An intervention resembling caloric restriction prolongs life span and retards aging in yeast[1]

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SPECIFIC AIMS

We addressed the hypothesis that caloric restriction acts at the cellular level to extend longevity and postpone senescence in eukaryotes, and provides one of multiple mechanisms of metabolic control in aging. The effect of progressive reduction in the glucose or amino acids concentration of the growth medium on the life span and aging of individual *Saccharomyces cerevisiae* cells has been examined, and the interaction of this caloric restriction effect with the retrograde response pathway, which signals the functional status of the mitochondrion and determines longevity, has been investigated.

PRINCIPAL FINDINGS

1. Lowering the glucose concentration increases mean and maximum life span of yeast cells in both broth and synthetic medium

Reduction of the glucose concentration in a modified broth routinely used for culturing yeast resulted in an increase in the life span of individual cells, measured by the number of daughters they produced, which was the more extensive the greater the reduction in nutrient levels. Corresponding increases in the mean and maximum life span were observed—up to 75% in some experiments. Similar results have been obtained with three different yeast strains. The effect on life span of reducing glucose levels was duplicated in a standard, chemically defined growth medium. Extension of life span by as much as 81% was found. However, the increased longevity was abrogated when glucose was reduced beyond a certain concentration, indicating that below this point glucose becomes limiting and malnutrition sets in.

2. Reduction of glucose levels postpones the development of a senescent phenotype during the yeast life span

One of the manifestations of aging in yeast is an increase in generation time, which is the interval between consecutive buddings of individual cells. The appearance of this age-related phenotype was delayed when life span was extended by lowering glucose levels in the medium, as measured by the rate of bud production. After a brief lag, there appeared to be a small increase in this rate at the lower glucose concentrations.

3. Extension of life span by adjustment of glucose levels does not depend on the retrograde response

Metabolic control plays a role in determining yeast life span, as evidenced by the increased longevity afforded by the induction of the retrograde response, a form of interorganelle communication that signals the functional status of the mitochondrion to the nucleus resulting in changes in the expression of nuclear genes that encode a variety of mitochondrial, cytoplasmic, and peroxisomal proteins. To ascertain whether the effect on life span of lowering glucose levels is mediated by the retrograde response, the expression of the *CIT2* gene, a diagnostic of the retrograde response, was assessed. Not only were *CIT2* transcript levels not increased under conditions that extend life span, they were reduced by 49%.

The *RTG* genes are required for the retrograde response. Rtg2p promotes the formation of an active heterodimeric Rtg1p-Rtg3p transcription factor by transducing mitochondrial signals that affect the phosphorylation state and subcellular localization of Rtg3p. Deletion of the *RTG2* gene did not suppress the life span extension caused by reduction of glucose levels (**Fig. 1*A***), suggesting the lack of a requirement for the retrograde response for life span extension. In fact, the small decrease in life span at high glucose concentrations that has been often observed

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on various growth media on deletion of this gene was eliminated at low glucose levels (Fig. 2*A*).

It is possible that the prolonged life span resulting from reduced glucose levels by-passed the requirement for Rtg2p but was still dependent on the Rtg1p-Rtg3p transcription factor. To examine this possibility, we deleted *RTG3*. This deletion did not prevent the extension of life span upon reduction of glucose concentration in the growth medium (Fig. 2*B*). There was a 55% increase in mean life span of the *rtg3*Δ strain even without a reduction in glucose levels. Thus, the expression of genes under the control of the Rtg1p-Rtg3p transcription factor suppresses longevity to an extent under these growth conditions, in contrast to the requirement of the Rtg pathway for optimal longevity when the retrograde response signal is present in yeast whose mitochondria are not fully functional. The increase in mean life span seen upon reduction of glucose levels (80%) was additive (123%) to that obtained upon deletion of *RTG3* (Fig. 2*B*), generating the largest increase in longevity described thus far in yeast. These results have been observed in two different yeast strains, where the details of the induction of the retrograde response differ but its effect on life span is the same.

4. Decreasing the amino acids concentration of the growth medium promotes an increase in the mean and maximum life span of yeast

The extension of life span detected on reduction of glucose concentration in broth was effective only when the levels of other nutrients were decreased, suggesting that manipulation of these nutrients, especially amino acids, might result in life span extension. Indeed, decreasing amino acids concentration while maintaining glucose levels resulted in an increased longevity (up to 95%) that was larger the greater the reduction in the nonessential amino acids. This life span extension does not operate through the retrograde response pathway, because it did not entail the induction of *CIT2* and it was not suppressed by deletion of *RTG2*. Thus, it is not the decreased availability of a specific nutrient but rather the restriction of the caloric content of the growth medium that plays a role in life span extension.

CONCLUSIONS

This study demonstrates that yeast life span can be modulated physiologically. The life span extension observed upon manipulation of nutritional status possesses many of the hallmarks of caloric restriction in mammals. First, this effect is continuous, operating like a rheostat—the greater the reduction in nutrient concentrations, the larger the life extension (up to a point). This evokes a mechanism involving changes in flux through a pathway rather than a threshold effect that triggers an all-or-none response. Second, the increased longevity is associated with a postponement in the appearance of an aging phenotype. Third, the effect is not due to limitation of a specific nutrient. On the basis of these similar features, we tentatively call this mechanism of life span extension in yeast caloric restriction.

The increase in life span upon reduction of glucose levels does not appear to be simply due to release of the cells from glucose repression, because the life span extension seen is continuous as glucose concentration is lowered progressively. Furthermore, the glucose concentrations at which the max-

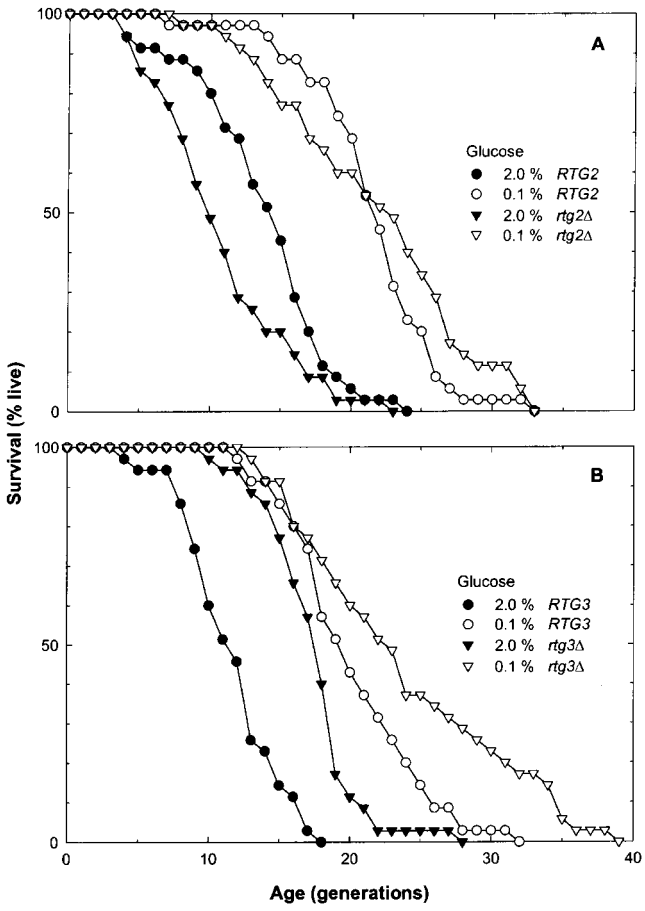

Figure 1. The retrograde response is not required for extension of life span by the reduction of glucose levels. A) Decreased glucose concentration caused an increase in mean and maximum life span ($P \ll 0.001$) in chemically defined medium. There was no significant effect on life span on 0.1% glucose of deletion of *RTG2* (P=0.74). Similarly, no effect on maximum life span was observed ($P > 0.05$). *B*) Deletion of *RTG3* increased mean life span 55% on 2% glucose in chemically defined medium. The life extension was significant ($P < 0.00001$). As before, growth on 0.1% glucose increased mean life span. The deletion of *RTG3* resulted in an additive increase in mean life span above the control. The additional enhancement of longevity was significant ($P < 0.01$). The corresponding differences in maximum life span were significant ($P \ll 0.001$).

imal effect on longevity was observed were quite different in broth and in chemically defined medium. Finally, life span extension by limitation of amino acids is clearly not the result of release from glucose repression.

A reduction in blood glucose levels is an inevitable, early response to caloric restriction in mammals. The fact that lowering the concentration of glucose available to individual yeast cells has such a marked effect on their longevity and aging suggests that a key component of the anti-aging effect of caloric restriction in mammals may be changes elicited by glucose at the cellular level. The reduction of glucose levels delayed the decline in budding rate normally seen during aging. The calorie-restricted yeast displayed a higher level of metabolic activity than the controls, all other things being equal, as indicated by an increased budding rate. This supports the notion that a change in the characteristics of fuel use underlies the caloric restriction effect.

Caloric restriction did not induce the retrograde response; in fact, a substantial reduction in the expression of the diagnostic gene for this response, *CIT2*, was found. This suggests that caloric restriction operates by down-regulating at least part of the retrograde response. Significantly, the extension of life span by caloric restriction did not require *RTG2*. Caloric restriction removed any need of this gene for a normal life span, suggestive of a positive relationship with certain features of the retrograde response. Deletion of *RTG3* also did not suppress the caloric restriction effect; instead, it potentiated a maximal increase in longevity that was additive to caloric restriction. Thus, the retrograde response and caloric restriction represent two distinct metabolic mechanisms of life extension (**Fig. 2**). The results suggest some separation of functions of the *RTG2* and *RTG3* mediators of the retrograde response. They are consistent with the hypothesis that caloric restriction diminishes or prevents certain aspects of the retrograde response and that certain aspects of the retrograde response have a negative effect on the increased longevity provided by caloric restriction, in effect preventing the full benefits of caloric restriction (Fig. 2). This does not rule out some overlap between the longevity effectors induced by the retrograde response and by caloric restriction.

There are similarities between the metabolic effects of the retrograde response in yeast and of the daf mutants of *Caenorhabditis elegans* that display extended adult longevities. These metabolic effects are reminiscent of some of the features of the respective dispersal forms of yeast and worms, the spore and the dauer larva, which represent evolutionarily conserved responses to environmentally unfavorable conditions. The retrograde response, however, appears to be a compensatory adaptation to the deleterious effects of the internal stress of mitochondrial dysfunction that can accumulate with age (Fig. 2). In contrast, caloric restriction may constitute an adjustment that prevents the deficits associated with aging. Although clues of its operation exist, evidence for the presence of the retrograde response in organisms other than yeast is currently lacking. A fundamental question concerning caloric restriction is whether the reduction in calories triggers responses that enhance survival, or whether it is the elimination of excess nutrients that have a negative effect on life span that is responsible.

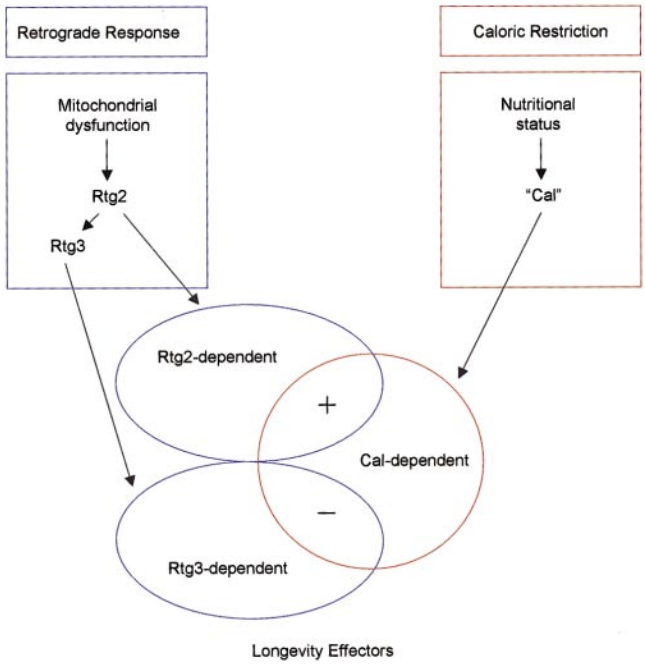

Figure 2. Multiple metabolic mechanisms of aging. The retrograde response and caloric restriction are two nonoverlapping pathways, which constitute a compensatory adaptation and a preventive adjustment to the dysfunction and deficits of aging, respectively. These pathways interact at the level of the longevity effectors. The interaction can be either positive or negative (see text). The extent of the overlap between the effectors of both pathways is not known at present. Rtg2 and Rtg3 are mediators of the retrograde response, and 'Cal' is a hypothetical mediator of the caloric restriction response.

The present study makes apparent the utility of yeast to elucidate the molecular mechanisms underlying caloric restriction, and it may bridge the gap between studies of aging across wide phylogenetic divisions. Our results provide evidence for multiple mechanisms of metabolic control in aging. Inasmuch as caloric restriction lowers blood glucose levels, this study raises the possibility that reduced glucose alters aging at the cellular level in mammals. FJ