

Correspondence

Increased Life Span due to Calorie Restriction in Respiratory-Deficient Yeast

Su-Ju Lin, Leonard Guarente

The Kaeberlein et al. paper in the November 2005 issue of *PLoS Genetics* [1] claimed that calorie restriction (CR) in yeast does not require respiration to extend life span of mother cells. This claim challenges our earlier finding that deleting *CYT1* (encoding cytochrome c1) prevented CR-associated longevity [2].

However, there is a fundamental difference in the two experiments: these authors typically use 0.05% glucose as their CR media instead of the 0.5% glucose in our experiments (compared with 2% glucose in controls). At their very low glucose levels, yeast cells are slow growing and show significant metabolic changes [3].

Moreover, the key for interpreting the effect of the *cyt1* deletion on CR is to examine the effect of the deletion on life span at a given reduced glucose concentration (compared with the usual 2%) and comparing this with the degree of life extension in the wild-type parental strain at that same reduced glucose concentration. The single experiment the authors present using our strain (PSY316) and our glucose concentration (0.5%) actually showed very little extension (at the margin of significance). Unfortunately, even this small effect is misleading because the experiment omits the wild-type control. In fact, an earlier study by Kaeberlein showed robust extension of life span at 0.5% glucose in the wild-type parental strain PSY316 [4]. The omission of this information from the experiment in Figure 3A obscures the key fact that deleting *CYT1* evidently did prevent most of the extension in life span by 0.5% glucose in their hands, which would agree with our previous findings.

The authors did find a much more robust extension at 0.05% glucose in the *cyt1* deletion in Figure 3A, a condition we did not examine [2]. The authors explain away the weak effect they see at 0.5% glucose in the *cyt1* mutant compared with the much larger effect at 0.05% glucose by saying a “non-optimal level of CR may have precluded detection of lifespan extension by CR in the *cyt1* deletion mutants in the prior study” [1]. But, as mentioned above, an earlier study by Kaeberlein showed a robust life extension in the wild-type parent PSY316 at 0.5% glucose, which was as great as that observed at 0.05% [4]. Indeed, 0.5% glucose was chosen as our standard in this strain because life span was maximal and the growth rate was reasonably rapid.

Therefore, the claims of Kaeberlein et al. that respiration and, by implication, *SIR2*-related genes are not required for CR-induced longevity are highly misleading. We previously showed the requirement for *SIR2* in PSY316 [2], and, moreover, Lamming et al. [5] recently showed the requirement for *SIR2* and related paralogs in the strain commonly used by Kaeberlein et al. (using 0.5% glucose). We think it is likely that different pathways are engaged at 0.05% compared with 0.5% glucose. Thus, their claim that respiration is not required for longevity may apply to their experimental conditions, but not to ours. In summary, we

think it is likely that differences in pathways identified by Kaeberlein et al. simply reflect their different experimental protocols and do not negate our earlier findings and interpretations. Furthermore, the fact that they changed the conditions we employed as CR and omitted relevant data have created confusion. ■

Su-Ju Lin

University of California Davis
Davis, California, United States of America

Leonard Guarente (leng@mit.edu)

Massachusetts Institute of Technology
Cambridge, Massachusetts, United States of America

References

1. Kaeberlein M, Hu D, Kerr EO, Tsuchiya M, Westman EA, et al. (2005) Increased life span due to calorie restriction in respiratory-deficient yeast. *PLoS Genet* 1: DOI: 10.1371/journal.pgen.0010069
2. Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, et al. (2002) Calorie restriction extends *Saccharomyces cerevisiae* life span by increasing respiration. *Nature* 418: 344–348.
3. Brauer MJ, Saldanha AJ, Dolinski K, Botstein D (2005) Homeostatic adjustment and metabolic remodeling in glucose-limited yeast cultures. *Mol Biol Cell* 16: 2503–2517.
4. Kaeberlein M, Andalis AA, Fink GR, Guarente L (2002) High osmolarity extends life span in *Saccharomyces cerevisiae* by a mechanism related to calorie restriction. *Mol Cell Biol* 22: 8056–8066.
5. Lamming DW, Latorre-Esteves M, Medvedik O, Wong SN, Tsang FA, et al. (2005) *HST2* mediates *SIR2*-independent life-span extension by calorie restriction. *Science* 309: 1861–1864.

Citation: Lin SJ, Guarente L (2006) Increased life span due to calorie restriction in respiratory-deficient yeast. *PLoS Genet* 2(3): e33.

Copyright: © 2006 Lin and Guarente. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The authors received no specific funding for this article.

Competing Interests: The authors have declared that no competing interests exist.

DOI: 10.1371/journal.pgen.0020033

Authors' Reply

As in our prior work showing that life span extension by calorie restriction (CR) occurs independently of *Sir2* [1], we have attempted to examine the putative role for respiration in life span extension in as comprehensive a manner as possible [2]. We have done this by measuring the effect of CR on life span across a range of glucose concentrations and by comparing the data derived from two different strain backgrounds [2]. We believe it is important to optimize life span extension by CR, in both wild-type and mutant backgrounds, in order to interpret genetic experiments involving CR. In contrast, Lin and colleagues have exclusively used 0.5% glucose for their CR experiments [3–5]. This was the case in their prior work [3], where the response of *cyt1Δ* cells to CR was tested using only one glucose concentration and one strain background. As we show [2], life span extension in the *cyt1Δ* mutant is not maximized at the level of restriction used by Lin et al. [3]. Thus, we speculated that their failure to test multiple glucose levels caused them to mistakenly report that CR does not increase the life span of *cyt1Δ* cells [3].

In their correspondence, Lin and Guarente [7] divert attention from our findings that Sir2 and respiration are not required for life span extension by CR in two ways. First, they misinterpret our data in the context of prior data from Kaeberlein et al. [6] showing that both 0.5% and 0.05% glucose increase life span to a similar extent in wild-type PSY316 cells. In fact, our data confirm the findings of Kaeberlein et al. [6]; however, these data are restricted to the wild-type case, and, as we demonstrate [2], it cannot be assumed that the optimal level of restriction will remain constant in various mutant backgrounds. Second, Lin and Guarente [7] suggest that CR at 0.5% glucose might increase life span by a different mechanism than CR at 0.05% glucose, yet they have presented no data to support this hypothesis. In contrast, we have shown that life span extension by CR is independent of Sir2 at either glucose concentration [1], and in our paper [2], we show that respiration is not required for life span extension at either glucose concentration. Since we have reported a statistically significant life span extension from CR at both glucose concentrations in *cyt1Δ* cells, the claims made by Lin and Guarente [7] are untenable.

In addition to responding to the statements of Lin and Guarente [7], we wish to point out that the single experiment they have chosen to focus on in their correspondence was of relatively minor importance in developing our conclusions. Lin and Guarente [7] do not address the primary finding of our paper [2]: respiration is not required for life span extension by CR. Even neglecting our data for PSY316 *cyt1Δ* cells, we show that CR at either glucose concentration significantly increases the life span of ρ^0 cells, which completely lack mitochondrial DNA [2]. Furthermore, we report here and elsewhere that CR does not result in activation of Sir2, and that Sir2-family proteins are not required for life span extension by CR [1,2,8,9]. In our opinion, these data represent substantial evidence that CR is not mediated by Sir2-family proteins and that increased respiration is not required in life span extension by CR. ■

Matt Kaeberlein (kaeber@u.washington.edu)

Di Hu

Emily O. Kerr

Mitsuhiro Tsuchiya

Eric A. Westman

Nick Dang

Stanley Fields

Brian K. Kennedy (bkenn@u.washington.edu)

University of Washington

Seattle, Washington, United States of America

References

1. Kaeberlein M, Kirkland KT, Fields S, Kennedy BK (2004) Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol* 2: DOI: 10.1371/journal.pbio.0020296
2. Kaeberlein M, Hu D, Kerr EO, Tsuchiya M, Westman EA, et al. (2005) Increased life span due to calorie restriction in respiratory-deficient yeast. *PLoS Genet* 1: DOI: 10.1371/journal.pgen.0010069
3. Lin SJ, Kaeberlein M, Andalis AA, Sturtz LA, Defossez PA, et al. (2002) Calorie restriction extends *Saccharomyces cerevisiae* life span by increasing respiration. *Nature* 418: 344–348.
4. Lin SJ, Ford E, Haigis M, Liszt G, Guarente L (2004) Calorie restriction extends yeast life span by lowering the level of NADH. *Genes Dev* 18: 12–16.
5. Lin SJ, Defossez PA, Guarente L (2000) Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* 289: 2126–2128.
6. Kaeberlein M, Andalis AA, Fink GR, Guarente L (2002) High osmolarity extends life span in *Saccharomyces cerevisiae* by a mechanism related to calorie restriction. *Mol Cell Biol* 22: 8056–8066.
7. Lin SJ, Guarente L (2006) Increased life span due to calorie restriction in respiratory-deficient yeast. *PLoS Genet* 2: DOI: 10.1371/journal.pgen.0020033
8. Kaeberlein M, McDonagh T, Heltweg B, Hixon J, Westman EA, et al. (2005) Substrate-specific activation of sirtuins by resveratrol. *J Biol Chem* 280: 17038–17045.
9. Kaeberlein M, Powers RW 3rd, Steffen KK, Westman EA, Hu D, et al. (2005) Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. *Science* 310: 1193–1196.

Citation: Kaeberlein M, Hu D, Kerr EO, Tsuchiya M, Westman EA, et al. (2006) Authors' reply. *PLoS Genet* 2(3): e34.

Copyright: © 2006 Kaeberlein et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The authors received no specific funding for this article.

Competing Interests: The authors have declared that no competing interests exist.

DOI: 10.1371/journal.pgen.0020034