Aging Cell (2011) **10,** pp1089–1091 Doi: 10.1111/j.1474-9726.2011.00742.x

SHORT TAKE

Sir2 deletion prevents lifespan extension in 32 long-lived mutants

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Summary

Activation of Sir2 orthologs is proposed to increase lifespan downstream of dietary restriction. Here, we describe an examination of the effect of 32 different lifespan-extending mutations and four methods of DR on replicative lifespan (RLS) in the short-lived *sir2Δ* yeast strain. In every case, deletion of *SIR2* prevented RLS extension; however, RLS extension was restored when both *SIR2* and *FOB1* were deleted in several cases, demonstrating that *SIR2* is not directly required for RLS extension. These findings indicate that suppression of the *sir2Δ* lifespan defect is a rare phenotype among longevity interventions and suggest that *sir2Δ* cells senesce rapidly by a mechanism distinct from that of wild-type cells. They also demonstrate that failure to observe lifespan extension in a short-lived background, such as cells or animals lacking sirtuins, should be interpreted with caution.

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Accepted for publication *19 August 2011*

Key words: ageing; replicative lifespan; longevity; yeast; epistasis.

Combining two or more longevity-altering interventions and determining the resulting effect on lifespan is a common method for examining the relationship between such interventions. An important subset of this type of analysis occurs when one of the factors under study promotes longevity, such as *daf-16* in *Caenorhabditis elegans* or *SIR2* in *Saccharomyces cerevisiae*. For both of these genes, several studies have combined a lifespan shortening null allele with an intervention that extends lifespan. A resulting lifespan similar to that of the short-lived single mutant has generally been interpreted as suggesting that the factors act in the same pathway. In contrast, an intervention extending the lifespan of the short-lived mutant has been interpreted as suggesting that the factors act in genetically distinct pathways. Specific examples of this type of comparison are studies in which dietary restriction (DR) fails to extend lifespan in yeast (Lin *et al.*, 2000), invertebrates (Rogina & Helfand, 2004; Wang & Tissenbaum, 2006) and mice (Li *et al.*, 2008) when Sir2-orthologs are mutated. These data have been, and continue to be, interpreted by some to support a model in which DR promotes longevity and healthspan through the activation of sirtuins (Baur *et al.*, 2010).

It has been previously reported that deletion of *SIR2* blocks replicative lifespan (RLS) extension from DR by reduction in glucose and in strains lacking *GPA2* or *HXK2*, two genetic mimics of DR, but not in a strain lacking the rDNA replication fork block protein, *FOB1* (Kaeberlein *et al.*, 2004). To examine the influence of deleting *SIR2* on RLS extension more generally, we generated 30 additional double mutant strains in which a RLS-extending deletion was combined with deletion of *SIR2*. We also tested three additional methods of DR involving growth on alternative carbon sources (ethanol, glycerol or raffinose). Strikingly, none of these interventions resulted in a significant RLS extension relative to *sir2Δ* cells (Figs 1 and S2; Table S1).

One possible interpretation of these data is that each of the RLS-extending interventions acts upstream of Sir2, perhaps by promoting Sir2 activity. Two observations are inconsistent with this model. First, at least eight single-gene deletions that increase wild-type RLS, and all four forms of DR, significantly extend the RLS of *sir2Δ fob1Δ* cells (Fig. S2; Table S1), demonstrating that *SIR2* is not absolutely required for RLS extension in these cases. Second, at least five long-lived deletion mutants show no indication of enhanced Sir2 activity *in vivo*, as measured by rDNA recombination or rDNA silencing (Fig. S3). A similar lack of increased Sir2 activity has been previously reported in cells subjected to DR (Kaeberlein *et al.*, 2005; Riesen & Morgan, 2009; Smith *et al.*, 2009). Interestingly, deletion of *TOR1* caused a significant decrease in rDNA recombination, but this effect was independent of *SIR2* (Fig. S3A).

An alternative explanation for these data is that loss of *SIR2* alters ageing such that molecular processes that do not limit RLS in wild-type cells become limiting in *sir2Δ* cells. Sir2 has multiple functions, including repression of extrachromosomal rDNA circle formation (Kaeberlein *et al.*, 1999), enhancing global rDNA stability and silencing (Gottlieb & Esposito,

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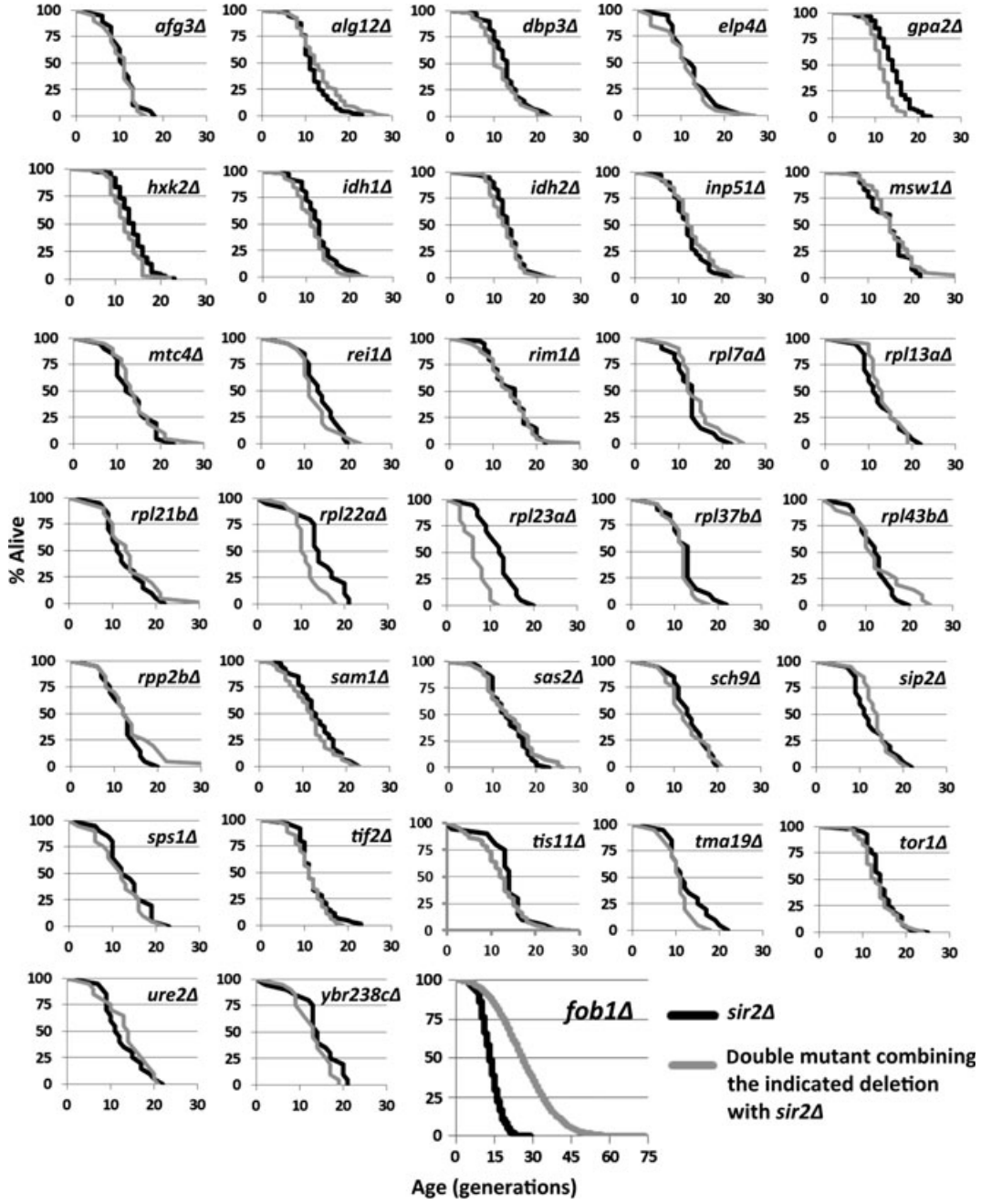

Fig. 1 Single-gene deletions that extend replicative lifespan (RLS) in wild-type cells do not extend RLS of *sir2Δ* cells. Replicative survival curves are provided for 33 double mutant strains combining a known long-lived gene deletion with deletion of *SIR2*.

1989; Smith & Boeke, 1997), promoting asymmetric inheritance of damaged proteins (Aguilaniu *et al.*, 2003) and maintaining telomeric chromatin during ageing (Dang *et al.*, 2009). Our observation that only deletion of *FOB1* is sufficient to suppress the short RLS of *sir2Δ* cells suggests that (i) the primary RLS-limiting defect in *sir2Δ* cells is likely related to rDNA instability and (ii) none of the 32 deletions tested that slow ageing in wild-type cells is able to overcome this defect. One prior study reported that overexpression of Hsp104 could also suppress the short RLS of *sir2Δ* cells (Erjavec *et al.*, 2007), raising the possibility that accumulation of damaged proteins in *sir2Δ* mother cells may also contribute to the reduced longevity.

While it is likely that many of the genes examined in this study do not require Sir2 for their effect on RLS, we do not believe that all of the 32 long-lived single-gene deletion mutants examined here necessarily act via Sir2-independent mechanisms. For example, deletion of *SAS2*, a histone acetyltransferase known to antagonize Sir2 effects on chromatin (Dang *et al.*, 2009), extends wild-type RLS but fails to extend the RLS of *sir2Δ fob1Δ* cells (Fig. S2B). Thus, both functional and genetic evidence suggest that Sas1 likely acts in the same longevity pathway as Sir2.

This study provides a clear demonstration of the challenges associated with interpreting longevity epistasis data. In particular, the failure of a

longevity intervention to extend lifespan in a short-lived background may not be informative regarding the mechanism of lifespan extension in the wild-type context. In the absence of strong evidence indicating that the lifespan shortening is caused by acceleration of the wild-type ageing process, caution is warranted when interpreting these types of data.

Acknowledgments

This work was supported by NIH Grant R01AG025549. JRD, GLS and SJ were supported by NIH Training Grant T32AG000057. JS was supported by NIH Training Grant T32ES007032. XL is supported by The National Natural Science Foundation of China (30672205, 30871440, 30900739, 30971620, 31101051), The Natural Science Foundation of Guangdong Province (7301506, 8452402301001450, 9252402301000002) and Key Foundation of Natural Science Research for Guangdong Universities (06Z015). MK is an Ellison Medical Foundation New Scholar in Aging.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1 Multiple forms of DR extend RLS in a Sir2- and Fob1-independent manner.

Fig. S2 Long-lived mutants extend RLS of *sir2Δfob1Δ* cells.

Fig. S3 rDNA recombination and silencing is not increased in long-lived strains.

Table S1 Summary of lifespan data presented in this study.

Table S2 Percent extension in replicative lifespan resulting from each gene deletion or intervention in the indicated genetic background.

Table S3 Genes examined in this study.

Table S4 Strains used in this study.

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