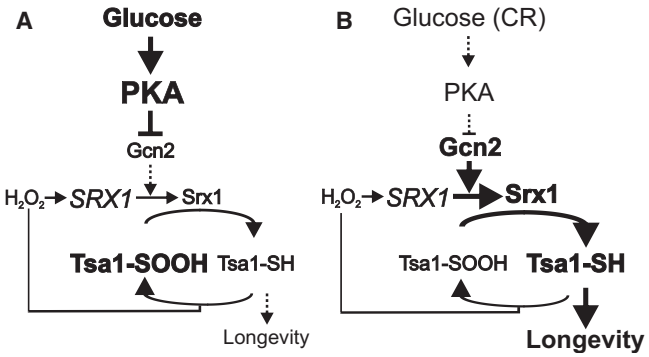

Figure 7. Model for How CR Elicits Tsa1/Srx1-Dependent H_2O_2 Resistance and Life Span Extension

(A) At a high concentration of glucose, leading to a high cAMP-PKA activity, H_2O_2 stress activates Yap1/Skn7-dependent transcription of the *SRX1* mRNA, but its translation is inhibited by PKA. As a consequence, Srx1 production is attenuated and Tsa1 hyperoxidized and inactivated.

(B) During CR, PKA activity is reduced, and this relieves the translational block of the *SRX1* mRNA in a Gcn2-dependent manner to provide more Srx1 protein and, as a consequence, more reduced, peroxidase-active Tsa1.

(eIF2) kinase Gcn2, relieves a cAMP-PKA-dependent inhibition in the translation of the *SRX1* gene (Figure 7).

In addition to being required for CR-induced H_2O_2 resistance, we show that Tsa1 and Srx1 are both necessary for a full life span extension by CR. Interestingly, we found that Tsa1 becomes hyperoxidized (sulfinylated) during aging, indicating that aged yeast cells have a limited ability to perform Srx1-dependent reduction of hyperoxidized and sulfinylated Tsa1. In support of this notion, providing the cells with an extra copy of the *SRX1* gene counteracted age-related hyperoxidation of Tsa1 and extended replicative life span by 15%–20% (Figure 5G, Figures S5A and S5C) in a *TSA1*-dependent manner (Figure 5H, Figure S5B). This life span extension by an extra copy of the *SRX1* gene is comparable to that achieved by a rapamycin-dependent reduction of TOR signaling (15.4%; Medvedik et al., 2007). Hyperoxidation of the mitochondrial PrxIII enzyme has been reported also in the liver of aged rats, indicating that Prx inactivation may be a common phenotype in aging organisms (Musicco et al., 2009).

Deleting the yeast *TSA1* causes a dramatic genome instability with gross chromosomal rearrangements and synthetic lethality in combination with deficiencies in Rad51-, Rad52-, and Rad6-mediated DNA repair (Huang and Kolodner, 2005). In addition, mice lacking the Tsa1 homolog Prdxl display increased genomic instability and an increased incidence of malignant tumors, and they age prematurely (Neumann et al., 2003). Similar results have been demonstrated for worms lacking the 2-Cys peroxiredoxin PRDX-2 (Olahova et al., 2008). The role of Tsa1 in protecting the genome and the fact that Tsa1 becomes hyperoxidized in aging cells is interesting in view of the fact that replicative aging of yeast mother cells encompasses a progressive decline in the maintenance of the genome. This decline includes accumulation of extrachromosomal rDNA circles (ERCs; Sinclair and Guarente, 1997) and a switch to a hyperrecombinational state (McMurray and Gottschling, 2003). McMurray and Gottschling (2004) suggested that the accumulation of damaged, oxidized, and aggregated proteins in aging cells (Erjavec et al., 2007; Liu et al., 2010) might lead to the loss of function of gene products critical for maintaining genome integrity. Our data are in line with this idea and suggest that Tsa1 might be one such gene product of special importance for the maintenance of the genome which itself, in addition to its partner Srx1, is inactivated during aging.

It has been difficult to establish if the cAMP-PKA or the TOR pathway predominates in regulating cellular responses to nutritional cues, including CR, because of the highly interconnected nature of these pathways (Zaman et al., 2008). Therefore, our observation of the distinct role of PKA and TOR in H_2O_2 resistance elicited by CR is somewhat surprising. As shown herein, reducing TOR signaling did not affect the ratio of reduced/oxidized Tsa1 (or Srx1 levels or H_2O_2 resistance) in cells with high PKA activity, whereas reducing PKA effectively increased this ratio regardless of TOR activity (Figure S3B, Figures 4C and 4D, Figure S2E). Moreover, TOR and PKA signaling affected Tsa1 synthesis in an opposite manner (Figures S3R and S3S; Figure 3A). Thus, with respect to stress-induced Tsa1 synthesis upon CR, the TOR and PKA pathways are antagonizing each other with the development of H_2O_2 tolerance being due to the reduction in PKA-dependent signaling. These results points to the importance of clarifying to what extent the beneficial effects of CR in yeast primarily act through PKA or TOR.

Prxs themselves have recently been shown to be involved in nutrient signaling affecting longevity. Specifically, a neuronal *Drosophila* Prx was identified as a downstream effecter for life span regulation and oxidative stress resistance of the insulin signaling-regulated transcription factor FOXO (Lee et al., 2009). In view of these results and the fact that *TSA1* displays such an important role for CR to extend yeast life span, we believe it might be warranted to elucidate the possible importance of Prx enzymes as a potential public, evolutionary conserved mechanism for life span extension by CR.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Conditions

The *Saccharomyces cerevisiae* strains used were grown and manipulated at 30°C using standard techniques and yeast media (Molin et al., 2007; for details, see the Supplemental Experimental Procedures). H_2O_2 resistance assays were performed as described in the Supplemental Experimental Procedures. Where indicated, rapamycin (Sigma, catalog number R0395-1MG) stock solution (1 mg/ml in 95% ethanol) or ethanol alone was added to the cultures.

Plasmids

Plasmids used are found in the Supplemental Experimental Procedures.

cAMP Measurement

cAMP was extracted from cells by a TCA extraction method as described in the Supplemental Experimental Procedures and measured using the LANCE cAMP 384 kit (Perkin-Elmer, cat# AD0262) as recommended by the supplier.

Radiolabeling and 2D-PAGE Analysis

For protein synthesis rate determinations, cells were labeled with 200 μCi ^{35}S-methionine for 20 min at 30°C. Cell harvesting, protein extraction, and 2D-PAGE were performed as described (Maillet et al., 1996).