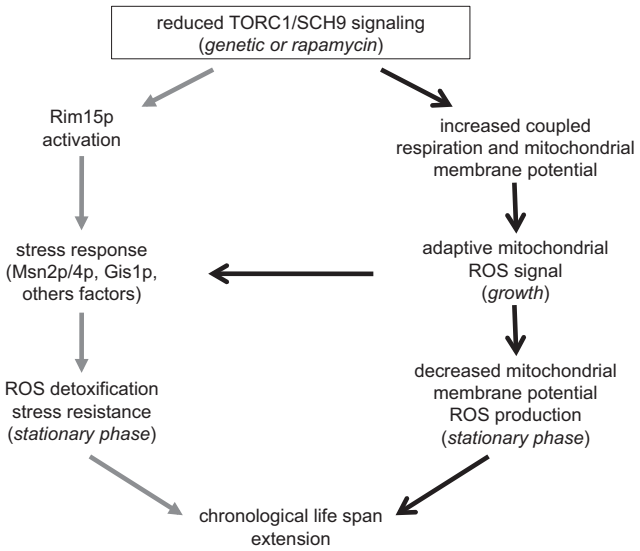

Figure 7. Adaptive Mitochondrial ROS Signaling and Activation of Rim15p-Dependent Stress Responses Collaborate to Mediate CLS Extension by Reduced TORC1 Signaling

A speculative model of how reduced TORC1 signaling extends CLS by activating both adaptive mitochondrial ROS signaling (right arm) and Rim15-dependent stress-resistance pathways (left arm). These responses would cooperate in CLS extension by enhancing ROS detoxification and stress resistance and by an adaptive response to elevated cellular superoxide and/or other ROS during growth that alters mitochondria function to decrease membrane potential and produce fewer ROS in stationary phase. This adaptive signal may activate some redox-sensitve factor that controls nuclear-encoded mitochondria and/or stress response genes (horizontal arrow), perhaps via epigenetic regulation, and results in altered respiration and enhanced stress responses in stationary phase. This model is meant to encapsulate those aspects of CLS extension by TOR inhibition involving ROS, mitochondria, and oxidative-stress resistance. We acknowledge that reduced TOR signaling has other effects on cell physiology that are also important for CLS, which are not pictured here.

Our results are consistent with a growing body of literature that mitochondrial ROS can act as mediators of adaptive/hormetic effects on yeast life span (Agarwal et al., 2005; Kharade et al., 2005; Piper et al., 2006), including a relevant recent study showing that caloric restriction elevates hydrogen peroxide in early stationary phase, which induces superoxide dismutase activity to help extend CLS (Mesquita et al., 2010; Weinberger et al., 2010). That we implicate superoxide as the adaptive/hormetic signaling molecule suggests that various ROS may promote adaptive signaling during different stages of growth (e.g., superoxide during logarithmic growth and H_2O_2 during stationary phase) or that there are mechanistic differences in life span extension induced by reduced TOR signaling and calorie restriction (CR). The observation that CR further extends the life span of *sch9Δ* strains supports the latter possibility (Wei et al., 2008; Weinberger et al., 2010). Furthermore, numerous studies in *C. elegans* provide strong evidence for mitochondria- and ROS-mediated adaptive/hormetic regulation of life span (Dillin et al., 2002; Gems and Partridge, 2008; Yang and Hekimi, 2010; Ristow and Zarse, 2010; Schulz et al., 2007). Finally, lower mitochondrial membrane potential, which we propose is a downstream consequence of adaptive mitochondrial signaling, correlates extremely well with longer life span in worms with genetic alterations in various longevity pathways (Lemire et al., 2009). In the context of these studies, our findings strongly suggest that pathways that conditionally regulate mitochondrial membrane potential and/or ROS production during specific growth or developmental windows, like TORC1, could be key regulators of longevity.

Media metabolites have also been shown to regulate yeast CLS (Aerts et al., 2009; Fabrizio et al., 2005; Wei et al., 2009). While our results (Figure 6A) are consistent with media acidification as one determinant of CLS (Burtner et al., 2009), we conclude from our media-swap experiments (Figures 6E and 6F) that reduced production or release of extracellular molecules does not underlie the extension of CLS in *tor1Δ* and *sch9Δ* strains, and hence the effects we describe in this study are cell-intrinsic. The additional finding that media neutralization reduces mitochondria membrane potential and cellular ROS (Figure 6) highlights the involvement of mitochondria in acid stress response and suggests that media acidification in yeast CLS experiments ultimately regulates general cell-intrinsic stress responses that are relevant to conserved mechanisms of aging. Finally, we observe a slight reduction in stationary-phase media ethanol levels in *tor1Δ* and wild-type cultures treated with rapamycin during growth (Figure S6). While our media-swap experiments indicate that differences in ethanol concentration at the time of swap do not determine CLS, we cannot exclude the possibility that enhanced extracellular ethanol depletion, via utilization as an alternate carbon source, in TOR-inhibited strains later in stationary phase contributes to CLS extension as proposed by others (Fabrizio et al., 2005). In fact, such metabolic reconfigurations may be part of the adaptive response in stationary phase to mitochondrial ROS signaling events during growth.

Reduced TORC1 signaling increases life span in organisms ranging from yeast to mammals and has other beneficial effects that are of potential therapeutic value for human disease (Fontana et al., 2010). Our results shed significant light on the involvement of mitochondrial adaptive/hormetic signaling in the TORC1 longevity pathway and therefore likely represent an important avenue that might be exploited in these regards.

EXPERIMENTAL PROCEDURES

Yeast Strains

All experiments were performed in the DBY2006 strain background (*MATα his3-Δ200 leu2-3,-112 ura3-52 trp1-Δ1 ade2-1*) or the BY4742 background (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) as indicated. The *tor1Δ* and *sch9Δ* strains have been described (Bonawitz et al., 2007; Pan and Shadel, 2009). The *RIM15* ORF in DBY2006 and in DBY2006 *sch9Δ* was deleted with a kanamycin cassette that was PCR amplified from the *rim15Δ* strain in the yeast DBY4742 knockout collection (Open Biosystems) with primers AATTA TCCCGGGTCCATATTGCCCTAGGTCTTG and AATTATCCCGGGGCCTCGA AATTGAGAAATGAA. Gel-purified amplicons were used in transformation and G-481-resistant colonies were selected. Successful integration was verified by PCR. The *sch9Δ* and *rim15Δ/sch9Δ* strains were transformed with an MSN4-GFP plasmid (Bonawitz et al., 2006). DBY2006 was transformed with the plasmid pYX142-SU9-GFP (Westermann and Neupert, 2000). All strains were grown in standard SD medium with appropriate nutrients (Sherman, 1991).

Oxygen Consumption and CLS Assays

Oxidative phosphorylation and coupling assays using purified mitochondria were performed as described (Ocampo et al., 2010). Cellular oxygen consumption was assayed as described (Bonawitz et al., 2007). Mean oxygen consumption as