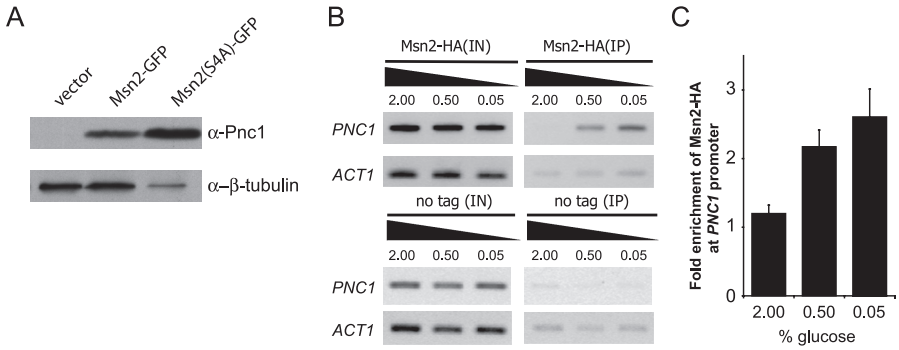

Figure 7. Msn2p Directly Regulates *PNC1*

(A) Nuclear localization of Msn2p is sufficient to induce *PNC1*.
(B) Chromatin immunoprecipitation analysis of Msn2p-HA binding to the *PNC1* promoter. IN, input; IP, immunoprecipitation.
(C) Relative enrichment of Msn2p-HA at the *PNC1* promoter shows increased promoter binding during CR.
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A previous study has shown that inhibition of TOR signaling can extend lifespan, even in the absence of *SIR2* [17]. In agreement with this data, we find that treatment with rapamycin can suppress rDNA recombination and extend lifespan in a *sir2*Δ *fob1*Δ strain (Figure S2). Yeast contain four additional sirtuin genes *(HST1–4)*, some of which can compensate for the lack of Sir2p during CR [15]. Under the conditions and with the strain used in this study, we have observed that rapamycin no longer lowers rDNA recombination or promotes longevity if both *SIR2* and *HST2* are deleted (Figure S2), implying that these two genes are primarily responsible for the effect. However, a W303 *sir2*Δ *hst2*Δ *fob1*Δ strain has a high rate of rDNA recombination and a short lifespan, which may serve to obscure the role of additional sirtuins or other mediators in the response to TOR inhibition. In fact, overexpression of *PNC1* in a wild-type strain lowers rDNA recombination more than in a strain lacking *MSN2/4*, which may indicate that genes downstream of *MSN2/4* besides *PNC1* also function to repress rDNA recombination. These alternative pathways may be especially important when glucose concentrations are extremely low [53] and may include pathways that directly regulate rDNA stability, such as *RPD3*-dependent loading of condensin onto the rDNA array in response to nutrient signaling [54]. TOR signaling also promotes the synthesis of ribosomal proteins, and downregulation of ribosomal biogenesis can extend the lifespan of both yeast [17] and *C. elegans* [22,23]. These data suggest that TOR signaling may act to promote lifespan via multiple pathways that act in parallel to promote longevity (Figure 8).

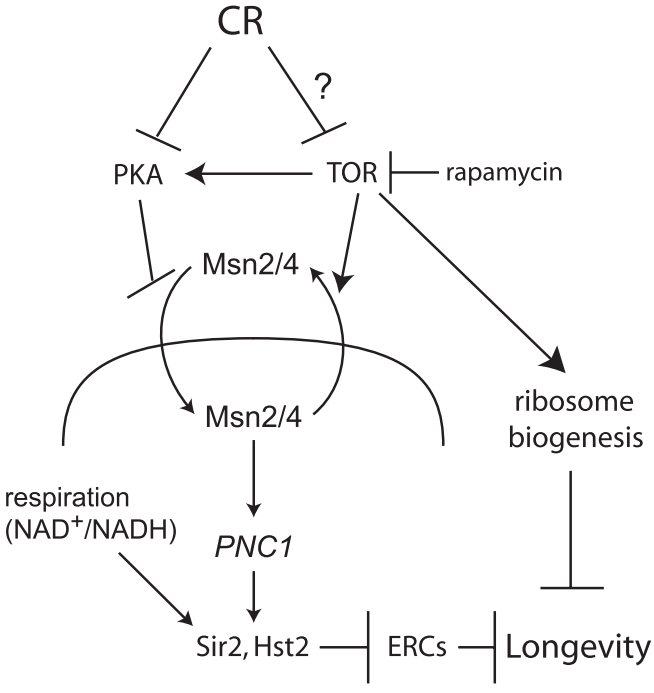

Figure 8. Model of How TOR Signaling and CR Regulate Yeast Replicative Lifespan

CR and TOR signaling regulate the nuclear localization of Msn2p/4p. When localized to the nucleus, Msn2p/4p promote the transcription of *PNC1,* a nicotinamidase. The removal of NAM by Pnc1p increases the activity of sirtuins, including Sir2p and Hst2p, which promote longevity by stabilizing the yeast rDNA array and preventing the formation of ERCs. TOR signaling may also regulate lifespan by sirtuin-independent pathways, such as the regulation of ribosomal biogenesis.
doi:10.1371/journal.pbio.0050261.g008

Our analysis of the responsiveness of STRE-containing genes found ten genes, including *PNC1,* that are upregulated more than 2-fold in response to a slight decrease in the glucose concentration (2% to 1.75%) (Table S1). In general, the genes in this category are highly sensitive to environmental stresses, including heat shock and osmotic stress [46]. We speculate that other genes in this category, which includes both metabolic and heat shock genes, may also play a role in lifespan extension. Heat shock proteins in particular have been shown to promote longevity in numerous organisms, and are upregulated during CR in rodents [55,56].

Interestingly, *MSN2/4* have also been shown to be required for the extension of yeast chronological lifespan [57]. *MSN2/4* are responsible for the activation of numerous stress-responsive genes, including the superoxide dismutase *SOD2,* a gene that promotes chronological lifespan [34]. Yet, overexpression of *SOD2* shortens replicative lifespan, and it has been demonstrated that deletion of *MSN2/4* can actually lead to increases in replicative lifespan [58]. Even though we saw no such effect (Figure 1A), perhaps because of a difference in strain background, there may be a reciprocal relationship between replicative and chronological lifespan. A recent study showed that deletion of *SIR2* can extend chronological lifespan in several strains [59], and we have