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Deposition of histone H2A.Z by the SWR-C remodeling enzyme prevents genome instability

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Abstract

The yeast SWR-C chromatin remodeling enzyme catalyzes chromatin incorporation of the histone variant H2A.Z which plays roles in transcription, DNA repair, and chromosome segregation. Dynamic incorporation of H2A.Z by SWR-C also enhances the ability of exonuclease I (Exo1) to process DNA ends during repair of double strand breaks. Given that Exo1 also participates in DNA replication and mismatch repair, here we test whether SWR-C influences DNA replication fidelity. We find that inactivation of SWR-C elevates the spontaneous mutation rate of a strain encoding a L612M variant of DNA polymerase (Pol) δ, with a single base mutation signature characteristic of lagging strand replication errors. However, this genomic instability does not solely result from reduced Exo1 function, because single base mutator effects are seen in both Exo1-proficient and Exo1-deficient *pol3-L612M swr1* strains. The data are consistent with the possibility that incorporation of the H2A.Z variant by SWR-C may stimulate Exo1 activity, as well as enhance the fidelity of replication by Pol δ**, the repair of mismatches generated by Pol δ, or both.**

Keywords

Chromatin remodeling; Genome Instability; Mismatch repair; Exonuclease 1; H2A.Z

1. Introduction

Chromatin remodeling enzymes promote DNA accessibility for nuclear processes by using the energy of ATP hydrolysis to unfold chromatin fibers, mobilize nucleosomes, evict

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Conflict of interest

None.

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histone components, or catalyze incorporation or removal of variant histones [1]. Many of these enzymes are recruited to DNA double strand breaks where they are believed to regulate accessibility of lesions to the DNA repair machinery [2]. Included among these chromatin remodeling enzymes [3] is *Saccharomyces cerevisiae* SWR-C, a large multi-subunit complex that promotes incorporation of the histone variant H2A.Z into chromatin [4]. Recently we found that resection of double stranded DNA ends by exonuclease 1 (Exo1) is blocked by nucleosomes containing histone H2A-H2B dimers, and that this resection barrier is relieved by SWR-C-dependent incorporation of H2A.Z [5]. This led us to wonder if SWR-C might also modulate Exo1 activity in other DNA transactions. For example, Exo1 can contribute to DNA flap removal during Okazaki fragment maturation of the nascent lagging strand [6], and it can excise replication errors during post-replication mismatch repair (MMR) [7], especially mismatches generated during lagging strand replication by DNA polymerase δ (Pol δ) and Pol α [8, 9]. Furthermore, several studies indicate that nucleosome assembly can regulate mismatch repair at replication forks. Nucleosomes assembled on mismatched DNA reduce the ability of MutSα to bind to a mismatch in DNA, to bind to ADP, to hydrolyze ATP [10] and to slide on DNA [11]. During MMR *in vitro*, CAF1-dependent deposition of histone H3-H4 protects the mismatch-containing DNA strand from excessive degradation [12]. Moreover, MutSα suppresses CAF1-dependent histone deposition in a mismatch-dependent manner [12, 13], and MutSα and CAF1 interact with each other via the N-terminus of Msh6 and the large p150 subunit of CAF1 [13].

These studies suggest that SWR-C-dependent incorporation of H2A.Z could modulate replication fidelity. This prompted us to examine if loss of the catalytic Swr1 subunit of the SWR-C complex elevates the rate of single base mutations typical of replication errors. Here we measure mutation rates after deleting *SWR1* from yeast strains that generate leading and lagging strand-specific replication errors at elevated rates due to active site mutations in each of the three major yeast replicative DNA polymerases (see [14] and references therein). When *SWR1* is deleted in a strain encoding a *pol3-L612M* variant of Pol δ, a single base mutator effect is observed that is characteristic of lagging strand replication infidelity. This mutator effect partially persists in an *exo1* strain, but it is not observed when *SWR1* is deleted from strains encoding wild type replicases or variants of Pol α or Pol ε. Several explanations for the observed genome instability are considered, including the possibility that H2A.Z deposition modulates the generation or repair of lagging strand DNA replication errors.

2. Materials and Methods

2.1. Yeast strains

The *Saccharomyces cerevisiae* strains used here are isogenic derivatives of strain |(-2)|-7B-YUNI300 (*MATa CAN1 his7-2 leu2- ::kanMX ura3- trp1-289 ade2-1 lys2- GG2899-2900 agp1::URA3-OR1*) [15]. Polymerase mutator alleles have been described previously [16-18]. Heterozygous *EXO1/exo1* , *SWR1/swr1* , *HTZ1/htz1* , and *MSH2/ msh2* diploids were generated in wild type or polymerase mutator backgrounds by PCR-based targeted gene deletion of the coding region. Deletion was verified by PCR, and

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haploids were obtained from tetrad dissection. Mutation rate data and sequencing analyses were performed using at least 3 independent haploids.

2.2. Spontaneous mutation rates and sequence analysis

Spontaneous mutation rates at *URA3* and *CAN1* were measured by fluctuation analysis as described [19]. Genomic DNA from independent 5-FOA-resistant colonies was isolated and the *URA3* gene was PCR-amplified and sequenced. Rates of various mutations were calculated by multiplying the proportion of each mutation type by the overall mutation rate for each strain.

2.3. Statistical analysis

Statistical analysis of comparisons between overall mutation rates was performed using a one-sided nonparametric Mann Whitney test in GraphPad Prism. Statistical analysis of mutation spectra was performed using two-sided Fisher's exact test to compare mutation spectra between the two *EXO1* strains (*pol3-L612M* versus *pol3-L612M swr1*) and between the two *exo1* strains (*pol3-L612M exo1* versus *pol3-L612M exo1 swr1*) [20]. The Benjamini-Hochberg (B-H) procedure was applied with a false discovery rate (FDR) of 0.05 in order to account for multiple tests [21].

3. Results

3.1. Mutation rates

Spontaneous mutation rates were measured in *SWR1* and *swr1* strains harboring either wild type replicase genes or alleles that affect Pol α (*pol1-L868M*), Pol ε (*pol2-M644G*) or Pol δ (*pol3-L612M*). Forward mutation rates were determined at two loci, *URA3* and *CAN1*, by monitoring the frequency of 5-FOA or canavanine resistance, respectively. Resistance to 5-FOA in the *pol3-L612M swr1* double mutant strain was 2-fold higher than for the *pol3-L612M* single mutant strain (Fig. 1A). This difference is significant as indicated by no overlap in the 95% confidence intervals for the two measurements, and by a similar 2-fold difference in the rate of mutation to canavanine resistance (Fig. 1B), again with no overlap in the 95% confidence intervals. No *SWR1*-dependent mutator effects were observed in the strain encoding wild type DNA polymerases, or in strains encoding variants of Pol α (*pol1-L868M*) or Pol ε (*pol2-M644G*).

We next determined whether the mutagenic effect of the *SWR1* deletion was due to lack of incorporation of the histone variant H2A.Z. Derivatives of the lagging strand mutator strains (*pol1-L868M* and *pol3-L612M*) were constructed that harbored a deletion of the *HTZ1* gene which encodes H2A.Z. Strikingly, *swr1* , *htz1* and *swr1 htz1* mutant derivatives all showed similar increases in *URA3* and *CAN1* mutation rates for the *pol3-L612M* mutator background. These results are consistent with the increased mutation rates being due to lack of H2A.Z deposition (Fig. 1). Interestingly, the *htz1* single mutant also led to a significant increase in mutation rate in the *pol1-L868M* strain. Notably, this increase was only observed at the *URA3* locus, not at *CAN1*. Since this increased mutation rate is not observed in the *swr1 htz1* double mutant, these data are consistent with previous genetics studies

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suggesting that the SWR-C enzyme can have inhibitory effects if its substrate, H2A.Z is absent [22].

We have previously shown that H2A.Z incorporation by SWR-C increases Exo1 resection activity [5]. Therefore, we investigated a possible relationship between SWR-C and Exo1 by performing similar mutation rate measurements in *pol3-L612M exo1* and *pol3-L612M swr1 exo1* strains. As expected, deletion of *EXO1* increased the mutation rates at both *CAN1* and *URA3* (Fig. 1C). Interestingly, inactivation of both Exo1 and Swr1 led to an even higher mutation rate, with 95% confidence intervals that do not overlap for canavanine resistance, but do overlap for resistance to 5-FOA (Fig. 1C). Thus, in these two mutational reporter genes, loss of *SWR1* leads to a mutator phenotype that is only partially dependent on Exo1, suggesting that Swr1 may function through both Exo1-dependent and Exo1-independent pathways. Likewise, we also analyzed whether Swr1-dependent mutation induction is directly related to mismatch repair by repeating these experiments in an *msh2* background. Deletion of *MSH2* caused *URA3* mutation rates to increase relative to the wild type strain (Fig. 1D). Importantly, deletion of *SWR1* did not cause a further increase in mutation rate, indicating that the mutator effect of Swr1 reflects loss of mismatch repair fidelity.

3.2. Analysis of mutational specificity

The mutation rate in the *pol3-L612M swr1* double mutant strain is 14-fold higher than the mutation rate in the *swr1* single mutant strain (4.4×10^{-8} versus 0.31×10^{-8}). Thus 93% of the mutations generated in the double mutant strain depend on L612M Pol δ. A similar comparison of rates in the corresponding *exo1* strains indicates the 91% of the mutations generated in the *pol3-L612M swr1 exo1* triple mutant strain depend on L612M Pol δ. Extensive evidence indicates that a primary function of Pol δ is to synthesize the nascent lagging strand during nuclear DNA replication [17, 23]. In doing so, L612M Pol δ generates a characteristic single base mutation signature [17, 23]. If loss of *SWR1* is promoting replication infidelity and/or reducing MMR of replication errors, this signature should be observed in the *pol3-L612M swr1* double mutant strain. If loss of *SWR1* is promoting genome instability by modulating some other DNA transaction in which Pol δ participates, a different mutational specificity might be observed.

To determine if the types of mutations observed in the *pol3-L612M swr1 EXO1* and *pol3-L612M swr1* exo1 strains are characteristic of errors made by L612M Pol δ during lagging strand replication, we sequenced the *URA3* gene in collections of independent, 5-FOA-resistant mutants, and compared the results to those observed in the corresponding *SWR1* strains. In all four strains, the majority of the mutations were single base changes (Table 1 and Supplemental Table S1). The mutations were non-uniformly distributed across the *URA3* open reading frame (Fig. 2), and many were at locations previously found to be characteristic of L612M Pol δ replication errors (see *ura3* spectrum in orientation 1 in Figure S3 of [24]. When the spectra of the *pol3-L612M* and *pol3-L612M swr1* double mutant strains were compared, they were significantly different, both in the *EXO1* ($p < 0.0001$) and *exo1* ($p < 0.0003$) backgrounds. The information in Table 1 and Figure 2 was also used to calculate site-specific mutation rates in each *pol3-L612M* strain at several

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positions. In the *EXO1* background (Fig. 3A), mutation rates in the *swr1* strain were significantly higher than in the *SWR1* strain at two positions, by 30-fold (p 0.0002) for a T to C substitution at base pair 95, and by 15-fold (p 0.027) for loss of a single A-T base pair in a homonucleotide run at base pair position 255. Consistent with a role for Swr1p in promoting the efficiency of Exo1-dependent MMR, mutation rates in the *swr1 exo1* strain were significantly higher than in the *SWR1 exo1* strain at two positions, by 16-fold (p 0.011) for a G to A substitution at base pair 345, and by 19-fold (p 0.0049) for a G to A substitution at base pair 768 (Fig. 3B).

4. Discussion

To our knowledge, this is the first demonstration that a defect in SWR-C-dependent chromatin remodeling reduces genome stability. This effect is specific for single base changes generated by L612M Pol δ. Hypothetically, these mutations could reflect a SWR-C-dependent defect in any of several cellular DNA transactions in which Pol δ participates, including replication, recombination and/or several types of excision repair. Because the effects reported here are on spontaneous mutation rates rather than damage-induced mutagenesis, and because the types and locations of the single base changes in *URA3* (Fig. 2) are typical of those previously found to be generated by L612M Pol δ during lagging strand replication, the current data favor the hypothesis that loss of *SWR1* is affecting lagging strand replication fidelity.

This study was motivated by the fact that SWR-C-dependent incorporation of H2A.Z facilitates resection of double stranded DNA ends by exonuclease 1 (Exo1) within nucleosomal arrays, and by the fact that Exo1 can contribute to replication fidelity by excising DNA flaps during maturation of Okazaki fragments, and by preferentially repairing mismatches generated during lagging strand replication [8, 9]. Indeed, a mutator effect was observed when SWR-C was inactivated in *EXO1* strains, consistent with a model wherein SWR-C-dependent incorporation of H2A.Z into chromatin may facilitate Exo1-dependent excision of mismatches generated by Pol δ in the newly replicated lagging strand. An effect of SWR-C on mismatch excision by Exo1 could be particularly important for repairing mismatches made by Pol δ as compared to those made by Pol α, for at least two reasons. On average, the mismatches generated by Pol δ will be further away from the 5′-ends of Okazaki fragments than are mismatches generated by Pol α as it initiates these fragments, thus possibly requiring more extensive resection by Exo1 to correct Pol δ errors. Also, errors made by Pol α might be repaired before histones H2A and H2B are reloaded behind the fork, obviating the need for SWR-C-dependent incorporation of H2A.Z to facilitate Exo1 resection.

The Exo1-independent mutator effects observed in the *pol3-L612M swr1 exo1* strain are consistent with two additional possibilities. One is that a defect in SWR-C-dependent incorporation of H2A.Z into chromatin may increase the rate at which single base mismatches are generated or escape proofreading at the replication fork, by a mechanism that is currently unknown but would need to account for the Pol δ specific effects seen here. A second, non-exclusive possibility stems from the fact that loss of Exo1 only partially reduces MMR (see [8, 9, 25] and references therein), indicating the existence of Exo1-

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independent MMR. The SWR-C-dependent incorporation of H2A.Z into chromatin may facilitate this MMR pathway. Given the need to coordinate nuclear DNA replication with the action of multiple histone chaperones ahead of and behind the replication fork and with multiple ATP-dependent chromatin remodeling enzymes, other genes involved in the latter processes can be anticipated to modulate genome stability. These enzymes may be partially redundant with SWR-C or act in parallel pathways to reduce mutagenesis, providing one explanation for why inactivation of SWR-C does not enhance mutation rates in strains with wild type DNA polymerases. Since inactivation of SWR-C does increase mutation rate in the L612M Pol δ lagging strand mutator variant, our data are also consistent with SWR-C working together with the replicative polymerases to reduce mutation rates (e.g., see [26]), and it remains a possibility that increased roles for SWR-C action may occur during conditions of replicative stress. Future studies will be needed to determine how these processes regulate mutagenesis and whether they are specific for normal or stressed replication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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• Highlights

- Deposition of histone H2A.Z facilitates DNA replication fidelity
- Lack of H2A.Z leads to increased mutations during lagging strand synthesis
- H2A.Z facilitates the function of Exo1 nuclease during DNA mismatch repair

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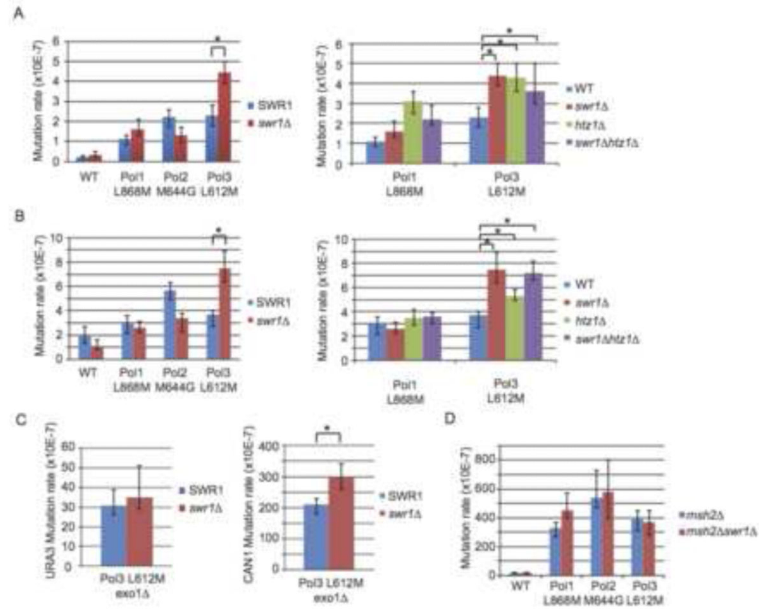

Fig. 1.

H2A.Z deposition by SWR-C reduces the mutation rate due to *pol3-L612M*. Spontaneous mutation rates to 5-FOA resistance (A) and canavanine resistance (B) with 95% confidence intervals are shown. Statistically significant differences (p<0.0001) in mutation rate are indicated by an asterisk. Deletion of *SWR1* or *HTZ1* separately and in combination increases *URA3* and *CAN1* mutation rate in a *pol3-L612M* mutator background. (C) *SWR1* was deleted in the *pol3-L612M* mutator strain in an *exo1* background. Mutation rates at the *URA3* (left panel) and *CAN1* loci (right panel) are shown as in (A). (D) The effect of *SWR1* deletion on *URA3* mutation rate in wild type (WT) and polymerase mutator strains was calculated in an *msh2* background. Mismatch repair is required for the mutagenic effect of *SWR1* deletion.

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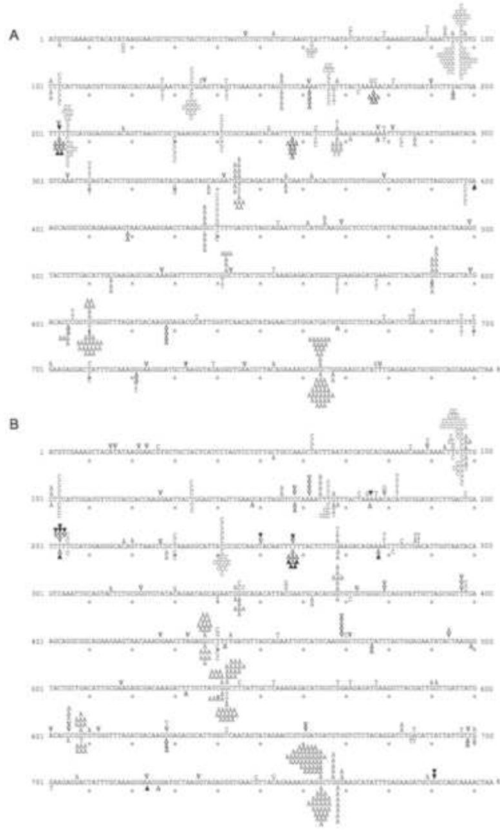

Fig. 2.

URA3 mutation spectra in *pol3-L612M* strains ± *SWR1* and/or *EXO1*. The coding strand of the 804 base pair *URA3* open reading frame (ORF) is shown with every tenth base indicated by a circle below the DNA sequence. Letters indicate single base substitutions, closed triangles indicate single base additions and open triangles indicate single base deletions. (A) Spectra for the *pol3-L612M SWR1 and pol3-L612M swr1* strains are depicted above and below the *URA3* ORF, respectively. (B) Spectra for the *pol3-L612M SWR1 exo1* and *pol3-L612M swr1 exo1* strains are depicted above and below the *URA3* ORF, respectively.

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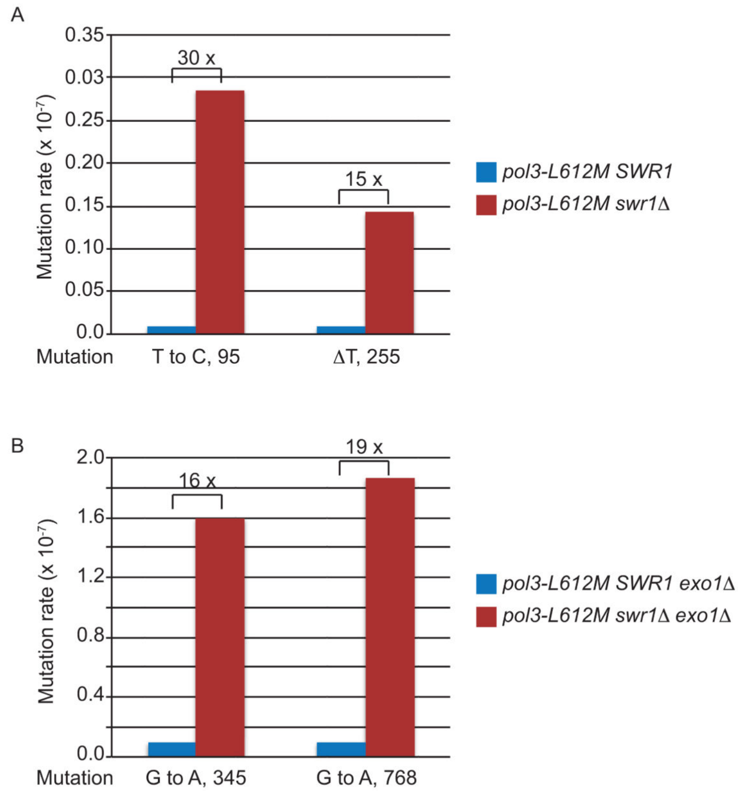

Fig. 3.

Mutation rates at four specific locations in *URA3*. (A) *pol3-L612M* ± *SWR1* strains. Rates for the *pol3-L612M SWR1* strain were calculated using data from [24] plus additional 5-FOA-resistant mutant sequences. No mutations were observed at *URA3* positions 95 or 255 in this strain, so rates were calculated as values had one event been observed. Rates for the *pol3-L612M swr1* mutant were calculated using data in Figure 2 and Table 1. (B) *pol3-L612M exo1* ± *SWR1* strains. Rates for the *pol3-L612M SWR1 exo1* strain were calculated using data from [9] plus additional 5-FOA-resistant mutant sequences (this study). No mutations were observed at *URA3* positions 345 or 768 in this strain, so rates were calculated as values had one event been observed. Rates for the *pol3-L612M swr1 exo1* mutant were calculated using data in Figure 2 and Table 1.

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Table 1

Sequence analysis of 5-FOA-resistant mutants *in pol3-L612M strains.*

Genotype (all *pol3-L612M*)	***SWR1*** ([a])	***swrl***	***SWRl exol*** ([b])	***swrl exol***
Mutation Rate ($\times 10^{-7}$)	2.3	4.4	29	48
Total Mutants Sequenced	245	277	290	180
Single Base Mutations	173	265	183	167
Others ([c])	72	12	80	12

[a] Data from [24] plus additional 5-FOA-resistant mutant sequences.

[b] Data from [9] plus additional 5-FOA-resistant mutant sequences (this study).

[c] Others include 5-FOA-resistant mutants that contained complex mutations, multi-base insertions/deletions or no change in the *URA3* open reading frame. The mutants with no sequence change in *URA3* were not investigated further, but they may result from epigenetic silencing, they may contain sequence changes in the promoter or 3' untranslated region of *URA3,* or they may contain mutations in other genes that result in 5-FOA resistance.