CellPress

Cell Metabolism
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Inactivation of Yeast Isw2 Chromatin Remodeling Enzyme Mimics Longevity Effect of Calorie Restriction via Induction of Genotoxic Stress Response

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http://dx.doi.org/10.1016/j.cmet.2014.04.004

SUMMARY

ATP-dependent chromatin remodeling is involved in all DNA transactions and is linked to numerous human diseases. We explored functions of chromatin remodelers during cellular aging. Deletion of *ISW2*, or mutations inactivating the Isw2 enzyme complex, extends yeast replicative lifespan. This extension by *ISW2* deletion is epistatic to the longevity effect of calorie restriction (CR), and this mechanism is distinct from suppression of TOR signaling by CR. Transcriptome analysis indicates that *isw2Δ* partially mimics an upregulated stress response in CR cells. In particular, *isw2Δ* cells show an increased response to genotoxic stresses, and the DNA repair enzyme Rad51 is important for *isw2Δ*-mediated longevity. We show that lifespan is also extended in *C. elegans* by reducing levels of *athp-2*, a putative ortholog of Itc1/ACF1, a critical subunit of the enzyme complex. Our findings demonstrate that the ISWI class of ATP-dependent chromatin remodeling complexes plays a conserved role during aging and in CR.

INTRODUCTION

The eukaryotic genome is packaged into a highly organized and largely repressive structure of chromatin; hence, DNA-based processes require remodeling of chromatin to expose or occlude DNA elements. ATP-dependent chromatin remodeling enzymes use the energy of ATP hydrolysis to alter chromatin states in chromatin assembly, DNA replication, recombination and repair, gene transcription and silencing, chromatin domain insulation, chromosomal dosage compensation, and chromosome segregation (Clapier and Cairns, 2009). Chromatin remodelers play critical roles in development, cell differentiation, and stem cell maintenance. Mutations in these enzymes contribute to cancer and congenital syndromes (Wu, 2012).

The four subfamilies of the chromatin remodeling ATPases (SWI/SNF, ISWI, CHD/NURD, and INO80) have distinct domain organization and catalytic mechanisms (Clapier and Cairns, 2009; Hota and Bartholomew, 2011). The ISWI subfamily alters nucleosome positioning, catalyzes chromatin assembly, and leads to chromosome condensation (Corona and Tamkun, 2004). These enzymes regulate DNA replication, transcription regulation, and cell fate determination (Yadon and Tsukiyama, 2011). The ISWI subfamily has a simple enzyme complex composition compared to other remodelers, but, nonetheless, forms distinct complexes with different subunits for varied functions. Notably, chromatin remodeling ATPases are highly conserved, and orthologs exist from yeast to humans (Clapier and Cairns, 2009). Despite considerable understanding, a role of ATPases in aging and lifespan regulation remains underexplored.

A hallmark of aging is the accumulation of deleterious cellular damage (including oxidized, misfolded, and/or aggregated proteins) dysfunctional organelles, and damaged DNA and chromatin structures (Feser and Tyler, 2011; Guarente, 2008; Kourtis and Tavernarakis, 2011; Sahin and Depinho, 2010). Various mechanisms reduce insults and remove damaged components in normal young cells, including enzymes to remove reactive oxygen species (Landis and Tower, 2005), heat shock proteins to remove misfolded proteins (Koga et al., 2011; Kourtis and Tavernarakis, 2011), recycling of damaged organelles (Green et al., 2011; Koga et al., 2011), and DNA repair and check point systems to fix DNA damage prior to replication (Langerak and Russell, 2011). These mechanisms comprise the cellular stress

response system, and genetic and environmental interventions often extend lifespan via enhanced stress responses (Kourtis and Tavernarakis, 2011). There are age-dependent changes in these stress response pathways (Gorbunova et al., 2007; Kourtis and Tavernarakis, 2011). However, the underlying mechanisms leading to altered stress responses during aging remain elusive.

Calorie restriction (CR), or more generally dietary restriction (DR), is the most robust and conserved intervention to extend lifespan (Mair and Dillin, 2008). Studies in model organisms indicate multiple pathways in mediating longevity and health benefits from DR, including reduced insulin-like growth factor (IGF) signaling (Mair and Dillin, 2008), downregulated target of rapamycin (TOR) signaling and ribosome abundance (Johnson et al., 2013), elevated sirtuin activity and reduced oxidative stress (Guarente, 2008), as well as improved DNA damage repair (Martins et al., 2011). For replicative aging of the budding yeast *Saccharomyces cerevisiae*, various CR conditions are proposed to mediate lifespan extension: enhancing Sir2 function through increased NAD/NADH ratio (Longo and Kennedy, 2006), repressing ribosome biogenesis through downregulation of TOR pathway kinases Tor1 and Sch9 (Johnson et al., 2013), and enhancing mitochondrial function and oxidative stress response (Molin et al., 2011; Ristow and Schmeisser, 2011).

Recently, chromatin remodeler SWI/SNF was linked to DAF-16-mediated longevity in *C. elegans* (Riedel et al., 2013). Here we investigate whether other remodelers impact aging in *S. cerevisiae*. We discovered that the Isw2 enzyme complex regulates lifespan through stress response pathways. Further, *ISW2* deletion results in a transcriptome and chromatin state that in part mimics CR conditions. Our findings reveal a conserved CR pathway for regulation of longevity that is distinct from previously identified TOR and sirtuin pathways.

RESULTS

Chromatin Remodeling Complex ISW2 Is an Aging Regulator

We screened chromatin regulators for lifespan alterations in the context of a large project quantifying replicative lifespan for single gene deletion strains from the yeast ORF deletion library (Kaeberlein et al., 2005a). Among nucleosome remodeling enzymes, only *isw2Δ* and *chd1Δ* were able to significantly extend lifespan, with *isw2Δ* robustly extending lifespan by 24% (Figures 1A and 1B). Deletion of *ISW2* extended both median and maximum lifespan (Figure 1A).

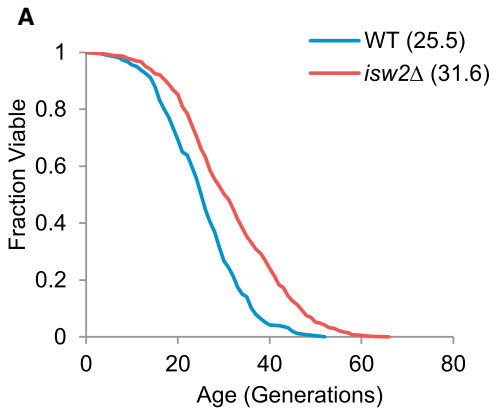
Since *isw2Δ* had a more significant effect, we chose to further investigate how it regulated lifespan, even though Chd1, a regulator of transcription elongation and chromatin assembly (Sims and Wade, 2011), might also be an interesting subject. Isw2 functions in a complex associated with three other subunits—Itc1, Dls1, and Dpb4—and we found that *itc1Δ* and *dls1Δ* also extended lifespan by 18% and 20%, respectively (Figure 1C; Figures S1A, and S1B available online). Deletion of *DPB4* did not extend lifespan (Figures 1C and S1C), likely because Dpb4 is also a subunit of DNA polymerase ε, and a defect in this enzyme may counterbalance any benefits from reduced Isw2 function (Iida and Araki, 2004). Isw2 shares a conserved ATPase domain with other members of the remodeler family; we found that a mutation in the conserved catalytic domain, K215R, also significantly extended lifespan by 15% (Figures 1C and S1D).

Because Isw2 regulates chromatin accessibility, we tested whether lifespan extension by *isw2Δ* required the histone deacetylase Sir2. An important cause of aging in yeast is the accumulation of extra-chromosomal rDNA circles (ERCs) in old cells, which is normally suppressed by Sir2 and requires Fob1, a replication fork protein (Longo and Kennedy, 2006). Homologs of Sir2 are found in all eukaryotes, collectively called sirtuins. Previous studies with *fob1Δ* mutants have revealed ERC-independent mechanisms that involve Sir2 in promoting longevity, such as telomeric heterochromatic silencing (Dang et al., 2009; Kaeberlein et al., 1999). We found that *isw2Δ* significantly extended lifespan in both *sir2Δ* and *sir2Δ fob1Δ* backgrounds (Figures 1D and 1E). The ability of *isw2Δ* to extend the short lifespan of *sir2Δ* cells is noteworthy, as it was recently reported that, of 33 gene deletions that extended lifespan, only *fob1Δ* had this property (Delaney et al., 2011a). This might suggest that effects of *isw2Δ* on lifespan are mediated by reduction of rDNA recombination. Hence, we further tested whether *isw2Δ* could reduce the accumulation of ERCs in old cells. Using quantitative real-time PCR targeting rDNA sequences, we observed increased rDNA copy number in old cells compared to young cells due to accumulation of ERCs. Interestingly, *isw2Δ* did not reduce the levels of ERCs in old cells compared to wild-type (WT) (Figure 1F), suggesting that *isw2Δ* did not extend lifespan by suppressing the formation and accumulation of ERCs. This is consistent with the results that *isw2Δ* extended lifespan in *sir2Δ fob1Δ* cells. Hence, we conclude that Isw2 regulates aging through a Sir2-independent and ERC-independent pathway.

Isw2 Functions in a Distinct CR Pathway

Since Isw2 requires ATP hydrolysis for its activity, we tested whether the altered cellular energy state in CR might be related to *isw2Δ*-mediated lifespan extension. As expected, lifespan was extended by limiting glucose concentrations to either 0.5% or 0.05%; however, these CR conditions were not able to extend lifespan when *ISW2* was deleted (compare Figures 2A to 2B and Figures 2C to 2D). Similarly, *isw2Δ* was unable to extend lifespan further under these CR conditions (Figures 2B and 2D). This is similar to findings that *tor1Δ* and *sch9Δ* are epistatic to CR (Kaeberlein et al., 2005b). Our observation suggests that *isw2Δ* and CR may share a common pathway to confer lifespan extension.

Therefore, we next tested how *isw2Δ* is related to known effectors of CR. Tor1 and Sch9 are kinases in the TOR signaling pathway that respond to nutrient availability and become inactivated in CR (Kaeberlein, 2010). A mechanism proposed for this pathway is that inactivation of TOR inhibits ribosome biogenesis and protein translation, which in turn induces the expression of Gcn4, a transcription factor activated in CR (Steffen et al., 2008). In order to determine if lifespan extension by *isw2Δ* is mediated by the same mechanism, we tested the lifespan of the *isw2Δ tor1Δ* double mutant. Interestingly, *isw2Δ tor1Δ* had a lifespan significantly longer than either of the single mutants (Figure 2E), suggesting that Isw2 and Tor1 regulate lifespan through distinct and parallel pathways. To verify that the lifespan extension by *isw2Δ* is independent of the TOR pathway and Gcn4, we tested lifespan epistasis between *isw2Δ* and *gcn4Δ*.

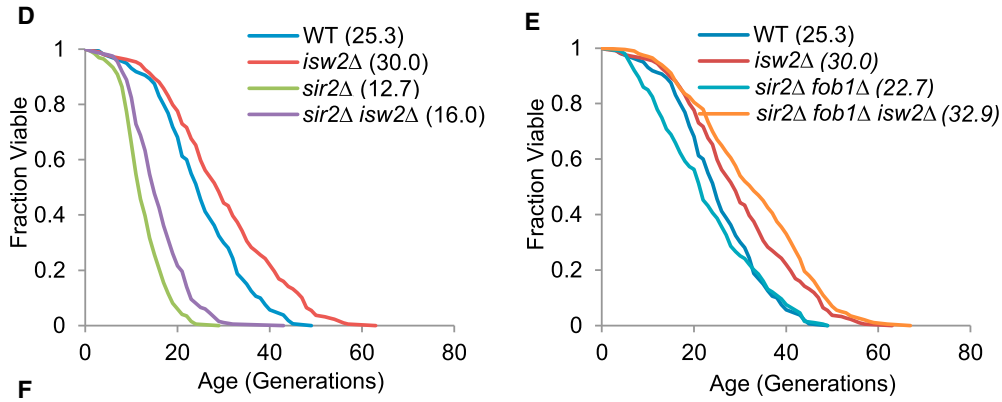


B

	WT	Mutant	*p*-value
snf2Δ	27.2 [105]	29.8 [104]	0.125
isw1Δ	27.3 [110]	26.4 [110]	0.643
isw2Δ	25.5 [405]	31.6 [521]	1.14×10^{-15}
chd1Δ	27.4 [144]	31.1 [145]	0.003
swr1Δ	23.0 [75]	23.8 [40]	0.726

C

	WT	Mutant	*p*-value
itc1Δ	31.6 [85]	37.2 [85]	5.59×10^{-4}
dls1Δ	28.7 [145]	34.4 [125]	7.48×10^{-6}
dpb4Δ	27.6 [130]	23.52 [130]	3.58×10^{-4}
isw2-K215R	26.1 [90]	30.6 [90]	0.010



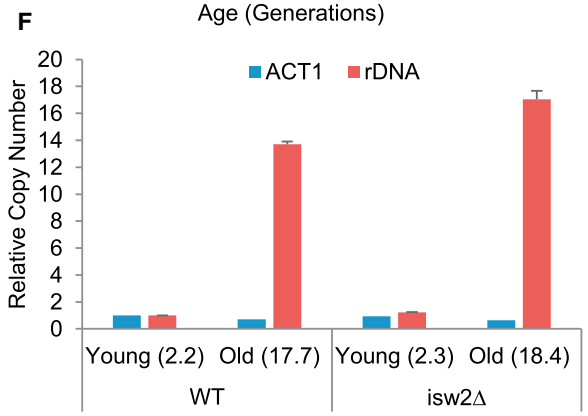


Figure 1. Disruption of ATP-Dependent Chromatin Remodeling Complex ISW2 Extends Yeast Replicative Lifespan

(A) Replicative lifespan for WT and *isw2Δ*. Values in parenthesis (here and hereafter) are mean lifespan.

(B) Mean replicative lifespan for strains deleted for chromatin remodeling ATPases. Values in brackets are the number of cells tested.

(C) Mean replicative lifespan for strains deleted for ISW2 complex subunits or via a catalytical mutant allele of *ISW2*.

(D and E) Replicative lifespan for *isw2Δ* in combination with either *sir2Δ* or *sir2Δ fob1Δ*.

(F) Quantitative real-time PCR analysis of rDNA copy number for young and old WT and *isw2Δ* cells. Values in parenthesis are mean age. Error bars, SEM. See also Figure S1and Table S1.

As predicted, *gcn4Δ* did not influence the longevity effect of *isw2Δ* (Figure 2F), further supporting the idea that Isw2 functions in a pathway different from TOR.

To further confirm this finding, we compared effects of these mutants directly on ribosomal gene expression, as well as their growth phenotypes. Ribosomal gene expression is strictly controlled by the cellular energy state (Lempiäinen and Shore, 2009). Nutrient deprivation can reduce ribosomal gene expression and ribosome assembly. Likewise, treatment with the mTOR inhibitor rapamycin, a CR mimetic, has a similar effect on ribosomal gene expression as CR (Jorgensen et al., 2004). As expected, *sch9Δ* and *tor1Δ* reduced gene expression of

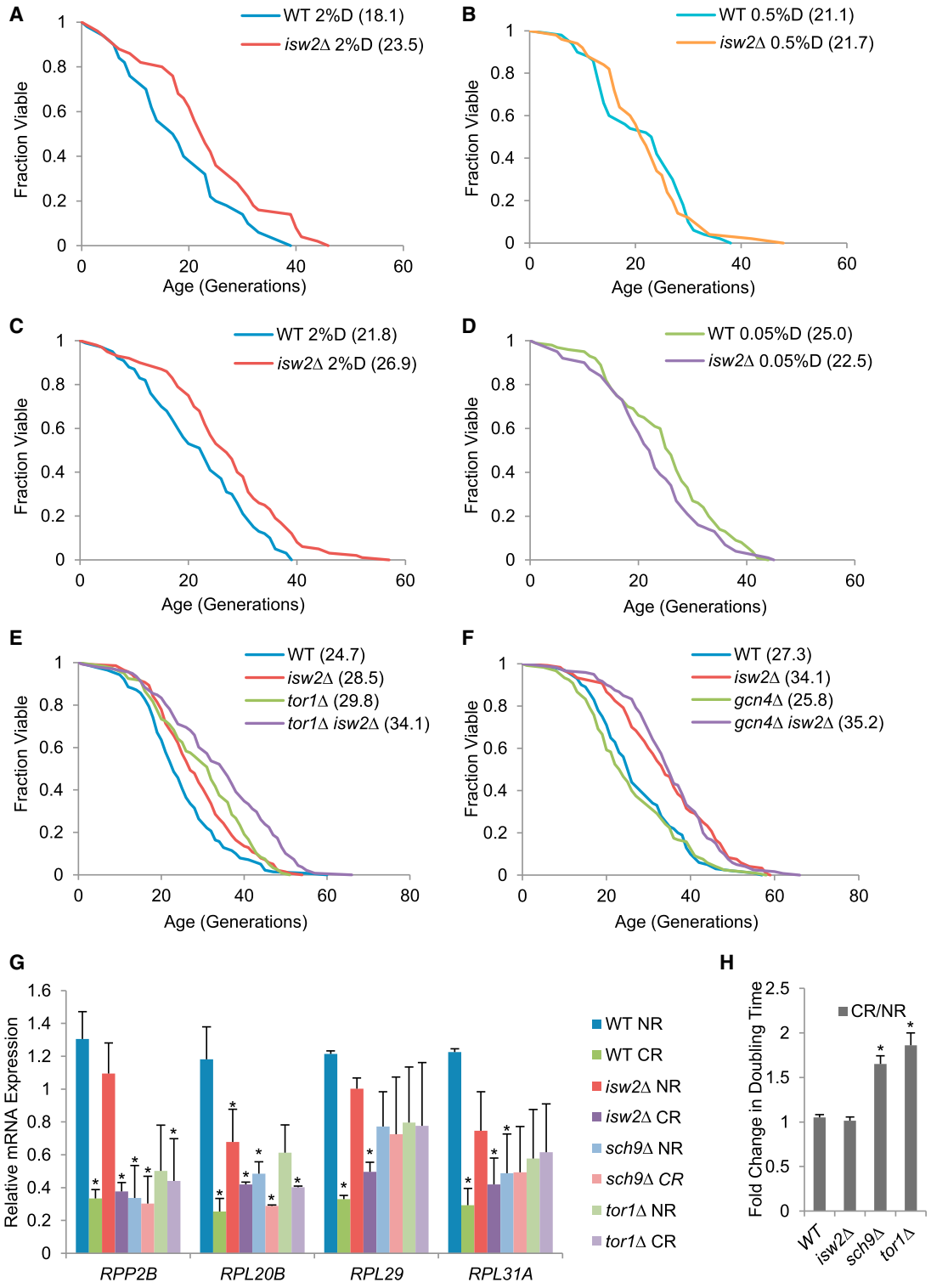


Figure 2. Deletion of *ISW2* Extends Lifespan through a Novel Mechanism Exploited by Calorie Restriction

(A and B) Replicative lifespan for *isw2Δ* under the moderate CR condition; YPD with 0.5% D-glucose (0.5% D), compared to the non-restricted (NR) condition, YPD with 2% D-glucose (2%D).

(C and D) Replicative lifespan for *isw2Δ* under the severe CR condition; YPD with 0.05% D-glucose (0.05% D), compared to NR.

(E and F) Replicative lifespan for *isw2Δ* in the *tor1Δ* (E) or *gcn4Δ* (F) background.

(G) Gene expression analysis by RT-qPCR for selected 60S ribosome subunit genes. NR: SC with 2% glucose. CR: SC with 0.05% glucose. Error bars, SEM.

(H) Fold change in doubling time during exponential growth in SC media. *p < 0.05 compared to WT NR. Error bars, SEM. See also Table S1.

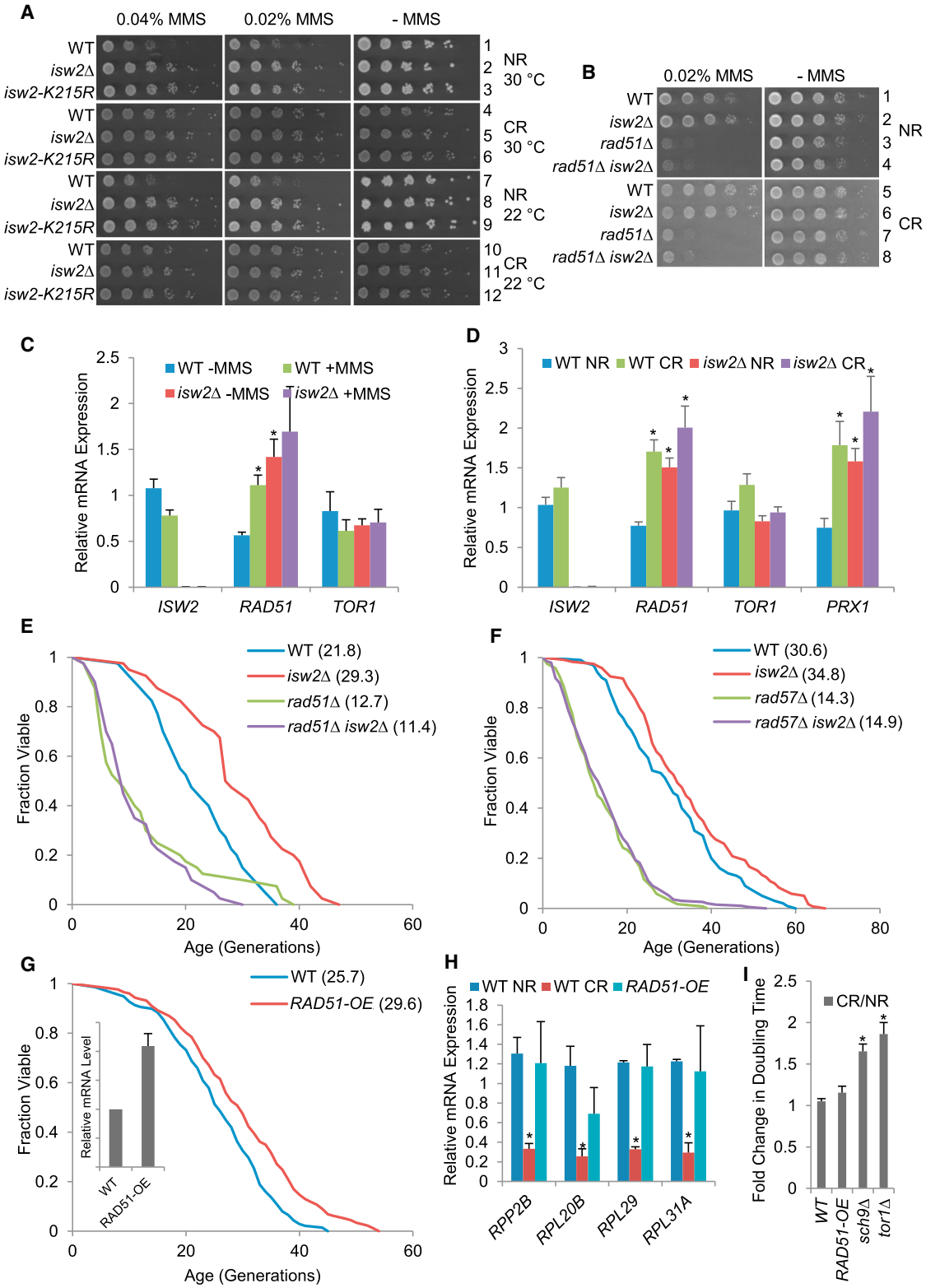


Figure 3. Isw2 Mediates Effects of Calorie Restriction through Homologous-Recombination-Based DNA Repair

(A and B) Designated strains were 5-fold serial diluted and spotted on SC with 2% glucose (NR) and SC with 0.05% glucose (CR).

(C and D) Gene expression by qPCR for WT and *isw2Δ* cells with or without MMS (C) and in NR and CR conditions (D). *p < 0.05 compared to WT NR. Error bars, SEM.

(legend continued on next page)

several ribosome large subunit proteins; however, *isw2Δ* did not show such an effect on ribosomal gene expression (Figure 2G), suggesting that *isw2Δ* did not reduce global mRNA translation and that *isw2Δ* extended lifespan through a mechanism distinct from that of ribosome regulation.

We then examined their growth effects. Deletion of *SCH9* extends lifespan by reducing the expression of ribosomal proteins, thus reducing the rate of protein synthesis, resulting in significant growth defects (Delaney et al., 2011b). We found that both *tor1Δ* and *sch9Δ* showed stronger growth defects under CR than normal growth conditions (Figure 2H); these mutants, which lack nutrient sensing capabilities, were apparently unable to cope with the nutrient deprivation stress. In contrast, both WT and *isw2Δ* showed no growth phenotype in CR compared to nonrestriction (NR) (Figure 2H). These distinct responses to CR between *isw2Δ* and *tor1Δ/sch9Δ* provided further evidence that Isw2 and Tor1/Sch9 function in distinct pathways.

Isw2 Regulates a Cohort of Stress Response Genes

Yeast Isw2 plays numerous important cellular roles, such as in retrotransposition, transcription, and DNA replication (Yadon and Tsukiyama, 2011). We next investigated functions of Isw2 relevant to lifespan extension under CR conditions.

Isw2 facilitates integration of retrotransposon Ty1 near tRNA genes (Gelbart et al., 2005), which could restrict lifespan by reducing tRNA levels. We tested Isw2 effects on Ty1, but did not uncover evidence that either *isw2Δ* or CR altered Ty1 genome copy number, Ty1 integration patterns, or adjacent tRNA expression (Figures S2A–S2C). In addition, Bdp1 recruits Isw2 to loci of Ty1 integration (Bachman et al., 2005); however, disruption of Isw2 recruitment by Bdp1 mutants failed to extend lifespan (Figure S2D), ruling out the possibility that lifespan extension by *isw2Δ* was mediated by Ty1 retrotransposon.

We then considered potential effects of Isw2 on antisense transcription (Whitehouse et al., 2007). We found that levels of antisense transcription were not globally altered in old cells compared to young (Figure S2E). In addition, we note that a global increase in antisense transcription via reduced Isw2 is not a plausible pathway to provide longevity benefits to cells.

We then considered effects on Isw2's function to promote DNA replication fork progression in parallel with the INO80 complex (Vincent et al., 2008). Given our recent findings of a correlation between short lifespan and S phase arrest in yeast terminal state (Delaney et al., 2013), it seems highly unlikely that a defect in DNA replication via reduced Isw2 would promote longevity.

Having ruled out these previously identified Isw2 functions as plausible explanations for the longevity effect of *isw2Δ*, we turned our attention to the characterized role of Isw2 in creating regular nucleosome spacing, leading to transcriptionally repressive chromatin (Fazzio et al., 2001). However, previous microarray analysis showed that only 35 genes were derepressed more than 2-fold by *ISW2* deletion (Fazzio et al., 2001). Hence, we re-examined this data set with a 1.5-fold cutoff, which was less stringent but still statistically relevant. Among the 281 genes derepressed more than 1.5-fold in *isw2Δ*, a majority of them, significantly greater than by chance, were also bound by Isw2 (Whitehouse et al., 2007) (Figure S2F). Using gene ontology (GO) analysis for these Isw2-regulated genes, we found a significant enrichment for stress response pathways (Figure S2G; Table S2). Examination of the published ChIP-chip data set (Whitehouse et al., 2007) confirmed that Isw2 localizes to these genes and promoters; response to abiotic stimulus/stress was again the second most significant GO cluster among Isw2-bound genes (Figure S2H). In summary, stress response genes are the most significant group both bound by Isw2 and derepressed in *isw2Δ*.

Activation of Homologous Recombination-Based DNA Damage Repair Promotes Longevity

We next examined which Isw2-regulated stress response pathway might be crucial for aging. *RAD51* was among the most derepressed genes in *isw2Δ* (Fazzio et al., 2001); genome-wide ChIP-chip showed a specific localization of Isw2 to the *RAD51* promoter (Whitehouse et al., 2007). We were able to reproduce these results for *RAD51* by RT-qPCR and ChIP-qPCR (Figures S3A and S3B).

We then turned to the specific function of Rad51, to unravel the role of Isw2. Rad51 is required for homologous recombination (HR) (Symington, 2002). Although both HR and non-homologous end joining (NHEJ) are major DNA double-strand break repair mechanisms (Polo and Jackson, 2011), HR, but not NHEJ, has been implicated in replicative lifespan in yeast (Kaeberlein et al., 1999; Park et al., 1999). Hence, we investigated whether the HR-based DNA repair pathway was responsible for the effects of *isw2Δ*.

We found that inactivation of *ISW2* improved the cellular response to genotoxic stress induced by DNA-damaging agents. Specifically, either *isw2Δ* or a catalytic mutation improved resistance to methyl methanesulfonate (MMS) and camptothecin (CPT) (Figure 3A rows 1–3; Figures S3C and S3D). Interestingly, CR also improved resistance to such stresses, and *isw2Δ* did not further increase the resistance, again suggesting epistasis between *isw2Δ* and CR (Figure 3A; compare rows 4–6 to rows 1–3). Notably, resistance to MMS under CR was not simply due to slow growth, since *isw2Δ* still improved MMS resistance when assayed at a lower temperature (Figure 3A, rows 7–12). Deletion of *RAD51* caused hypersensitivity to MMS and CPT; however, additional deletion of *ISW2* did not restore resistance to these agents (Figures 3B and S3D), indicating that Rad51 was required for elevated resistance to genotoxic stress in *ISW2* mutants.

To better understand the molecular mechanism underlying the improved genotoxic response, we further investigated *RAD51* expression under these conditions. In cells either treated with MMS or deleted for *ISW2*, *RAD51* expression levels were significantly increased (Figure 3C); little further induction was seen

(E and F) Replicative lifespan for *isw2Δ* in the *rad51Δ* (E) or *rad57Δ* (F) backgrounds.
(G) Replicative lifespan for *RAD51* overexpression strain (*RAD51-OE*). Inset, relative *RAD51* expression level. Error bars, SEM.
(H) Gene expression analysis by RT-qPCR for selected 60S ribosome subunit genes. NR: SC with 2% glucose. CR: SC with 0.05% glucose. Error bars, SEM.
(I) Fold change in doubling time during exponential growth in SC. *$p < 0.05$ compared to WT NR. Error bars, SEM. See also Figures S2 and S3 and Tables S1and S2.

when *isw2Δ* cells were treated with MMS, suggesting that *isw2Δ* alone was sufficient to mimic the elevated *RAD51* expression induced by genotoxic stress. In contrast, no significant change in *TOR1* expression was observed under these conditions (Figure 3C), further distinguishing these pathways. Since *isw2Δ* showed epistasis to CR, we then compared the effect of *RAD51* induction between *isw2Δ* cells and cells grown under CR conditions. Again, either CR or *isw2Δ* significantly induced *RAD51* expression, while there was insignificant further induction when growing *isw2Δ* cells in CR conditions, and again, there was no significant change of *TOR1* expression (Figure 3D). These observations are consistent with the epistasis between *isw2Δ* and CR. Since Isw2 regulates a cohort of stress response genes, we examined another gene showing the most upregulation in *isw2Δ*: the *PRX1* gene encodes the mitochondria peroxiredoxin, whose expression is activated by oxidative stress and is derepressed in *isw2* by nearly 2-fold (Fazzio et al., 2001). Similar to *RAD51*, *PRX1* expression was also significantly elevated under CR to an extent similar to *isw2Δ* cells (Figure 3D).

We next examined whether epistasis between *isw2Δ* and *rad51Δ* also occurs in the context of aging. As shown previously, lifespan was shortened by deletion of *RAD51* (Figure 3E), and we found that *isw2Δ* was unable to extend the *rad51Δ* short lifespan (Figure 3E), indicating that Rad51 was required for longevity regulated by Isw2. Rad57 is a critical facilitator protein for HR that forms a heterodimer with Rad55 and promotes the assembly of Rad51 at sites of DNA double-strand breaks (Symington, 2002). To further verify that it was the HR-based DNA damage repair that was required for the longevity extension by *isw2Δ*, we tested epistasis between *isw2Δ* and *rad57Δ*. Consistent with the *rad51Δ* results, *isw2Δ* was not able to extend lifespan in the *rad57Δ* (Figure 3F), confirming that *isw2Δ* required a functional HR pathway to extend lifespan.

Since *RAD51* expression was elevated in *isw2Δ* cells, we next tested the longevity effect of a strain carrying an extra copy of the *RAD51* gene integrated in its genome. Overexpression of *RAD51* is known to suppress certain mutations in the DNA double-strand break repair pathways and does not show deleterious effects in WT cells (Klein, 2008). Strikingly, lifespan was extended 24% by overexpressing *RAD51* (Figure 3G). These data showed that lifespan extension by *isw2Δ* required *RAD51* and that upregulation of *RAD51* alone could promote longevity.

To investigate whether the longevity benefit of *RAD51* overexpression was mediated by Tor1/Sch9, we again examined both ribosomal gene expression and growth phenotype. We found *RAD51* overexpression did not cause a significant decrease in ribosomal gene expression, as opposed to the case for CR (Figure 3H) and for *tor1Δ* and *sch9Δ* (Figure 2G). In addition, *RAD51* overexpression did not lead to a slow growth phenotype under CR, again differing from *tor1Δ* and *sch9Δ* (Figure 3I). These observations provided further evidence that the extended lifespan by *RAD51* overexpression was not the result of an altered Tor1/Sch9 signaling pathway.

ISW2 Deletion Partially Mimics CR Effects and Potentiates Stress Response in Old Cells

For several phenotypes described above, *isw2Δ* showed genetic epistasis to CR, and stress response pathways appeared to be similarly upregulated by *isw2Δ* and CR treatment. To obtain a broader view, we compared transcriptome changes between *isw2Δ* and CR cells. Two biological replicates of RNA-seq experiments were carried out for WT cells grown in synthetic complete (SC) media containing 2% glucose (NR) or 0.05% glucose (CR). There was strong agreement between the two replicates, with Pearson correlation coefficients of 0.97 and 0.91 for NR and CR samples, respectively. For all 6,437 annotated protein-coding genes with RNA-seq data for all samples, we plotted the $\log_2$(CR/NR) data in a histogram, which followed a normal distribution (Figure S4). Genes that had a change in $\log_2$(CR/NR) greater than 1 SD from the mean were selected for GO analysis, which included 775 upregulated and 853 downregulated genes. This 1-SD cutoff represented over 2-fold change in either direction (Table S3).

GO clustering analysis for these genes showed characteristic changes expected for calorie-restricted cells (Lee and Lee, 2008; Sharma et al., 2011; Steffen et al., 2008; Wang et al., 2010). These changes included downregulated ribosome biogenesis and protein translation; purine nucleotide biosynthesis; and glycolysis—as well as upregulated respiration; mitochondria function; and carbohydrate, fatty acid, and protein metabolic processes (Figures 4A and 4B). Notably, response to abiotic stimulus, which largely overlaps with response to stress, was one of the most significantly enriched functional clusters among upregulated genes (Figure 4B).

Since the response to abiotic stimulus/stress cluster was upregulated in both *isw2Δ* cells and CR-treated cells, we compared the lists of genes present in this functional cluster from the two data sets. Among all genes in the stress response cluster, there was a statistically significant overlap (14 genes) between those upregulated in CR cells (81 genes) and in *isw2Δ* cells (61 genes) (Figure 4C; Table S4). These data suggested that *isw2Δ* cells partially resembled CR-treated cells at the transcriptome level. By RT-qPCR, we validated the transcriptome analysis for a majority of 14 genes elevated in both *isw2Δ* and CR-treated cells (Figure 4D). In addition, we found that *isw2Δ* did not alter the expression of ribosomal genes, as was the case for CR and *tor1Δ* (Figures 4D and 2G). Taken together, we conclude that *isw2Δ* and CR similarly increase expression of a cohort of stress response genes, and this pathway is distinct from the Tor1 pathway that regulates protein synthesis.

To further evaluate the contribution of *isw2Δ* to the stress response specifically associated with aged cells, we compared expression changes by RT-qPCR for selected stress response genes in WT and *isw2Δ* cells during aging. We found that a number of stress response genes were moderately upregulated in aged WT cells, compared to young cells (Figure 4E), consistent with elevated stress levels in old cells. Interestingly, the induction of stress response genes in the aged population was much greater in *isw2Δ* cells (Figure 4E). Hence, it is likely that derepression of stress response pathways in young *isw2Δ* cells establishes a responsive state poised to antagonize age-associated elevation of cellular stress. Hormesis of this nature has been previously proposed with respect to aging (Martins et al., 2011).

CR Produces a Chromatin Conformation Similar to *isw2Δ* Cells

Isw2 alters nucleosome positioning in vivo (Whitehouse and Tsukiyama, 2006). Published data sets showed that *isw2Δ*

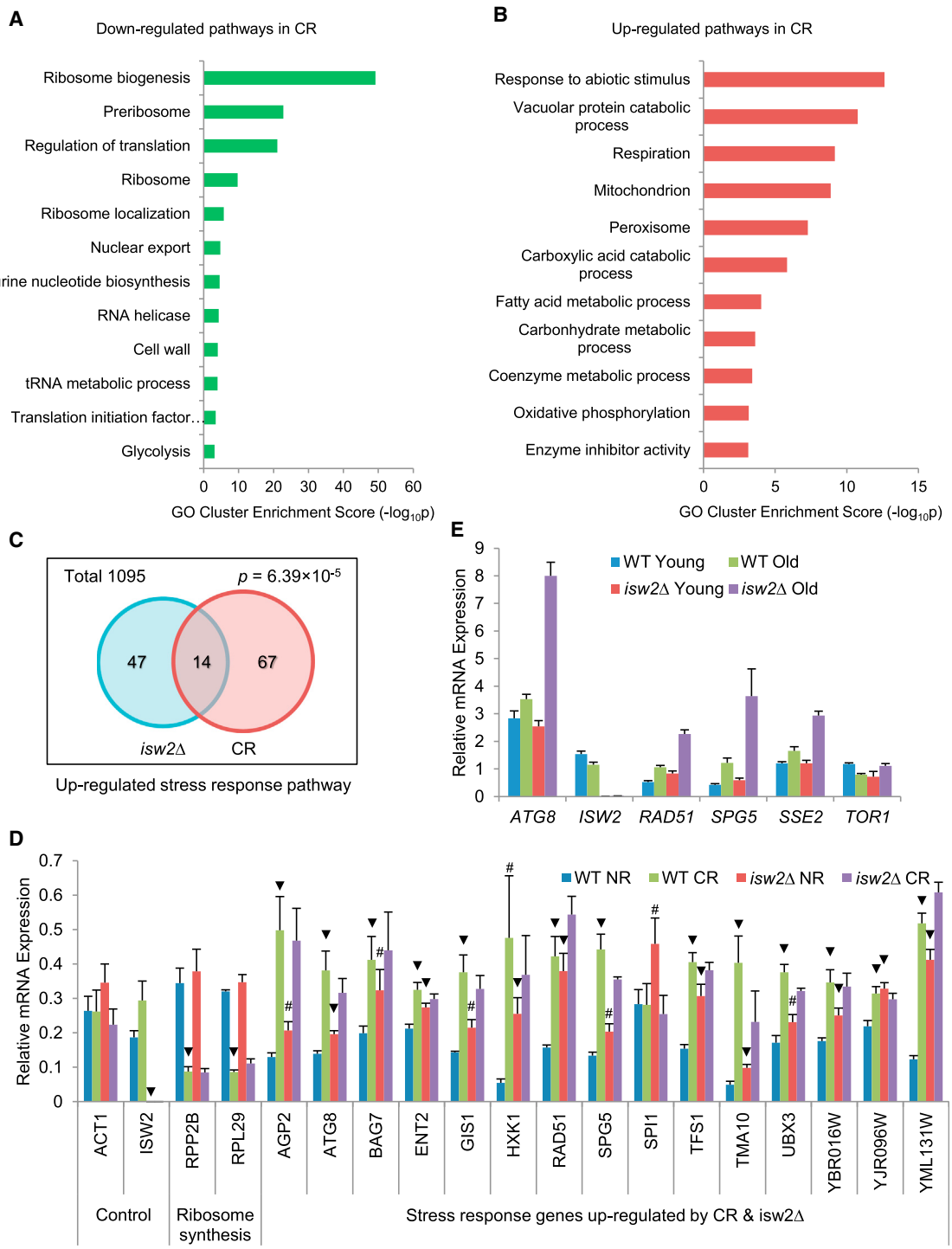


Figure 4. *ISW2* Deletion Partially Mimics CR and Potentiates Stress Response in Old Cells

(A and B) GO clustering analysis for downregulated (A) and upregulated (B) genes in CR. GO categories with $p < 0.001$ were included in enrichment score.

(C) Venn diagram showing statistically significant overlap between upregulated stress/abiotic stimulus response pathways in *isw2Δ* cells (61 genes) and in cells grown in CR conditions (81 genes).

(D) Validation of gene expression by qPCR for overlapped genes. #$p < 0.05$, ▼$p < 0.01$, compared to WT NR. Error bars, SEM.

(E) Gene expression for young and old, WT, and *isw2Δ* cells. Error bars, SEM. See also Figure S4 and Tables S3 and S4.

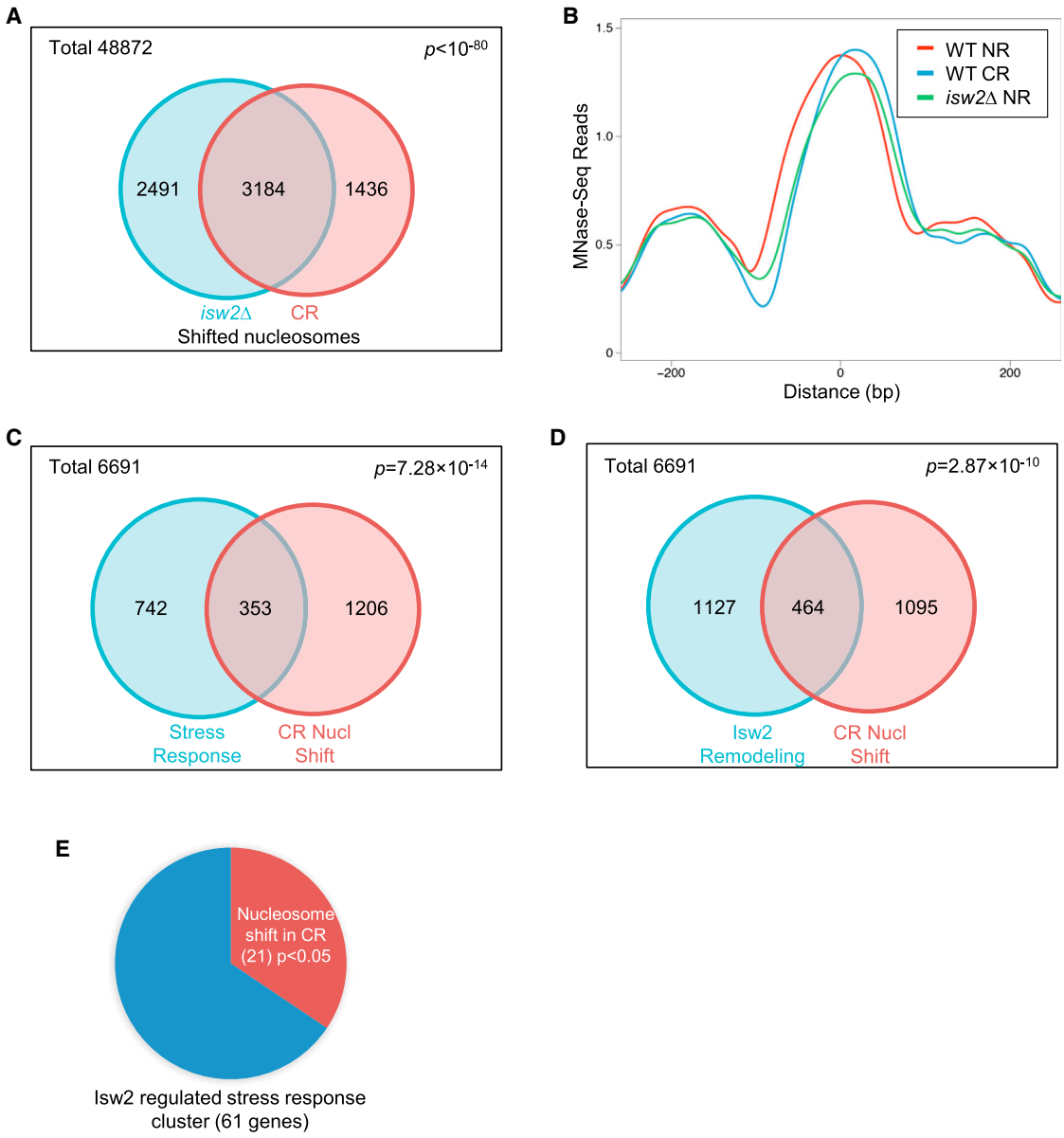


Figure 5. Genome-Wide Nucleosome Mapping for Calorie-Restricted Cells Shows Nucleosome Positioning Shifts Also Found in *isw2Δ* Cells

(A) Venn diagram showing statistically significant shifted promoter and ORF nucleosomes in both *isw2Δ* NR (SC with 2% glucose) cells and WT CR (SC with 0.05% glucose) cells.

(B) Profiles of 3,184 nucleosomes at promoters and ORFs shifted in both WT CR cells and *isw2Δ* NR cells.

(C) Statistically significant enrichment for response to stress/abiotic stimulus GO cluster (left circle; 1,095 genes) in genes showing nucleosome positioning shifts in CR (right circle; 1,559 genes).

(D) Statistically significant overlap between genes showing nucleosome positioning shifts in CR (left circle; 1,591 genes) and genes with Isw2 remodeling activities (right circle; 1,559 genes).

(E) Pie chart showing the fraction of Isw2-regulated stress response genes also bearing shifted nucleosomes in CR. See also Figure S5.

caused nucleosome positioning changes at 1,187 gene loci across the genome, more than half of which were specifically bound by Isw2 (Whitehouse et al., 2007). Since we found that CR led to transcriptional induction of stress response genes similar to those induced in *isw2Δ* cells, we then tested whether comparable chromatin conformation changes occur in CR cells and *isw2Δ* cells. Using a similar method of MNase/ExoIII digestion of chromatin (Whitehouse et al., 2007) followed by next generation sequencing, we found nucleosome positioning shifts (20–100 bp) at 1,291 genes in cells grown under CR conditions, compared to NR. Similar shifts were also seen in *isw2Δ* cells grown under NR conditions. Specifically, of 4,620 nucleosomes showing changed positioning at promoters and ORFs in these 1,291 genes in WT CR cells, a significant portion of them, 3,814 nucleosomes, also shifted in *isw2Δ* cells under NR conditions (Figure 5A). On average, these nucleosomes altered their

positions by 40.3 bp (Figure 5B). GO analysis showed a strong enrichment for stress response genes (Figure 5C). We then compared genes showing nucleosome shifts in CR with genes containing the previously defined Isw2 remodeling regions (Whitehouse et al., 2007) and found a significant overlap (Figure 5D). To further focus on the 61 stress response genes upregulated in *isw2Δ* (Figure 4C), we found that a significant portion (21 genes, 34%) also showed nucleosome positioning shifts under CR conditions (Figure 5E). Examples of similarly altered nucleosomes in CR as in *isw2Δ* NR are shown from (1) stress response genes upregulated in both CR and *isw2Δ* cells identified in our study (Figure S5A), (2) Isw2-regulated genes (Fazzio et al., 2001) (Figure S5B), and (3) genes showing Isw2-dependent chromatin remodeling (Whitehouse et al., 2007) (Figure S5C). These observations suggest that CR generated a chromatin conformation partially resembling the alterations caused by *isw2Δ*, supporting the model that Isw2 is one of the downstream effectors of CR.

Implications of a Conserved Longevity Mechanism in Complex Eukaryotes

We next investigated the effect of Isw2 on aging and stress response pathways in other eukaryotes. A dramatic difference between yeast replicative aging and human aging is that yeast telomeres do not shorten with replicative age (Laun et al., 2007). However, yeast telomerase mutants (e.g., *tlc1Δ* and *est2Δ*) senesce in response to progressive telomere shortening, providing a model for human cellular senescence (Teixeira, 2013). Hence, we tested whether *isw2Δ* could delay replicative senescence in *tlc1Δ* cells. Telomere shortening in *tlc1Δ* mutants leads to a crisis involving the death of most cells, followed by emergence of rare survivors of the crisis, which use telomere recombination to relengthen chromosome ends (Figure 6A, dark blue). Intriguingly, *isw2Δ* delayed replicative senescence in *tlc1Δ* cells, extending the crisis from 60 population doubling (PD) to 70 PD (Figure 6A, dark green; see also Figures S6A and S6B). Deletion of *RAD52*, a gene facilitating Rad51 and required for HR-based DNA repair (Symington, 2002), led to much faster crisis, at 45 PD, as reported previously (Figure 6A, dark red) (Le et al., 1999), and the more severe crisis in *rad52Δ* was not significantly extended by *isw2Δ* (Figure 6A, dark purple), similar to the findings in the replicative aging model (Figure 3E). These results indicate that the mechanism of lifespan extension conferred by *isw2Δ* also has a role in a genetic model where telomere length limits lifespan and thus suggest possible relevance in mammalian cellular senescence.

In order to gain more insights into the role of the ISWI class of enzymes in aging of metazoans, we determined how the lifespan of *C. elegans* was affected by RNAi-mediated knockdown of a key subunit of the putative complex homologous to the yeast ISW2 complex (Figure 6B, insert). The worm ISWI class gene *isw-1* has been characterized genetically, together with *nurf-1*, an ortholog of *NURF301* in the fruit fly *D. melanogaster* (Andersen et al., 2006). However, knocking down *isw-1* did not affect worm lifespan (Curran et al., 2009). In the yeast ISW2 complex, the auxiliary subunit Itc1 is required for its in vivo function (Gelbart et al., 2001) and is homologous to ACF1 in the *Drosophila* CHRAC/ACF complex. A search for worm homologs of yeast Itc1 and fruit fly ACF1 led us to the worm gene *athp-2* (H20J04.2), which shares a maximum of 42% identity (62% similarity) to ACF1 and 28% identity (47% similarity) to Itc1 in amino acid sequence, with the best E values of 3×10^{-34} and 9×10^{-6}, respectively. Multiple alignment of worm ATHP-2 with yeast Itc1, fly ACF1, and mammalian BAZ1A confirms their homology (Figure S6C). We hypothesized that, similar to fruit fly, worm ISW-1 also forms multiple complexes, including an orthologous NURF complex with NURF-1 and an ACF/CHRAC complex with ATHP-2. We therefore focused on the specific orthologous complex and knocked down *athp-2* with the RNAi clone H20J04.f (Figure 6B; insert shows mRNA levels were reduced compared to control). We found that *athp-2* knockdown significantly extended worm lifespan by 15% (Figure 6B) when the DNA synthesis inhibitor FUdR (5-fluorodeoxyuridine) was used in the worm lifespan assay to prevent the growth of progeny (Gandhi et al., 1980). Since FUdR has documented effects on lifespan for certain mutants (Aitlhadj and Stürzenbaum, 2010), we performed worm lifespan assays without FUdR and observed consistent lifespan extension (Figure 6C). These results suggested that the function of ISW2/ACF/CHRAC in aging regulation might be evolutionarily conserved.

To investigate possible functional conservation of the ISW2/ACF/CHRAC orthologous complex in a mammalian system in repressing stress response genes, we knocked down the human Itc1/ACF1 ortholog *BAZ1A* in IMR90 cells (primary fetal lung fibroblasts) by lentivirus-based shRNA (Figure 6D, left side). Using two validated shRNAs, #34279 and #34282, compared to a non-targeting control (NT), we tested expression changes for a list of 23 stress response genes. These genes included ten human homologs of yeast stress response genes upregulated in both *isw2Δ* and CR (Table S4), as well as 13 stress response genes whose promoters are bound by the catalytic subunit SMARCA5 (hSNF2H). These 13 genes were selected from a significantly enriched stress response GO cluster consisting of 54 genes from a GO analysis for all gene promoters bound by SMARCA5 (Table S6). We found that four out of ten human homologs of yeast stress response genes (Figures 6C, marked with #, and S6D; Table S4), as well as six out of the 13 SMARCA5-bound stress response genes, showed significantly elevated expression in the *BAZ1A* knockdown cells compared to the control, as measured by RT-qPCR (Figures 6C and S6E; Table S6). Overall, among the 23 tested genes, ten were significantly activated upon *BAZ1A* knockdown, corresponding to a false discovery rate of 0.115. This observation provides further evidence for a possible conserved role of the Isw2 orthologous complex in repressing stress response pathways.

DISCUSSION

Using the yeast replicative aging model, we demonstrate a novel longevity regulation mechanism mediated by the ATP-dependent chromatin remodeling enzyme Isw2. Deletion or enzymatic inactivation of Isw2 extends lifespan. This longevity effect is the result of derepressing a cohort of stress response genes—in particular, *RAD51* in the HR-mediated DNA damage repair pathway. Further, we find that these changes in transcriptome partially mimic the stress response state of calorie-restricted cells.

Our findings provide one of the first lines of evidence that ATP-dependent chromatin remodeling complexes play direct roles in aging regulation. Indeed, as this manuscript was in preparation,

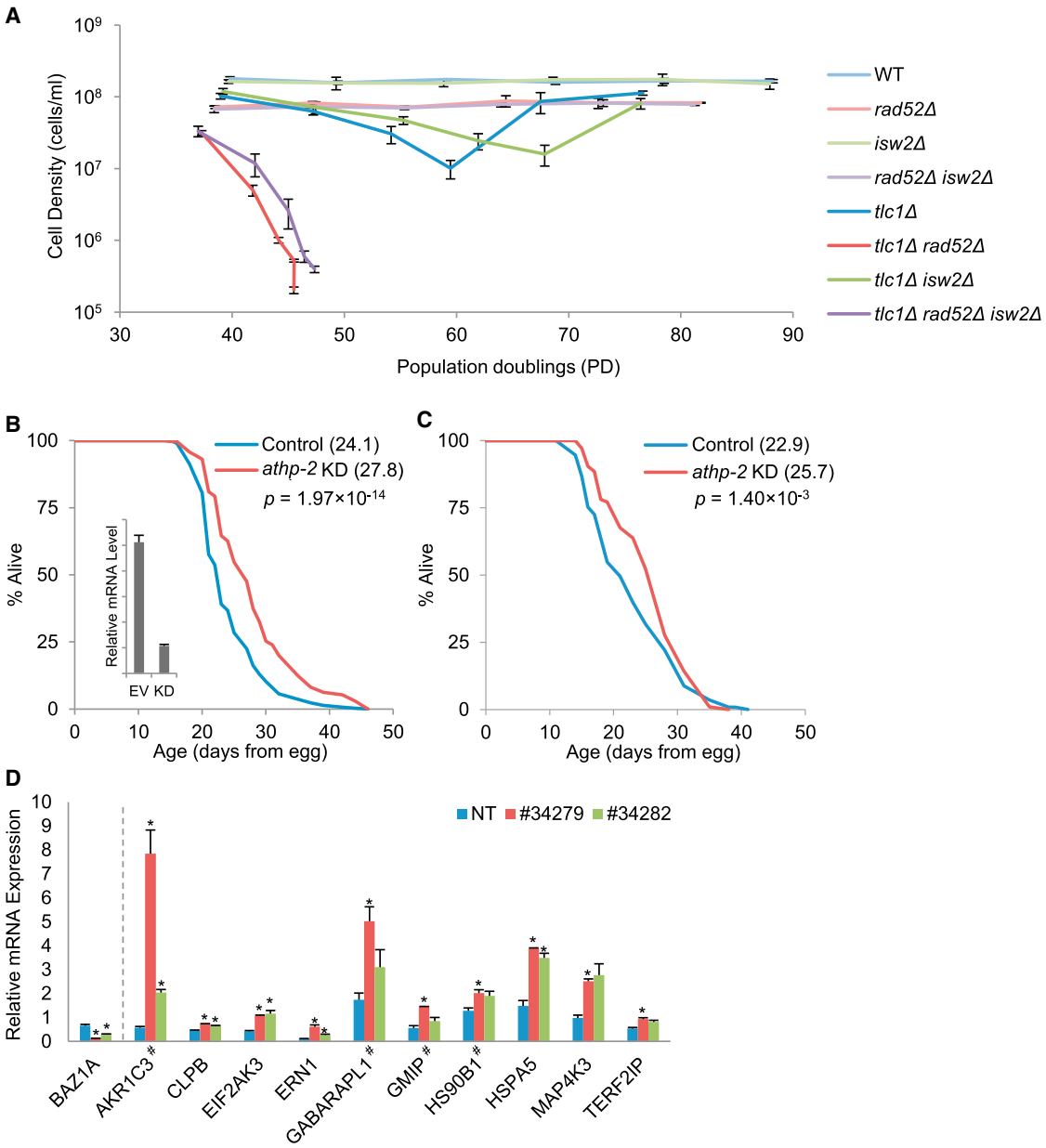


Figure 6. Functional Conservation in Other Eukaryotic Model Systems

(A) Yeast replicative senescence assay. Cells of the indicated genotypes were sporulated from a *TLC1/tlc1Δ ISW2/isw2Δ RAD52/rad52Δ* diploid. The mean values for the indicated number of spore products are shown: n = 2 for WT, *rad52Δ*, *isw2Δ*, and *isw2Δ rad52Δ*; n = 6 for *tlc1Δ*, *tlc1Δ isw2Δ*, and *tlc1Δ rad52Δ*; n = 9 for *tlc1Δ isw2Δ rad52Δ*. Significance test: *tlc1Δ isw2Δ versus tlc1Δ*, p = 0.04; *rad52Δ tlc1Δ versus rad52Δ tlc1Δ isw2Δ*, p = 0.37. Error bars, SEM.

(B and C) Mean lifespan (in parenthesis) for *C. elegans* treated with RNAi targeting *athp-2* (KD) or empty vector control (EV). Experiments were performed with (B) or without (C) FUdR. Inset in (B) shows knockdown efficiency tested by RT-qPCR. Error bars, SEM.

(D) Relative mRNA expression for selected stress response genes in human fibroblast IMR90 (harvested at estimated population doublings of 38–40) treated with lentiviruses carrying either non-targeting control (NT) or shRNA (#34279 and #34282) targeting BAZ1A. *p < 0.05. The symbol "#" indicates genes homologous to yeast stress response genes upregulated in both *isw2Δ* and CR. See also Figure S6 and Tables S4–S6. Error bars, SEM.

Riedel et al. (2013) showed that the SWI-SNF complex acts as a transcription cofactor for DAF-16/FOXO in *C. elegans* and is essential for DAF-16-mediated stress response and longevity. Here, we demonstrate that the distinct ISWI subfamily of the ATP-dependent remodeling enzymes negatively regulates longevity through stress response pathways and contributes to the longevity effect of CR (Figures 7A and 7B). In addition, we also provide evidence that this aging modulation mechanism might be evolutionarily conserved among eukaryotes. Since deletion of *ISW2*, or inhibition of enzymatic activity, provided longevity benefits, Isw2 could potentially be further explored as a pharmaceutical target for clinical applications.

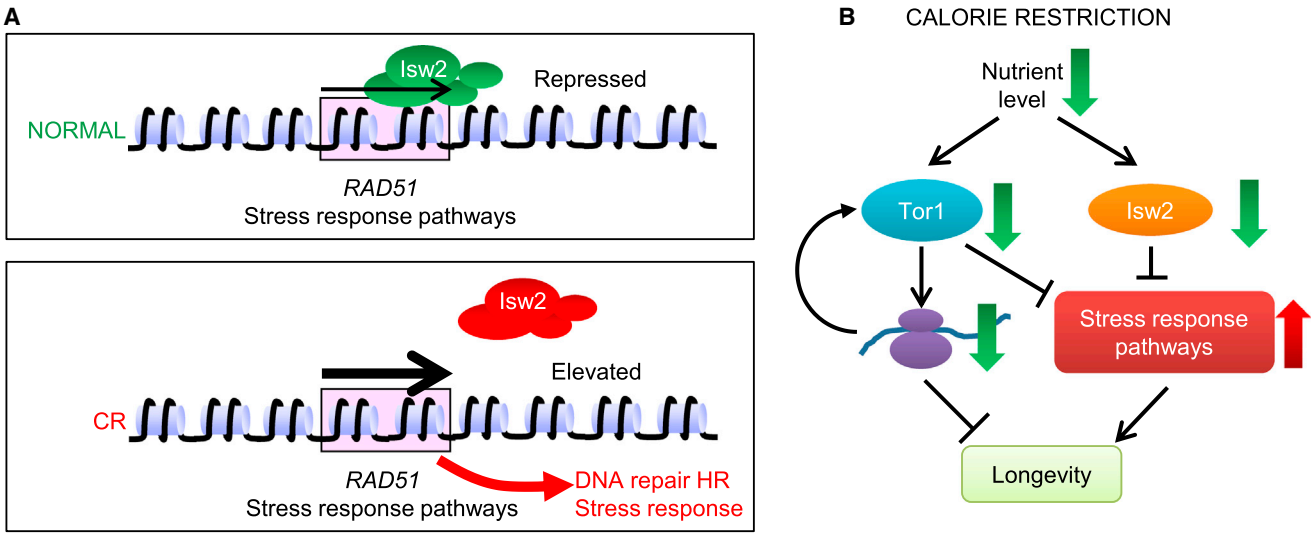


Figure 7. Models of the Isw2-Regulated Pathway during CR

(A) Effect of ISW2 complex on its stress response gene targets in NR (upper) and in CR cells (lower). We found that Isw2 represses a cohort of stress response genes, including *RAD51*, which are induced either by *isw2Δ* or CR.

(B) During CR, reduced nutrient levels are signaled to TOR signaling pathway and the Isw2-regulated pathway, resulting in downregulation of both pathways. Decreased TOR signaling results in reduced ribosome biosysthesis and protein translation. Downregulated Isw2 causes derepression of stress response genes, including *RAD51*, as shown in (A). Both reduced protein synthesis and elevated stress response promote longevity.

Inactivation of Isw2 Potentiates Stress Response during Aging

After ruling out a number of possible Isw2 functions that may extend lifespan when removed, we found that Isw2 moderately represses a cohort of stress response genes (Figures S2F and S2G). Thus, upon inactivation of Isw2, these genes become activated, rendering cells in a constitutive moderately stress-responsive state (Figure 7A, lower panel). Pre-exposing cells to mild stress states has been shown to be beneficial for surviving future stronger stresses, such as heat shock, osmotic, oxidative, and genotoxic stresses, which is a phenomenon known as hormesis (Rattan, 2008). Our findings are consistent with the idea that reduction of Isw2 activity induces an elevated stress response state to mimic a hormetic effect, providing increased resistance to more severe genotoxic stresses during aging (Figure 4E).

We show in this study that *isw2Δ* increases resistance to genotoxic stresses by activation of the HR-based DNA repair mechanism (Figures 3 and S3), which in turn promotes longevity (Figure 7B). In addition, Isw2 represses a number of other stress response pathways, including oxidative stress, heat shock, osmotic stress, autophagy, etc. (Table S2). However, no phenotype was obvious for these stress conditions (data not shown); thus, either the appropriate test condition was not found or the elevated response for other stress pathways by *isw2Δ* was subtle. Indeed, the increased resistance to MMS and CPT by *isw2Δ* was modest (Figures 3A, 3B, S3C, and S3D), providing a potential explanation as to why the phenotype was not observed in previous tests (Vincent et al., 2008). Hence, it appears that loss of Isw2 results in slight constitutive elevation of numerous stress pathways to extend lifespan.

Robust cellular stress response capability has been associated with longevity. An excellent example is the naked mole rat, which lives for nearly 30 years in captivity and is the longest-lived rodent. Fibroblasts obtained from these animals show much stronger resistance to various stressors compared to mouse (Lewis et al., 2012). In support of this paradigm, our results show that *isw2Δ* extends yeast replicative lifespan by activating cellular stress response pathways, especially HR-based DNA damage repair. Furthermore, old *isw2Δ* cells exhibit much higher expression of stress response genes when compared to old WT cells (Figure 4E). This suggests that their derepression resulting from inactivation of Isw2 can lead to a more potent response to stress when needed during aging, hence conferring longevity benefits.

Isw2 Regulates Stress Response Pathways Exploited by CR

Downregulation of the TOR signaling pathway by CR through Tor1 and Sch9, and the downstream ribosome biogenesis, has been extensively studied (Figure 7B) (Steffen et al., 2008). In fact, yeast has evolved a sophisticated ribosome biogenesis regulation system called ribi regulon that precisely adjusts ribosome abundance to availability of nutrients (Lempiäinen and Shore, 2009). Thus, TOR signaling plays a critical role in mediating the effects of CR. One important experiment highlighting this mechanistic pathway is the epistasis of *tor1Δ* to CR, that is, *tor1Δ* cells under CR conditions show the same lengthened lifespan as either *tor1Δ* or CR alone (Kaeberlein, 2010). Our study shows that *isw2Δ* displays a similar relationship to CR (Figures 2A–2D), suggesting that Isw2 regulates an aging pathway downstream of CR.

We also show that *isw2Δ* and *tor1Δ* have a combinatorial effect on lifespan extension (Figure 2E), suggesting parallel pathways. This would seem to be conflicting with the epistasis of either *tor1Δ* or *isw2Δ* with CR. To explain this, we hypothesize that *tor1Δ* and *isw2Δ* phenocopy two separate events, each

promoted by CR, and that CR may exert an effect from each pathway to a lesser extent compared to the gene deletion. Thus, both pathways act downstream of CR to mediate lifespan extension in yeast. In addition, although *tor1Δ* and *isw2Δ* each mimic CR, the presence of both *TOR1* and *ISW2* genes may be important to realize the benefits of CR. Hence, deletion of either one blocks the CR longevity effect.

It has been well established that CR provides cellular protection and stress resistance (Lee and Longo, 2011). Since *isw2Δ* showed a partial overlap with CR-regulated stress response, we compared the correlation at the chromatin level and found similar nucleosome positioning shifts between *isw2Δ* and CR for a gene set that is also enriched for stress response (Figures 5A–5C), a significant fraction of which appear to be attributable to changes in Isw2 activity under CR conditions (Figure 5D). However, not all Isw2-regulated genes showed similar nucleosome positioning shifts under CR (Figure S5), which is likely due to additional transcription regulation for Isw2 target genes. Indeed, *isw2Δ* alone has a very modest effect on gene transcription in general, and other transcription repressors, such as Rpd3 and Fkh2, are known to work in parallel with Isw2 (Fazzio et al., 2001; Sherriff et al., 2007) and to regulate stress responses (Postnikoff et al., 2012; Ruiz-Roig et al., 2010). However, neither *rpd3Δ* nor *fkh2Δ* extended replicative lifespan (data not shown), suggesting that Isw2 is important in conferring the longevity effect. Overall, our evidence supports the model that regulation of stress response by Isw2 is one of the downstream effects of CR that contributes to longevity.

Implications to Aging in Higher Eukaryotes

Yeast Isw2 and the ISWI class of chromatin remodeling ATPases are evolutionarily conserved. Enzyme complexes homologous to ISW2 have been characterized in plants, worms, flies, mice, and humans. However, the degree of diversity and complexity associated with this class of enzymes also increases significantly; hence, genetic and longevity investigations in these more complex organisms are difficult. We sought to use several eukaryotic model systems to test functional conservation of Isw2 orthologous enzyme complexes.

The nematode *C. elegans* is a popular animal model for aging research due to its short lifespan, easy lifespan determination, and the availability of RNAi libraries. Studies in worm aging have led to discoveries of many aging regulation and CR pathways, such as insulin/IGF signaling, autophagy, mitochondria, and histone methylation (Tissenbaum, 2012). Thus, we tested the worm aging phenotype. The observed lifespan extension by knocking down the Itc1/ACF1 ortholog *athp-2* suggests that the ISW2/ACF/CHRAC complex may function in a conserved mechanism to regulate aging in eukaryotes (Figure 6B).

Although mammalian cellular senescence has long been thought to be a cancer-suppression mechanism, recent evidence also links senescence to aging (Campisi, 2013). Therefore, it is intriguing to test whether elevated stress responses in *isw2Δ* cells can mitigate the stress associated with cellular senescence. The yeast *tlc1Δ* telomerase mutant is valuable as a cellular senescence model to initially test this idea. Our data shows that *isw2Δ* delays replicative senescence and is also dependent on HR (Figure 6A). This result suggests that the elevated stress response in *isw2Δ* antagonizes senescence caused by telomere shortening, leading to the hypothesis that a similar pathway might function in mammalian senescence and aging.

Homozygous knockout of mammalian *SMARCA5* (*SNF2H*), the closest homolog to yeast Isw2, is embryonic lethal (Stopka and Skoultchi, 2003). Adding to this complexity, SMARCA5 is a component of, at minimum, five distinct complexes. Hence, we instead focused on the *BAZ1A* gene, which encodes a subunit orthologous to yeast Itc1 and fruit fly ACF1, and knocked down expression in human primary lung fibroblasts IMR90 in their early passages. The moderate increase in several stress response genes upon *BAZ1A* knockdown (Figure 6D) supports the hypothesis of a conserved mechanism of stress response regulation by ISW2/ACF/CHRAC chromatin remodeling enzyme complexes. Future studies will establish whether this pathway may be one of the antiaging mechanisms in mammals that can be exploited by DR and, potentially, clinical intervention in age-associated human diseases.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media

All yeast mutant strains were derived from BY4741/2, except as noted. Strains YWD781/2 were made by integrating plasmid pWD210, containing the *RAD51* gene and flanking sequences. See Table S7 for strain details. Standard SC solid or liquid media were used in yeast experiments, except as noted.

Yeast Replicative and Senescent Lifespan

Assays were performed as described (Kaeberlein et al., 1999; Kozak et al., 2010).

Yeast Phenotype Assay

Equal amounts of cells were serially diluted, spotted on SC agar media with 2% (NR) or 0.05% (CR) D-glucose and indicated compounds, and incubated at 30°C or 22°C for 2–5 days.

Isolation of Young and Old Cells

Young and old cells were purified as described (Dang et al., 2009) with the following changes: SC media with 2% (NR) or 0.05% (CR) D-glucose were used; cell density was maintained within logarithmic growth phase (OD_{600} < 2.0 for NR and OD_{600} < 0.2 for CR); bud scars were stained with WGA Alexa Fluor 488 (Life Technologies).

Worm Lifespan Assay with *athp-2* RNAi Knockdown

RNAi feeding bacteria were obtained from the Ahringer RNAi library and verified by sequencing. *C. elegans* strain N2 was used in lifespan experiments conducted at 20°C as described (Sutphin and Kaeberlein, 2009). Worms prepared in parallel were harvested for RNA analysis. In experiments without FUdR, adult animals were transferred to fresh RNAi plates at each scoring when eggs or larvae were present.

IMR90 Culturing and Lentivirus Knockdown

IMR90 cells were cultured in standard conditions with 3% O_2. Control (non-mammalian target) and knockdown (TRC #34279 and #34282 targeting human BAZ1A) shRNA lentiviruses were produced by standard methods. Infected cells were selected with puromycin and harvested after two passages.

RNA Extraction, RT-PCR, and Quantitative Real-Time PCR Analysis

Yeast cells were lysed in QIAzol (QIAGEN) by beadbeating with 0.5 mm diameter zirconia/silica beads (BioSpec) four cycles (1 min beating, 2 min pause on ice). Total RNA was purified with miRNeasy Mini Kit (QIAGEN). Worm RNA was prepared similarly, except using 1.0 mm diameter beads. IMR90 cell pellets were lysed with QIAshredder (QIAGEN) and purified with RNeasy Mini Kit (QIAGEN). TaqMan Reverse Transcription Reagents (Life Technologies) were used to synthesize cDNA from 1 μg purified RNA with random hexamers. Relative changes in mRNA levels were determined by

quantitative real-time PCR. Yeast gene expression data were normalized to *ACT1*; worm data were normalized to the ribosomal RNA *rrn-1.1*; human data were normalized to the average of β-actin and GAPDH. See real-time PCR primers in Table S8.

Ribosomal DNA Copy Number

Yeast genomic DNA was extracted by standard methods and diluted 10-fold before real-time PCR. Relative copy number was estimated by normalizing to an intergenic region in chromosome V.

Preparation of RNA-seq and Nucleosome-seq Libraries and Next-Gen Sequencing

Polyadenylated RNA was enriched from 10 μg purified total RNA with Poly(A) Purist Kit (Life Technologies) and treated with Fragmentation Reagents (Life Technologies). Strand-specific RNA-seq libraries were prepared with Small RNA Sample Preparation Kit (Illumina). Sequencing was done in 50 bp single-end format on Illumina HiSeq 2000.

For nucleosome-seq, chromatin was digested with MNase and ExoIII as described (Whitehouse et al., 2007), and mononucleosome-sized DNA was purified by agarose gel electrophoresis. Sequencing libraries were prepared with NEBNext DNA Library Prep Master Mix Set for Illumina (NEB) and Multiplexing Sample Preparation Oligonucleotide Kit (Illumina). Sequencing was done in 100 bp paired-end format on Illumina HiSeq 2000.

Bioinformatics and Data Analysis

Sequencing read mapping and quantification of gene expression in FPKM by RNA-seq was performed with Bowtie and Cufflink, respectively, with default settings. Subsequent RNA-seq analysis was based on the geometric mean of FPKM from two biological replicates and included genes that scored in both replicates.

An upper and lower 1-SD cutoff was used to generate gene lists for GO analysis by DAVID functional annotation tool (http://david.abcc.ncifcrf.gov/). The Functional Annotation Clustering option was used to group similar categories. An enrichment score for each cluster was given as a mean of $-\log_{10}p$ of member GO categories with $p < 0.01$ or 0.001. Nucleosome positions were called from the nucleosome-seq data using NucPosSimulator (Schöpflin et al., 2013). Nucleosomes were called that significantly shifted positions in WT_CR from WT_NR (20–100 bp). The average shift of nucleosomes was calculated over the promoter and gene body regions. Meta-nucleosome analysis included only shifts found in both WT_CR and *isw2Δ*.

Fisher's exact test was used to assess the null hypothesis (i.e., that the proportion of genes in common for two independent observations is less than or equal to random chance). Yeast replicative lifespan and worm lifespan p values were determined by the Wilcoxon rank-sum test. Statistical significance p values for non-high-throughput data and non-lifespan data were calculated by unpaired two-tailed Student's t test. All error bars are SEM.

ACCESSION NUMBERS

The GEO accession number for the RNA-seq and nucleosome-Seq data in this study is GSE53721.

SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures, eight tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2014.04.004.

ACKNOWLEDGMENTS

We thank Dr. Matteo Cesaroni in the R.S. lab and Dr. Wei Liu in the W.D. lab for technical assistance. We also thank members of the B.K.K. and M.K. labs who participated in yeast lifespan experiments. University of Pennsylvania PGFI provided Next-Gen Sequencing service. The Wistar Institute Molecular Screening Facility provided shRNA lentivirus. This work was supported by NIH grants (P01AG031862 to S.L.B. and F.B.J.; K99AG037646 to W.D.; R01AG039390 to M.K.; R01AG043080 to B.K.K.; and T32AG000057 for G.L.S.) and an Ellison Medical Foundation grant (S.L.B.).

Received: November 1, 2012
Revised: May 30, 2013
Accepted: March 31, 2014
Published: May 8, 2014

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