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LETTER TO THE EDITOR

Does resveratrol activate yeast Sir2 *in vivo*?

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In the February issue of *Aging Cell*, Yang *et al*. (2007) describe resveratrol derivatives with improved stability and report that they increase yeast replicative lifespan. The authors refer to these drugs as 'sirtuin activating compounds', implying that lifespan is increased due to activation of the Sir2 histone deacetylase. We applaud the development of more stable and potent resveratrol analogs. We wish to point out, however, that whether resveratrol and related compounds activate Sir2 *in vivo* remains unknown and further experimental validation is necessary.

The Yang *et al*. (2007) study as well as a prior report (Howitz *et al*., 2003) are complicated by the use of the PSY316AT yeast strain background. Among the strains commonly used in yeast aging research, PSY316AT is the only known case where overexpression of Sir2 fails to increase lifespan (Kaeberlein *et al*., 2004, 2005b). While it is possible that a sirtuin agonist could increase Sir2 activity in a manner not achieved by overexpression of the enzyme, it also needs to be considered that resveratrol (and its analogs) could affect longevity in a Sir2-independent manner. Resveratrol is known to interact with a variety of proteins that have yeast homologs, such adenosine monophosphate (AMP) kinase, protein kinase C and mitochondrial adenosine triphosphate (ATP) synthase, among others (Kaeberlein & Rabinovitch, 2006). Any of these proteins could potentially mediate effects of resveratrol on yeast cells or in other organisms.

Another complicating factor is the use of replicative lifespan as the sole measure of drug activity. While longevity is of clear interest, increased lifespan does not imply increased Sir2 activity, as several single-gene mutations are known to increase lifespan in a Sir2-independent manner (Kaeberlein *et al*., 2004, 2005c). Sir2 activity can be measured more directly, for example, by monitoring transcription of marker genes at telomeres, silent mating loci and rDNA (Smith & Boeke, 1997; Roy & Runge, 2000; Sandmeier *et al*., 2002; Kaeberlein *et al*., 2005a). In fact, the PSY316AT strain used by Yang *et al*. was engineered for such a purpose and contains a subtelomerically integrated *ADE2* marker. Activation of Sir2 by overexpression of the enzyme results in decreased *ADE2* transcription, which in turn causes accumulation of a red pigment in this strain (Kaeberlein *et al*., 2005b). Visual inspection of the colonies in the lifespan assays would reveal this pigmentation and it would have been of interest for Yang *et al*. to comment whether the resveratrol derivatives increased Sir2 activity by this assay.

It would also be of great interest to assess the effect of resveratrol and related compounds on Sir2-dependent silencing and histone acetylation at each of the loci Sir2 is known to silence. If locus-specific effects are observed, they could be compared between drug treatments and Sir2 overexpression, which would provide additional evidence of whether Sir2 enzymatic activity is enhanced *in vivo* by these compounds. The utility of these experiments extends beyond simple verification of these compounds as Sir2 agonists. In a prior study (Howitz *et al*., 2003), it was suggested that resveratrol activated Sir2 in a selective fashion, decreasing rDNA recombination without increasing rDNA silencing. Increased expression of Sir2, in contrast, enhances rDNA silencing (Fritze *et al*., 1997; Gallo *et al*., 2004). Thus, the reported locus-specific activity of resveratrol remains unexplained, but is of great interest when considering potential therapeutic applications in humans. *In vitro* assays have, however, also produced ambiguous results: while resveratrol activates Sir2 to deacetylate a fluorescent peptide substrate, it does not activate either Sir2 or the human ortholog SIRT1 towards more native peptide substrates (Borra *et al*., 2005; Kaeberlein *et al*., 2005b).

We greet the development of more stable resveratrol analogs with optimism. Thus far, attempts to replicate the reported effects of resveratrol on yeast replicative lifespan (Howitz *et al*., 2003) or rDNA recombination have been unsuccessful (Kaeberlein *et al*., 2005b). One possible explanation is that resveratrol may be unstable over the time course required to carry out these assays. Fortunately, if resveratrol and related compounds do activate Sir2 *in vivo*, this activation should be easily detected and independently verified using the more stable derivatives, and most importantly, more specific assays for Sir2 activity. It is our hope that a more thorough examination of the *in vivo* effects of resveratrol and related compounds in yeast will resolve these interesting questions.

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