

DISCUSSION

Growth signaling and superoxide anions in the chronological aging model

Our findings reveal that under a variety of experimental conditions, an inverse relationship exists between budding yeast CLS and intracellular levels of O_2^- (summarized in Table 2) that points to O_2^- accumulating downstream of growth signaling as a primary cause of chronological aging. A role for growth signaling-induced O_2^- in chronological aging is consistent with earlier reports that CR extends CLS in part by downregulating Tor1p-, Ras2p- and Sch9p-dependent growth signaling pathways that inhibit the Rim15p kinase and its induction of oxidative stress defenses ([7]; Figure 6). Our findings also indicate that the Rim15p-independent extension of CLS by CR reported earlier [7] is related to the induction of H_2O_2 that reduces O_2^- (Figure 1G) by activating SODs [8] independently of Rim15p (Figure 6).

Burtner et al. [12] recently proposed that the primary cause of chronological aging in budding yeast is toxic effects of acetic acid that are not caused by oxidative stress, and that inactivation of Sch9 or Ras2 protect against acetic acid toxicity through unknown mechanisms rather than reduced growth signaling. Our data are consistent with a role for acetic acid toxicity as a determinant of CLS in 2% glucose medium. However, acetic acid causes O_2^- to accumulate in stationary phase cells, because buffering SC medium to a higher pH, which extends CLS, reduces levels of O_2^- [18]. O_2^- levels are similarly reduced in cells in YPD (Figure S3), which in addition to maintaining a higher medium pH (Table 1) exhibit a longer CLS (Figure 3; [13]). O_2^- accumulating in stationary phase cells is toxic, because experimental manipulations that directly elevate O_2^- levels (inactivation of Sod2p or exposure to NAC) shorten CLS (Figure 5).

Acetic acid [41] and/or intracellular acidification [42] induce the same TOR- and RAS-dependent growth signaling pathways induced by glucose, and the induction of O_2^- by acetic acid is likely a consequence of acetic acid-induced growth signaling. A role for RAS-dependent growth signaling in acetic acid toxicity is consistent with an earlier report that the enhanced stationary phase viability of *ras1Δ* and/or *ras2Δ* cells cultured in SC medium is absent in YPD cultures or in SC cultures buffered to a higher pH [43]. Our finding that CLS extension in *sch9Δ* compared to wild type cells cultured in 2% glucose SC (Figure 1B; [3]) is similarly absent when these cells are cultured in 2% glucose YPD, (Figure 3F), which also maintains a

higher pH, suggests that acetic acid also triggers Sch9p-dependent growth signaling pathways. Therefore, the protective effects against acetic acid toxicity in unbuffered 2% glucose medium associated with inactivating Ras1p, Ras2p and Sch9p are likely due to downregulation of growth signaling by acetic acid and consequent upregulation of SODs and other oxidative stress defenses by Rim15p (Figure 6).

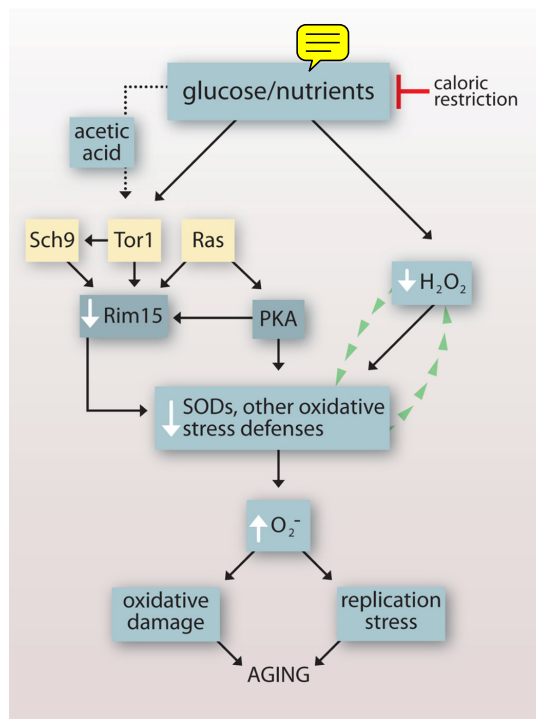


Figure 6. Impact of growth signaling pathways and caloric restriction on chronological lifespan in budding yeast. Glucose and other nutrients signal growth through conserved Sch9p-, Tor1p- and Ras-dependent pathways that inhibit Rim15p and its induction of oxidative stress defenses, leading to elevated O_2^- that cause oxidative damage and DNA replication stress. Acetic acid induces O_2^- by activating the same pathways. Caloric restriction attenuates signaling through these pathways and also induces H_2O_2 that activates SODs and reduces levels of O_2^- independently of Rim15p. In caloric restriction conditions, H_2O_2 that accumulates as a byproduct of increased SOD activity might stimulate SOD activity further by a self-amplifying mechanism.

Burtner et al. also proposed that the effects of acetic acid on CLS are specific for this form of acid [12]. However, deletion of *RAS1* and *RAS2* also protects against acid stress induced by hydrochloric acid [43]. Furthermore, Ras2p-dependent growth signaling is triggered by the acidifying protonophore 2,4-