maintaining the cells in expired culture medium, and periodically determining viability for a subset of the population based on ability to resume mitotic growth under appropriate conditions (e.g. rich culture medium) [37].

CR has been shown to extend both chronological and replicative life span in yeast [35]. In both aging paradigms, CR is generally accomplished by reducing the glucose concentration of the culture medium from 2% to either 0.5% or 0.05%. For simplification, we have chosen to limit our discussion here to the yeast replicative aging paradigm; however, similar considerations apply equally to the yeast chronological aging paradigm.

Although the use of yeast as a model for human aging has been questioned [38], studies in yeast have proven pivotal in the discovery of more than two dozen conserved aging genes [39]. In fact, two of the most widely studied longevity pathways, the target of rapamycin (TOR) pathway and the sirtuin family of protein deacetylases, were first shown to play a role in aging from studies performed in yeast [40,41]. The method of inducing CR in yeast (reducing glucose 4 to 40-fold) is clearly different from the primary method of inducing CR in mammals, which most often involves a 20–40% reduction in overall caloric intake; however, there is
reason to believe that at least some of the same downstream responses to CR are shared. In particular, life span extension in response to CR in yeast is mediated, at least in part, through reduced TOR signaling [42], which also appears to be the case in both nematodes and fruit flies [15,43]. As in yeast, CR in mice results in inhibition of the mammalian TOR (mTOR) [44], and inhibition of mTOR is sufficient to extend life span in mice [45]. Thus, although many aspects of human aging are not shared with yeast and other non-mammalian species, there is no question that yeast has already proven to be an invaluable tool for the discovery and study of conserved aging pathways.

The importance of genotype in the effect of CR on replicative life span in yeast is evidenced by two observations. First, as in other model organisms, specific mutations have been identified that alter the effect of CR on life span. For example, deletion of the sirtuin histone deacetylase Sir2 completely prevents replicative life span extension from CR, while deletion of Sir2 combined with deletion of the replication fork block protein Fob1 enhances life span extension from CR [46–48] (Fig. 2). Second, even among different “wild type” laboratory strains, the same CR regimen can have dramatically different effects on replicative life span (Table 1).

In order to begin to clarify the molecular and cellular processes that influence the effect of genotype on life span extension from CR, we have initiated a large-scale analysis of the effect of CR at 0.05% glucose on life span across more than 150 single-gene deletion mutants. Our approach is based in part on experience gained from our nearly completed analysis for more than 4500 single-gene deletion strains contained in the haploid yeast ORF deletion collection [50]. We are measuring replicative life span for individual single-gene deletion strains within the same experiment under both control (YEPD, 2% glucose) and CR (YEPD, 0.05% glucose) and quantifying the change in life span in response to this level of CR. The design of this study is, in principle, similar to that of Liao et al. [30] who examined the effect of 40% CR on life span in recombinant inbred mouse lines. There are a few important differences, however, which are described next.

3.1. Genetic variation

The study performed by Liao et al. [30] utilized 41 recombinant inbred lines that were generated from eight different inbred mouse strains. Each strain is expected to differ from every other strain at multiple loci, with the genetic variation limited to alleles present in the initial inbred strains. Our study utilizes non-essential single gene deletion strains from the yeast haploid ORF deletion collection [50]. As a consequence, our design is restricted to null alleles; however, since each gene is non-essential, it is likely that many of the deletion mutations are only partially defective for essential cellular processes. For example, deletions that completely prevent glycolysis will be lethal, while deletions that only partially impair glycolysis and yield a viable cell can be assayed. A major advantage of this approach is that we know the identity, and usually the function, of the deleted gene. Thus, it will be possible to assess the response to CR based on functional grouping and GO Terms and, perhaps, identify specific molecular processes or pathways that show a characteristic differential response. This, in turn, should allow for the formulation of specific hypotheses regarding underlying molecular mechanisms.

3.2. Sample size

Liao et al. [30] measured life span for 10 animals per strain (5 male/5 female) fed ad libitum or subjected to CR across 41 strains. Our initial design involves measuring replicative life span for a minimum of 20 cells per genotype under control or CR conditions across more than 150 unique single-gene deletion strains. Life span is then determined for additional cells on a case-by-case basis. A major advantage of the yeast system is the relatively short time frame required to perform longevity assays. This, combined with the initially larger sample size for each genotype and larger number of genotypes examined, should allow us to have confidence in the initial observations and to rapidly validate and test specific hypotheses in follow-up studies.
3.2. Testing multiple levels of restriction

For initial screening purposes, we have chosen to utilize 0.05% glucose as our method of CR. This level was chosen because it has provided the most reproducible replicative life span extension in our hands in prior studies [47,51,52], and is similar to the approach taken by Liao et al. [30] in that only one level of restriction (40%) was examined for the recombinant inbred mouse lines. In select cases, however, we will perform additional experiments examining the full response profile for CR in those genotypes by measuring replicative life span at additional glucose concentrations (e.g. 1%, 0.5%, 0.1%, 0.01% 0.005% glucose), similar to what has been previously done for sirtuins [52]. Such an analysis is essential in order to interpret differential responses to CR, as some genotypes may simply show a shifted nutrient response profile while others may show true differential response to nutrient restriction across all levels of restriction (Fig. 2).

3.4. Epistasis with additional longevity factors

Another powerful feature of yeast as a model system for aging-related research is that there is a large body of literature on pathways that influence replicative and/or chronological life span and their interaction with CR [35,37,53,54]. For example, deletion of SIR2 blocks replicative life span extension at all levels and methods of restriction tested; however, CR extends replicative life span to a greater extent when both SIR2 and FOB1 (which encodes a protein involved in rDNA stability) are deleted, compared to wild type cells [47]. CR can also extend replicative life span in wild type cells in the absence of a functional mitochondrial respiratory chain, at least under certain conditions [55]. Thus, combining select deletions that show a differential response to CR with mutations in SIR2, FOB1, or mitochondrial respiration is likely to be informative. Likewise, mutations that decrease signaling through the TOR/Sch9/PKA pathways, which are thought to mimic CR in the wild type context [42,56,57], may also show differential longevity effects depending on the genetic context.

3.5. Model system

The relevance of yeast as a model for human aging has been debated elsewhere [58–60], but cannot truly be assessed until it is possible to experimentally quantify rates of human aging through reliable biomarkers. Clearly, some aspects of the CR response in mammals, such as the marked reduction in inflammation [61–63], will not be captured in the yeast model. There is reason to believe, however, that analysis of the genetic and molecular mechanisms underlying the response to CR in yeast may yield insight into similar mechanisms in mammals. It has now been quantitatively demonstrated that there is conservation of genetic control of longevity between the yeast replicative aging paradigm and aging in C. elegans [39]. Furthermore, the identification of conserved genetic pathways (e.g., TOR) and environmental manipulations (e.g., CR) that regulate lifespan across an evolutionary diverse set of organisms, coupled with the numerous advantages of yeast as a model organism (ease of genetic manipulation, fast growth, etc.) provides an impetus for continued utilization of yeast as a model in aging research.

Table 1

<table>
<thead>
<tr>
<th>Strain background</th>
<th>0.05% Glucose</th>
<th>0.5% Glucose</th>
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<tbody>
<tr>
<td></td>
<td>Median percent change</td>
<td>Range</td>
</tr>
<tr>
<td>BY4742</td>
<td>15</td>
<td>10 to 48</td>
</tr>
<tr>
<td>PSY316</td>
<td>25</td>
<td>18 to 51</td>
</tr>
<tr>
<td>W303AR5</td>
<td>6</td>
<td>-8 to 23</td>
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4. Conclusion

The impact of genetic variation on the response to CR has been clearly demonstrated in laboratory animals, but the mechanisms underlying these effects remain unknown. It also remains unclear to what degree individual genetic variation will influence the effects of CR in outbred natural populations of common model organisms. In addition, there is only limited data on the health consequences of long-term CR in humans. Thus, it is difficult to predict the degree to which genetic variation will lead to differential responses to CR in people. As the likelihood of widely available CR mimetics becomes a reality, it will become increasingly more important to answer these questions. Yeast provide a powerful model system to begin doing so. By better defining the molecular processes that modulate the relationship between genotype and CR in yeast, we can begin to develop hypotheses that can be tested in more complex organisms and may ultimately provide important information for individuals considering practicing CR or using a putative CR mimetic drug.

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