Genome Instability Is Promoted by the Chromatin-Binding Protein Spn1 in *Saccharomyces cerevisiae*

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ABSTRACT Cells expend a large amount of energy to maintain their DNA sequence. DNA repair pathways, cell cycle checkpoint activation, proofreading polymerases, and chromatin structure are ways in which the cell minimizes changes to the genome. During replication, the DNA-damage tolerance pathway allows the replication forks to bypass damage on the template strand. This avoids prolonged replication fork stalling, which can contribute to genome instability. The DNA-damage tolerance pathway includes two subpathways: translesion synthesis and template switch. Post-translational modification of PCNA and the histone tails, cell cycle phase, and local DNA structure have all been shown to influence subpathway choice. Chromatin architecture contributes to maintaining genome stability by providing physical protection of the DNA and by regulating DNA-processing pathways. As such, chromatin-binding factors have been implicated in maintaining genome stability. Using *Saccharomyces cerevisiae*, we examined the role of Spn1 (Suppresses postrecruitment gene number 1), a chromatin-binding and transcription elongation factor, in DNA-damage tolerance. Expression of a mutant allele of *SPN1* results in increased resistance to the DNA-damaging agent methyl methanesulfonate, lower spontaneous and damage-induced mutation rates, along with increased chronological life span. We attribute these effects to an increased usage of the template switch branch of the DNA-damage tolerance pathway in the *spn1* strain. This provides evidence for a role of wild-type Spn1 in promoting genome instability, as well as having ties to overcoming replication stress and contributing to chronological aging.

KEYWORDS yeast; genome instability; DNA-damage tolerance; methyl methanesulfonate

MAINTEINING the genome of a cell is of fundamental importance. Instability within the genome contributes to cancer, aging, and genetic diseases (Aguilera and Garcia-Muse 2013; Vijg and Suh 2013). Point mutations, deletions, duplications, and translocations are all forms of genome instability (Aguilera and Garcia-Muse 2013; Skoneczna *et al.*

2015). Overlapping and conserved DNA-repair pathways, DNA-damage activated cell cycle checkpoints, proofreading DNA polymerases, and chromatin structure are some of the ways in which the cell minimizes changes to the genome (Kawasaki and Sugino 2001; Aguilera and Garcia-Muse 2013; Polo and Almouzni 2015; Chatterjee and Walker 2017). It is important to note that some level of genome instability is necessary to fuel genetic diversification and evolution (Skoneczna *et al.* 2015).

DNA base lesions, breaks, strand cross-links and gaps, secondary structures, and strongly bound proteins are obstacles for the replication machinery (Hustedt *et al.* 2013; Brambati *et al.* 2015; Chatterjee and Walker 2017). Replication stress caused by these structures can result in genome instability and/or cell death (Hustedt *et al.* 2013; Zeman and Cimprich 2014). Furthermore, intermediate steps of

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DNA-damage repair can be detrimental to the cell if performed without proper coordination with replication fork progression (Ulrich 2011). For example, the cleavage of the phosphate backbone in the single-stranded DNA (ssDNA) template would result in a double-stranded break, putting the cell at risk for chromosomal rearrangements (Branzei and Szakal 2016). The DNA-damage tolerance (DDT) pathway provides mechanisms for cells to circumvent blocks to the DNA replication fork (Ulrich 2011; Bi 2015; Xu *et al.* 2015; Branzei and Psakhye 2016; Branzei and Szakal 2016). DDT is different from other repair pathways since the initial damage is not immediately repaired. The DDT pathway includes two subpathways: the translesion synthesis (TLS) branch (error prone) and a template switch branch (error free) (Lee and Myung 2008; Bi 2015; Xu *et al.* 2015; Branzei and Szakal 2016).

TLS utilizes polymerase switching to overcome replication blocks using the lower-fidelity polymerases POL ζ (Rev3/Rev7) and Rev1 (Prakash *et al.* 2005; Xu *et al.* 2015). The TLS branch is considered error prone as it can potentially introduce a mismatched dNTP via the low-fidelity polymerase. TLS can contribute to upwards of one-half of the point mutations accumulated by a cell at each division cycle (Stone *et al.* 2012). Template switch (the error-free subpathway) utilizes the newly synthesized sister strand as a template for DNA synthesis past an obstruction. This requires homologous recombination (HR) factors for strand invasion and downstream DNA-processing factors to resolve recombination intermediates (Branzei *et al.* 2008; Branzei and Szakal 2016). The error-free subpathway has been determined to be different from traditional recombination repair pathways through genetic studies (Branzei and Szakal 2016). How the cell determines which DDT subpathway to use is still unclear; however, post-translational modification of PCNA and histone tails, cell cycle phase, and local DNA structure have all been shown to influence subpathway choice (Daigaku *et al.* 2010; Gonzalez-Huici *et al.* 2014; Meas *et al.* 2015; Xu *et al.* 2015; Branzei and Szakal 2016; Hung *et al.* 2017).

Spn1 (Suppresses postrecruitment gene number 1) is a transcription elongation and chromatin-binding factor (Fischbeck *et al.* 2002; Krogan *et al.* 2002; Li *et al.* 2018). SPN1 is essential for cellular viability and is conserved from yeast to humans (Fischbeck *et al.* 2002; Liu *et al.* 2007; Pujari *et al.* 2010). Both yeast and human Spn1 consist of two intrinsically disordered tails with an ordered central core domain (Pujari *et al.* 2010) (Figure 1A), and have been shown to associate with chromatin (Kubota *et al.* 2012; Alabert *et al.* 2014; Dungrawala *et al.* 2015). Human Spn1 recruits the HYPB/Setd2 methyltransferase required for H3K36 trimethylation (H3K36me3) (Yoh *et al.* 2008). Through Setd2 activity, H3K36me3 has been shown to recruit HR factors to double-stranded breaks (Pfister *et al.* 2014). Yeast Spn1 binds histones, DNA, and nucleosomes (Li *et al.* 2018), and associates with RNA Polymerase II (RNAPII) and the histone chaperone Spt6 (Diebold *et al.* 2010; McDonald *et al.* 2010; Pujari *et al.* 2010; Li *et al.* 2018). Spn1 maintains repressive chromatin in

Figure 1 Expression of Spn1¹⁴¹⁻³⁰⁵ suppresses sensitivity to the DNA-damaging agent, methyl methanesulfonate (MMS). (A) Schematic of Spn1 and Spn1¹⁴¹⁻³⁰⁵. (B) Ten-fold serial dilutions of cells expressing Spn1 or Spn1¹⁴¹⁻³⁰⁵ were spotted onto YPD and YPD plates containing camptothecin (CPT), hydrogen peroxide (H₂O₂), or increasing concentrations of MMS. For UV damage, cells were spotted onto YPD plates and exposed to UV. (C) Quantification of immunoblot showing H2A S129 phosphorylation levels before and after exposure to 0.1% MMS in cells expressing Spn1 or Spn1¹⁴¹⁻³⁰⁵. H2A S129 phosphorylation signal is normalized to TBP signal as a loading control. The resulting ratio is used to calculate an average ratio per strain in the tested condition. Four to five biological replicates were assessed per strain in each condition; averages and SD were normalized to the wild type strain grown in YPD, which is scaled to 1.

human cells (Gérard *et al.* 2015), and loss of the DNA-, histone- and nucleosome-binding functions in yeast results in increased nucleosome occupancy at the activated *CYC1* locus (Li *et al.* 2018). Yeast Spn1 has been shown to genetically and/or physically interact with the ATP-dependent chromatin remodelers INO80 (Costanzo *et al.* 2016) and SWR-C/SWR1 (Collins *et al.* 2007), both of which are involved in DNA double-strand break repair (van Attikum *et al.* 2007). In addition, SPN1 genetically interacts with genes whose protein products are involved in DNA repair, such as Rad23

(Prakash and Prakash 2000; Collins *et al.* 2007), Pol ϵ (Dubarry *et al.* 2015), and the histone chaperones CAF-1, Asf1 (Kim and Haber 2009; Li *et al.* 2018), and FACT (C. A. Radebaugh, unpublished results; Dinant *et al.* 2013). Together, these observations raise the possibility that Spn1 may influence DNA repair functions.

In this study, we examined a role for Spn1 in genome instability in *Saccharomyces cerevisiae*. We utilized an allele, *spn1*¹⁴¹⁻³⁰⁵, which encodes a derivative that lacks the intrinsically disordered tails (Figure 1A) (Fischbeck *et al.* 2002; Li *et al.* 2018). Expression of the mutant protein Spn1¹⁴¹⁻³⁰⁵ led to increased resistance to the DNA-damaging agent methyl methanesulfonate (MMS). We tested genetic interactions between *SPN1* and genes involved in base excision repair (BER), nucleotide excision repair (NER), HR, and the DDT pathway. Through the analyses of these genetic interactions, the resistance to MMS observed in the *spn1*¹⁴¹⁻³⁰⁵ strain was determined to be dependent on the DDT pathway and HR factors. Furthermore, the *spn1*¹⁴¹⁻³⁰⁵ strain displayed decreased spontaneous and damage-induced mutation rates, and increased chronological longevity. Taken together, our results indicate a role for Spn1 in promoting genome instability by influencing DDT subpathway selection.

Materials and Methods

Yeast culturing and strains

All strains were grown and experiments were performed in yeast peptone dextrose (YPD; 2% dextrose) media at 30° unless otherwise indicated. A description of the yeast strains, plasmids, and DNA primers utilized in this study are provided in Supplemental Material, Tables S1, S2, and S3.

The wild-type strain BY4741 (*MATa his3 Δ 1 ura3 Δ 0 leu2 Δ 0 met15 Δ 0*) and deletion strains were purchased from Thermo Scientific Open. Strains were created as described (Zhang *et al.* 2008; Li *et al.* 2018). Briefly, strains were transformed with a covering plasmid expressing Spn1. Endogenous *SPN1* was replaced by a *LEU2* fragment flanked by *SPN1* promoter sequences by HR. Plasmids containing *SPN1* or *spn1*¹⁴¹⁻³⁰⁵ were introduced into strains by plasmid shuffling.

DNA damage exposure phenotypic assays

To assess the growth phenotypes and possible genetic interactions between *SPN1* and other mutant strains, yeast cells were grown overnight in YPD. Cultures were diluted, grown to log phase, collected by centrifugation, washed with sterile water, and diluted. Ten-fold serial dilutions were spotted onto the indicated solid media and incubated at 30°. Images of plates were taken daily. MMS, camptothecin, and hydrogen peroxide (H₂O₂) plates were made 24–48 hr before each experiment. It is important to note that some deletion strains are sensitive to MMS and hydroxyurea (HU), thus lower concentrations were used for growth assessment (as indicated in the figures). UV exposure was performed with a UVP UVLMS-38 light source at a wavelength of 254 nm. To

test if *SPN1* is dominant, strains were grown using SC-His dropout media to maintain selection for the plasmid expressing Spn1 or Spn1¹⁴¹⁻³⁰⁵. Phenotypic growth assays were performed with a minimum of three independent biological replicates and representative spots are shown in the figures.

Fluctuation analysis

Fluctuation analyses were performed to determine the rates of spontaneous and damage-induced forward mutation in the *CAN1* gene. The indicated strains were patched and grown for 24 hr on YPD. Strains were streaked to single colonies onto YPD plates and grown for 48 hr. Multiple colonies of each strain were inoculated and allowed to grow for 24 hr in 5 ml of YPD. Cells were pelleted and washed in sterile water, and appropriate dilutions were plated on YPD and SC-Arg dropout media containing 60 μ g/liter of canavanine. Colonies were counted after 2- (YPD, permissive) and 3-day (SC-Arg dropout + Can, selective) growth. Mutation rates were calculated through the Lea-Coulson method of the median (Lea and Coulson 1949) in using the FALCOR web application (Hall *et al.* 2009). Statistical significance was determined using the Mann-Whitney nonparametric Student's *t*-test using GraphPad Prism software. For damage-induced mutation rates, the same protocol was followed, except that strains were streaked onto YPD plates containing 0.001% MMS and inoculated into liquid YPD containing 0.005% MMS. Strains deleted for *MMS2* (*mms2 Δ*) were inoculated in YPD liquid containing 0.001% MMS, due to this strain's extreme sensitivity to MMS. Plates containing MMS were made < 24 hr before use.

Budding index

Strains were grown overnight in YPD media, and cultures were then diluted and grown to log phase. The cultures were split and MMS was added to one-half of the cells for a final concentration of 0.03% MMS. Cultures were incubated for an additional 30 min at 30°. The cells were washed and fixed with formalin. At least 300 cells were evaluated for each strain; cell cycle phase was determined by bud size analysis.

Immunoblotting analysis

Cells were harvested at log phase and suspended in 0.1 M NaOH for 5 min. The NaOH was removed, the cell pellet was resuspended in lysis buffer (120 mM Tris-HCl pH 6.8, 12% glycerol, 3.4% SDS, 200 mM dithiothreitol, and 0.004% bromophenol blue), and the cell suspension was incubated at 95° for 5 min. Insoluble cell debris was removed by centrifugation and total protein was separated on a 15% SDS-PAGE gel. Proteins were transferred to a nitrocellulose blotting membrane (0.2 μ m; Protran, Amersham, Piscataway, NJ) and blocked with 3% milk. The following antibodies were utilized: anti-TBP (1:5000), anti-H2AS129 phosphorylation (#ab15083, 1:500; Abcam), and anti-rabbit (#925-32211, 1:15,000; Li-COR). Protein bands were visualized

using the Li-COR Odyssey CLx and quantified using Image Studio.

Chronological aging assay

Chronological aging assays were performed as described (Parrella and Longo 2008). Briefly, strains were inoculated in synthetic dropout media and grown overnight. Cultures were diluted to an OD of 0.1 and grown in synthetic dropout media for 3 days (72 hr) to ensure that cultures had reached stationary phase (T_0). To determine viability, dilutions of each biological replicate were plated every other day onto YPD plates. Dilutions and plating were carried out in triplicate, and averaged for each biological replicate. Four to five biological replicates for each strain were averaged to determine the % viability. The % viability is the ratio of viable colonies at a specific time (T_x) over the number of viable colonies at T_0 .

Data availability

Strains and plasmids are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, tables, and supplemental material. Supplemental data are available at Figshare as one PDF. Figure S1 shows further analysis of the MMS resistance, Figure S2 shows growth phenotypes after UV exposure, Figure S3 diagrams the DDT subpathways, and Figures S4 and S5 present the growth phenotypes after exposure to MMS or HU in the indicated deletion background strains. Tables S1–S3 list the strains, plasmids, and DNA primers utilized in this study, respectively. Supplemental material available at Figshare: <https://doi.org/10.25386/genetics.7096262>.

Results

Expression of *Spn1*¹⁴¹⁻³⁰⁵ results in increased resistance to MMS

To test if *Spn1* functions in DNA-damage repair, we utilized the *spn1* mutant allele *spn1*¹⁴¹⁻³⁰⁵ (Figure 1A). The *Spn1*¹⁴¹⁻³⁰⁵ protein consists of only the conserved ordered core domain, and lacks the N- and C-terminal regions present in full-length *Spn1* (Fischbeck *et al.* 2002; Krogan *et al.* 2002; Li *et al.* 2018). Cells expressing *Spn1*¹⁴¹⁻³⁰⁵ were grown on media containing various DNA-damaging agents and, interestingly, the *spn1*¹⁴¹⁻³⁰⁵ strain displayed resistance to MMS (Figure 1B). The observed resistance is specific to MMS since sensitivity to the other DNA-damaging agents was not altered (Figure 1B). Coexpression of plasmid-borne *Spn1*¹⁴¹⁻³⁰⁵ in a strain carrying a chromosomal copy of wild-type *Spn1* did not result in increased resistance to MMS (Figure S1A), suggesting that *spn1*¹⁴¹⁻³⁰⁵ is recessive.

To determine whether cells expressing *Spn1*¹⁴¹⁻³⁰⁵ are able to trigger a DNA damage response after exposure to MMS, the levels of histone H2A serine 129 phosphorylation (H2A S129Ph) in *SPN1* and *spn1*¹⁴¹⁻³⁰⁵ cells were measured by immunoblot analysis. H2A S129 is phosphorylated in response

to DNA damage (Downs *et al.* 2000; Foster and Downs 2005). A ~2.5-fold increase in the amount of H2A S129Ph after MMS exposure was observed in both the *SPN1* and *spn1*¹⁴¹⁻³⁰⁵ strains (Figure 1C). The levels of H2A S129Ph were similar in the two strains prior to and after exposure to MMS (Figure 1C). In addition, we examined the cell cycle-phase distribution between the two strains after exposure to MMS (Figure S1B). No difference was observed between the cell cycle-phase distributions of the two strains. Since both the H2A S129Ph DNA-damage response and cell cycle progression were not altered in the *spn1*¹⁴¹⁻³⁰⁵ strain, we reasoned that the DNA damage checkpoints remained intact.

Mec1 and *Tel1* are evolutionarily conserved phosphatidylinositol-3 kinase-related protein kinases and transduce a kinase cascade, which activates DNA-damage repair, cell cycle arrest, transcription programs, dNTP synthesis, and replication fork stabilization in response to cellular stress (Craven *et al.* 2002; Toh and Lowndes 2003; Enserink 2011). Serine 23 (S23) of *Spn1* is phosphorylated in response to exposure to MMS in a *Mec1*- and *Tel1*-dependent, and *Rad53*-independent, manner (Chen *et al.* 2010; Bastos de Oliveira *et al.* 2015). We investigated whether cells expressing *Spn1*¹⁴¹⁻³⁰⁵ combined with the loss of *Tel1* or *Rad9* (a mediator kinase that activates *Rad53*) remain MMS-resistant. Expression of *Spn1*¹⁴¹⁻³⁰⁵ in either deletion strain does not result in resistance when grown on MMS (Figure S1C). Since *Tel1* and *Rad9* affect many downstream factors, we wanted to test if the loss of *Spn1* S23 phosphorylation is sufficient for MMS resistance. Phospho-mimetic (*spn1*^{S23D}) and phospho-deficient (*spn1*^{S23A}) strains were created and grown on MMS. The *spn1*^{S23D} and *spn1*^{S23A} strains grew similarly to the *SPN1* strain (Figure S1D). Taken together, this indicates that MMS resistance observed in the *spn1*¹⁴¹⁻³⁰⁵ strain is dependent on *Tel1* and *Rad9* activity, and that the loss of S23 phosphorylation of *Spn1* is insufficient for MMS resistance.

Removal of methyl lesions through *Mag1* glycosylase is necessary for MMS resistance

To investigate the resistance to MMS, we introduced *spn1*¹⁴¹⁻³⁰⁵ into strains deficient for BER, the primary pathway to repair DNA damage caused by MMS (Memisoglu and Samson 2000). As such, strains deleted for gene products important for BER, such as *Mag1* and *Apn1*, are sensitive to MMS (Prakash and Prakash 1977; Bennett 1999). *Mag1* is the DNA glycosylase responsible for the removal of the toxic N3-methyladenine adducts resulting in an abasic site (Prakash and Prakash 1977; Chen *et al.* 1989). *Apn1* is the major endonuclease responsible for cleavage of the phosphate backbone at the abasic site, which is subsequently repaired through long- or short-patch BER (Ramotar *et al.* 1991; Memisoglu and Samson 2000; Odell *et al.* 2013). Cells expressing *Spn1*¹⁴¹⁻³⁰⁵ in the *mag1Δ* background were sensitive to MMS (Figure 2A). In contrast, cells expressing *Spn1*¹⁴¹⁻³⁰⁵ in the *apn1Δ* background were resistant to MMS compared to the *apn1Δ* strain (Figure 2A). This indicates that cells bearing *spn1*¹⁴¹⁻³⁰⁵ are able to retain resistance with a defective

Figure 2 Resistance to methyl methanesulfonate (MMS) is dependent on removal of the lesion by Mag1 and independent of nucleotide excision repair (NER). Ten-fold serial dilutions of cells expressing Spn1 or Spn1¹⁴¹⁻³⁰⁵ in (A) base excision repair and (B) NER background strains. Cells were grown on YPD and YPD plates containing MMS. Concentrations of MMS varied, depending on the sensitivity of the deletion strain. Strains are: *spn1*¹⁴¹⁻³⁰⁵, *mag1*Δ, *mag1*Δ*spn1*¹⁴¹⁻³⁰⁵, *apn1*Δ, *apn1*Δ*spn1*¹⁴¹⁻³⁰⁵, *rad26*Δ, *rad26*Δ*spn1*¹⁴¹⁻³⁰⁵, *rad14*Δ, and *rad14*Δ*spn1*¹⁴¹⁻³⁰⁵.

BER pathway if the damaging lesion can be processed by Mag1.

Resistance to MMS is independent of the NER pathway

Since *Spn1* is involved in transcription and mRNA processing (Fischbeck *et al.* 2002; Krogan *et al.* 2002; Yoh *et al.* 2007, 2008), it seemed possible that *Spn1* could be functioning in transcription-coupled NER (TC-NER). Additionally, NER has been shown to compete with BER endonucleases in the repair of abasic sites (Torres-Ramos *et al.* 2000). Genetic analyses were performed with the introduction of *spn1*¹⁴¹⁻³⁰⁵ into the *rad26*Δ and *rad14*Δ backgrounds. *Rad26* is a DNA-dependent ATPase involved in TC-NER (Guzder *et al.* 1996a; Prakash and Prakash 2000), and *Rad14* is a subunit of NER factor 1 (NEF1), which is required for TC-NER and global genomic NER (Guzder *et al.* 1996b; Prakash and Prakash 2000). Resistance to MMS was observed when *Spn1*¹⁴¹⁻³⁰⁵ was expressed in the *rad26*Δ or *rad14*Δ strains (Figure 2B), indicating that this phenotype is not dependent on either NER pathway. Furthermore, expression of *Spn1*¹⁴¹⁻³⁰⁵ in the wild-type, *rad26*Δ and *rad14*Δ strain backgrounds did not result in a resistance phenotype after exposure to UV (Figure S2, A and B), which is primarily repaired by NER.

Figure 3 Resistance to methyl methanesulfonate (MMS) is dependent on the error-free branch of the DNA-damage tolerance pathway. Ten-fold serial dilutions of cells expressing Spn1 or Spn1¹⁴¹⁻³⁰⁵ in (A) translesion synthesis subpathway deletion background strains and (B) error-free subpathway deletion background strains. Strains were grown and spotted onto YPD and YPD plates containing MMS. Strains are: *spn1*¹⁴¹⁻³⁰⁵, *rev7*Δ, *rev7*Δ*spn1*¹⁴¹⁻³⁰⁵, *rev1*Δ, *rev1*Δ*spn1*¹⁴¹⁻³⁰⁵, *rev3*Δ, *rev3*Δ*spn1*¹⁴¹⁻³⁰⁵, *rad5*Δ, *rad5*Δ*spn1*¹⁴¹⁻³⁰⁵, *mms2*Δ, *mms2*Δ*spn1*¹⁴¹⁻³⁰⁵, *ubc13*Δ, and *ubc13*Δ*spn1*¹⁴¹⁻³⁰⁵.

This further supports the idea that the resistance phenotype is specific to MMS and is not due to enhancement of the NER pathway.

Resistance is dependent on the error-free subpathway of DNA damage tolerance

DDT provides mechanisms (Figure S3) for cells to bypass blocks to the DNA replication fork (Ulrich 2011; Bi 2015), including lesions caused by exposure to MMS. The primary signal for entry into the TLS subpathway of DDT is dependent

Table 1 Damaged-induced and spontaneous mutation rates of the indicated strains

Strain	Mutation rate $\times 10^{-7a}$	95% C.I.	Fold change ^b	Number of replicates	One-tailed ^c
MMS-induced mutation rate					
<i>SPN1</i>	22.76	19.12–25.83	1	21	
<i>spn1¹⁴¹⁻³⁰⁵</i>	15.46	12.4–15.98	0.68	21	< 0.0001
<i>rev3Δ</i>	11.43	7.63–15.09	1	20	
<i>rev3Δ spn1¹⁴¹⁻³⁰⁵</i>	4.72	3.25–9.31	0.41	21	0.0041
<i>mms2Δ</i>	289.63	250.24–391.05	1	21	
<i>mms2Δ spn1¹⁴¹⁻³⁰⁵</i>	240.62	180.9–334.16	0.83	21	0.0815
<i>dot1Δ</i>	90.01	77.06–102.78	1	14	
<i>dot1Δ spn1¹⁴¹⁻³⁰⁵</i>	63.13	58.29–67.8	0.70	13	0.0002
Spontaneous mutation rate					
<i>SPN1</i>	1.20	0.96–1.59	1	21	
<i>spn1¹⁴¹⁻³⁰⁵</i>	0.64	0.44–0.84	0.53	21	< 0.0001
<i>rev3Δ</i>	2.02	1.36–2.49	1	7	
<i>rev3Δ spn1¹⁴¹⁻³⁰⁵</i>	0.95	0.65–3.57	0.47	7	0.0189
<i>mms2Δ</i>	25.55	22.96–31.87	1	14	
<i>mms2Δ spn1¹⁴¹⁻³⁰⁵</i>	23.08	14.00–13.31	0.90	12	0.1344
<i>dot1Δ</i>	2.86	1.53–7.23	1	7	
<i>dot1Δ spn1¹⁴¹⁻³⁰⁵</i>	2.06	1.73–2.63	0.72	7	0.0318

^a Mutation rates were calculated through the Lea-Coulson method of the median (Lea and Coulson 1949) using the FALCOR web application (Hall *et al.* 2009).

^b Reported fold change is the comparison of the mutation rate of cells expressing *Spn1¹⁴¹⁻³⁰⁵* compared to wild-type *Spn1* in the corresponding strain background.

^c Statistical significance was determined using the Mann-Whitney nonparametric Student's *t*-test using GraphPad Prism software.

on the monoubiquitination of PCNA through the actions of the Rad18/Rad6 complex (Ulrich 2011; Bi 2015). Additional polyubiquitination through the actions of the Ubc13/Mms2 and Rad5 complex is the primary signal for error-free bypass (Ulrich 2011; Bi 2015). It has been shown that abolishment of the DDT pathway results in extreme sensitivity to MMS (Huang *et al.* 2013). Since we observe resistance to MMS in the *spn1¹⁴¹⁻³⁰⁵* strain, we predicted that the DDT pathway must function. Consistent with this, deletion of *RAD6* or *RAD18* resulted in the loss of MMS resistance in strains expressing *Spn1¹⁴¹⁻³⁰⁵* (Figure S4A).

MMS resistance has been shown to correlate with changes in DDT subpathway selection (Conde and San-Segundo 2008; Conde *et al.* 2010). Genetic analyses between *SPN1* and *REV3/REV7/REV1*, TLS polymerases, and *RAD5/MMS2/UBC13*, a complex responsible for error-free subpathway signaling, were performed. Cells expressing *Spn1¹⁴¹⁻³⁰⁵* retained resistance to MMS in all tested TLS gene-deletion backgrounds (Figure 3A). In contrast, loss of MMS resistance was observed in the error-free deletion strains (Figure 3B). These data suggest that cells expressing wild-type *Spn1* are utilizing the TLS subpathway, whereas cells expressing *Spn1¹⁴¹⁻³⁰⁵* shift the response toward the error-free subpathway.

To test if the expression of *Spn1¹⁴¹⁻³⁰⁵* confers resistance to other forms of replication stress, we examined cellular growth after the addition of HU. Exposure to HU results in slowed or stalled replication forks due to depleted levels of dNTPs (Koç *et al.* 2004), while MMS causes damage-induced replication stress (Wyatt and Pittman 2006). In contrast to resistance to MMS, the *spn1¹⁴¹⁻³⁰⁵* strain displayed a slight sensitivity to HU (Figure S4B). HU sensitivity was exacerbated in the DDT deletion strains (Figure S4B). This suggests that *Spn1* is important for overcoming replication stress

caused by HU and that the stress induced by HU cannot be overcome by the expression of *Spn1¹⁴¹⁻³⁰⁵*.

Spn1 contributes to spontaneous and damage-induced genome instability

The TLS polymerases can be responsible for upwards of 50% of point mutations in a genome (Stone *et al.* 2012). Thus, we predicted that if the *spn1¹⁴¹⁻³⁰⁵* strain does not utilize the TLS subpathway, then we would observe a difference in the damage-induced mutation rates between the *SPN1* and *spn1¹⁴¹⁻³⁰⁵* strains. To determine levels of damage-induced mutations, a fluctuation assay detecting forward mutations occurring within the *CAN1* locus in the presence of MMS was performed. Cells expressing *Spn1¹⁴¹⁻³⁰⁵* had a significant decrease in the damage-induced mutation rate compared to wild-type cells (Table 1). The *spn1¹⁴¹⁻³⁰⁵* strain also displayed a decrease in the spontaneous mutation rate (Table 1). These results indicate that *Spn1* contributes to genome instability, regardless of the presence of a DNA-damaging agent.

To test if the decreased mutation rate observed in the *spn1¹⁴¹⁻³⁰⁵* strain is dependent on the error-free subpathway, damage-induced mutation rates in *mms2Δ* were examined. We predicted that the introduction of *Spn1¹⁴¹⁻³⁰⁵* into the *mms2Δ* strain would result in the mutation rate increasing and returning to levels observed in *mms2Δ* expressing wild-type *Spn1*. As predicted, the deletion of *MMS2* combined with the *SPN1* mutant resulted in wild-type damaged-induced mutation rates (Table 1). Furthermore, deletion of *rev3Δ* (a component of the TLS pathway) in the *spn1¹⁴¹⁻³⁰⁵* strain still produced a decrease in mutation rate (Table 1). Taken together, this indicates that the mutation rate decrease in the *spn1¹⁴¹⁻³⁰⁵* strain is dependent on the error-free subpathway.

The deletion of the histone methyltransferase *Dot1* results in resistance to MMS through the loss of inhibition of the TLS

Figure 4 Resistance to methyl methanesulfonate (MMS) is dependent on the homologous recombination factors. Ten-fold serial dilutions of cells expressing Spn1 or Spn1¹⁴¹⁻³⁰⁵ in (A) *rad51Δ*, *rad55Δ*, and *rad57Δ* backgrounds, and (B) *sgs1Δ* and *rmi1Δ* backgrounds. Strains spotted on YPD and YPD plates containing MMS or HU. Strains are: *spn1*¹⁴¹⁻³⁰⁵, *rad51Δ*, *rad51Δspn1*¹⁴¹⁻³⁰⁵, *rad55Δ*, *rad55Δspn1*¹⁴¹⁻³⁰⁵, *rad57Δ*, *rad57Δspn1*¹⁴¹⁻³⁰⁵, *sgs1Δ*, *sgs1Δspn1*¹⁴¹⁻³⁰⁵, *rmi1Δ*, and *rmi1Δspn1*¹⁴¹⁻³⁰⁵.

subpathway (Conde and San-Segundo 2008). The resistance in the *spn1*¹⁴¹⁻³⁰⁵ strain is due to use of the error-free subpathway, and thus we predicted that Spn1 and Dot1 are acting in parallel pathways (Figure S3). To test this, a genetic analysis between *SPN1* and *DOT1* was performed. Interestingly, combining the deletion of *DOT1* with *spn1*¹⁴¹⁻³⁰⁵ resulted in enhanced growth compared to *dot1Δ* alone on YPD and MMS plates (Figure S4C). Expression of Spn1¹⁴¹⁻³⁰⁵ in the *dot1Δ* strain resulted in significantly decreased mutation rates (Table 1), although we observed higher damage-induced mutation rates when *DOT1* was deleted, which is consistent with previously reported data (Conde and San-Segundo 2008). The increase in MMS resistance and the mutation rate observed in the *dot1Δ spn1*¹⁴¹⁻³⁰⁵ strain suggests a deregulation of both subpathways of DDT.

Resistance to MMS is dependent on the HR machinery

Factors involved in HR are utilized for template switching in the error-free subpathway (Branzei *et al.* 2008; Bi 2015; Branzei and Szakal 2016); thus, we predicted that the MMS

Figure 5 Expression of Spn1¹⁴¹⁻³⁰⁵ increases chronological life span. Each time point represents the average cell viability at the number of days indicated for *SPN1* (squares; *n* = 5) and *spn1*¹⁴¹⁻³⁰⁵ (triangles; *n* = 4).

resistance seen in the *spn1*¹⁴¹⁻³⁰⁵ strain would require various HR factors like Rad51, Rad55, and Rad57. Rad51 forms a nucleoprotein filament when it binds ssDNA, and Rad55 and Rad57 work as a heterodimer to stabilize the association of Rad51 with the ssDNA (Symington *et al.* 2014; Hanamshet *et al.* 2016). Deletion of *RAD51*, *RAD55*, or *RAD57* combined with *spn1*¹⁴¹⁻³⁰⁵ resulted in a loss of MMS resistance (Figure 4A), indicating that the observed resistance to MMS is dependent on functional HR factors.

DNA intermediates are processed through Sgs1 and Rmi1 in *spn1*¹⁴¹⁻³⁰⁵

During error-free DDT and HR, DNA crossover intermediates are a result of strand invasion. The functions of topoisomerases, helicases, and nucleases aid in resolving these intermediates (Mitchel *et al.* 2013; Campos-Doerfler *et al.* 2018). Sgs1, Rmi1, and Top3 work as a complex to aid in resolving Holliday junctions (Mullen *et al.* 2005; Bernstein *et al.* 2009). Genetic analysis revealed that the deletion of *SGS1* or *RMI1* is synthetically lethal with *spn1*¹⁴¹⁻³⁰⁵ on MMS (Figure 4B). This was also observed when the strains were grown on HU (Figure S5). Together, these observations suggest that cells expressing Spn1¹⁴¹⁻³⁰⁵ may commit to recombination pathways and are therefore dependent on a functional Sgs1/Rmi1 complex to resolve recombination intermediates.

Spn1¹⁴¹⁻³⁰⁵ expression results in increased chronological longevity

Decreased mutation rates and inactivation of the TLS pathway have been linked to increased chronological longevity (Longo and Fabrizio 2012). Since cells expressing Spn1¹⁴¹⁻³⁰⁵ have decreased mutation rates, we asked whether they would have an increased chronological life span. A dramatic difference in the chronological life span between cells expressing Spn1 and Spn1¹⁴¹⁻³⁰⁵ was observed (Figure 5).

Figure 6 Summarization of DDT pathway selection in *spn1* strains. Cells tightly regulate the balance between error-free (template switch) and error-prone (TLS) DDT subpathways. In wild-type cells, utilization of both subpathways results in wild-type levels of genome instability. We predict that *Spn1*¹⁴¹⁻³⁰⁵ shifts the regulation, such that cells utilize the error-free subpathway more than wild-type cells, resulting in MMS resistance, HU sensitivity, decreased level of spontaneous and damage-induced mutation rates, and increased chronological longevity. BER, base excision repair; DDT, DNA-damage tolerance; HR, homologous recombination; NER, nucleotide excision repair; TLS, translesion synthesis.

At the termination of the assay (19 days), the *spn1*¹⁴¹⁻³⁰⁵ culture maintained 85% viability, while the control *SPN1* culture was ~5%. This suggests a link between *Spn1*, genome instability, and chronological aging.

Discussion

Here, we have investigated the role of the chromatin-binding factor *Spn1* in the DDT response and genome instability by undertaking an analysis of a mutant allele, *spn1*¹⁴¹⁻³⁰⁵. Expression of *Spn1*¹⁴¹⁻³⁰⁵ covers the essential functions of wild-type *Spn1* when cells are grown in rich culturing conditions (Fischbeck *et al.* 2002; Li *et al.* 2018). However, *Spn1*¹⁴¹⁻³⁰⁵ has lost the ability to bind DNA, nucleosomes, and histones, although it retains the ability to bind *Spt6* and RNAPII (McDonald *et al.* 2010; Li *et al.* 2018). Upon exposure to the DNA-damaging agent MMS, we observed increased resistance in cells expressing *Spn1*¹⁴¹⁻³⁰⁵. MMS treatment results in the methylation of single- and double-stranded DNA (Yang *et al.* 2010). The methyl group is primarily transferred to a double-bonded nitrogen on adenine, cytosine, and guanine with varying frequencies (Wyatt and Pittman 2006). Not all lesions induced by MMS are toxic; however, N3-methyl-adenine is toxic to cells as it creates a barrier for the replication machinery (Chang *et al.* 2002). Our genetic analyses revealed that the resistance observed in the *spn1*¹⁴¹⁻³⁰⁵ strain was dependent on the error-free subpathway of DDT. Resistance was lost upon deletion of any of the genes responsible for polyubiquitination of *PCNA* (*RAD5/MMS2/UBC13*), the major signal for entry into the error-free subpathway and deletion of HR factors (*RAD51*, *RAD55*, *RAD57*, *SGS1*, and *RMI1*), which are utilized for the template switching step of the subpathway (Bi 2015; Branzei and Psakhye 2016; Branzei and Szakal 2016; Hanamshet *et al.* 2016). We conclude that expression of *Spn1*¹⁴¹⁻³⁰⁵ shifts the regulation of DDT toward the error-free subpathway (Figure 6). This shift in the regulation of DDT subpathway selection is advantageous after exposure to MMS; however, when cells are

exposed to HU, the effects of expression of *Spn1*¹⁴¹⁻³⁰⁵ are detrimental to cell growth. This could be due to the amount and/or type of replication stress that a cell is experiencing during exposure to MMS vs. HU. As expected, in cells expressing *Spn1*¹⁴¹⁻³⁰⁵, the shift toward the error-free subpathway results in a significant decrease in mutation rates. This decrease is lost upon the deletion of *MMS2* in cells expressing *Spn1*¹⁴¹⁻³⁰⁵, indicating that the decreased mutation rates are dependent on the error-free subpathway of DDT.

Increased dependence on the error-free subpathway and decreased mutation accumulation could contribute to the increased chronological life span observed in cells expressing *Spn1*¹⁴¹⁻³⁰⁵. As yeast age, there is an increase in the frequency of all types of mutations (Madia *et al.* 2007; Longo and Fabrizio 2012). These accumulated mutations are linked to a cell's ability to process damaged DNA (primarily oxidative damage), the activity of the TLS polymerases, the control of mitotic recombination rates, and the regulation of metabolism (Madia *et al.* 2009). Cells lacking *Sch9*, a protein kinase, had increased chronological longevity (Longo and Fabrizio 2012), which is linked to the inactivation of the Rev1-Polζ polymerases and decreased damage accumulation (Madia *et al.* 2009; Longo and Fabrizio 2012). Taken together, our results indicate that wild-type *Spn1* contributes to genome instability and increased mutation rates. As noted previously, some level of genome instability is critical to fuel genetic diversification and evolution (Skoneczna *et al.* 2015).

Chromatin structure, histone tail modification, and DNA topography all influence DDT subpathway selection (Gonzalez-Huici *et al.* 2014; Meas *et al.* 2015; Hung *et al.* 2017). Deletion of the H3K79 methyltransferase *Dot1* also results in resistance to MMS (Conde and San-Segundo 2008). The loss of TLS inhibition resulting in MMS resistance in *dot1Δ* was determined to be due to the loss of the methylase activity (Conde *et al.* 2010). MMS resistance is maintained in the *dot1Δspn1*¹⁴¹⁻³⁰⁵ strain, suggesting deregulation of both subpathways of DDT, possibly due to aberrant chromatin structure. Chromatin in cells expressing *Spn1*¹⁴¹⁻³⁰⁵

is more resistant to micrococcal nuclease digestion during activated *CYC1* transcription than wild-type cells (Li *et al.* 2018), and *Spn1* prevents the chromatin remodeler SWI/SNF from being recruited during the repression of *CYC1* transcription (Zhang *et al.* 2008). *Spn1* has nucleosome assembly *in vitro*, which is lost upon deletion of the disordered tails (Li *et al.* 2018), and *Spn1* is speculated to be involved in the quality assessment of the assembled nucleosomes (McCullough *et al.* 2015).

The error-free subpathway of DDT is proposed to occur primarily during S phase of the cell cycle (Branzei and Szakal 2016). Chromatin structure is disrupted during S phase to allow the replisome access to the DNA template for DNA replication. Following passage of the replication fork, the DNA double helix must reassociate with histones to form proper the chromatin structure of the newly synthesized sister chromatids. Maturation of chromatin after histone nucleosome assembly involves establishing the proper histone code, the association of linker histones, and the establishment of higher-order chromatin structure (MacAlpine and Almouzni 2013; Alabert *et al.* 2014; Bellush and Whitehouse 2017). CAF-1, along with histone chaperone *Asf1*, aids in the proper assembly of newly formed chromatin after DNA synthesis (MacAlpine and Almouzni 2013). Genetic interactions have been shown between mutant alleles of *SPN1* and the replicative histone chaperone complexes CAF1 (Li *et al.* 2018) and FACT (C. A. Radebaugh, unpublished results). Both yeast and human *Spn1* associate with chromatin throughout the cell cycle (Kubota *et al.* 2012; Alabert *et al.* 2014; Dungrawala *et al.* 2015). Additionally, human *Spn1* was determined to be an early arriving chromatin component factor after the DNA was replicated (Alabert *et al.* 2014). Thus, a possible mechanism is that wild-type *Spn1*, which is capable of binding DNA, histone, and nucleosomes, aids in the creation and/or maintenance of chromatin architecture during S phase that provides a balance between the error-free and error-prone (TLS) subpathways. Loss of the intrinsically disordered tails of *Spn1* results in an altered chromatin structure, which tips this balance toward the error-free subpathway, and the outcome is a decrease in genome instability.

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