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Brief Communication

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 swr1Δ* 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.
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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 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 *exo1Δ* strain, but it is not observed when *SWR1* is deleted from strains encoding wild

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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 *S. cerevisiae* strains used here are isogenic derivatives of strain Δ|(−2)|-7B-YUNI300 (*MATa CAN1 his7-2 leu2-Δ::kanMX ura3-Δ trp1-289 ade2-1 lys2-ΔGG2899-2900 agp1::URA3-OR1*) [15]. Polymerase mutator alleles have been described previously [16–18]. Heterozygous *EXO1/exo1Δ*, *SWR1/swr1Δ*, *HTZ1/htz1Δ*, and *MSH2/msh2Δ* 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 haploids were obtained from tetrad dissection. Mutation rate data and sequencing analyses were performed using at least three 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 swr1Δ*) and between the two *exo1Δ* strains (*pol3-L612M exo1Δ* versus *pol3-L612M exo1Δ swr1Δ*) [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 *swr1Δ* 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 swr1Δ* double mutant strain was 2-fold higher than for the *pol3-L612M* single mutant strain (Fig. 1A). This difference is significant as

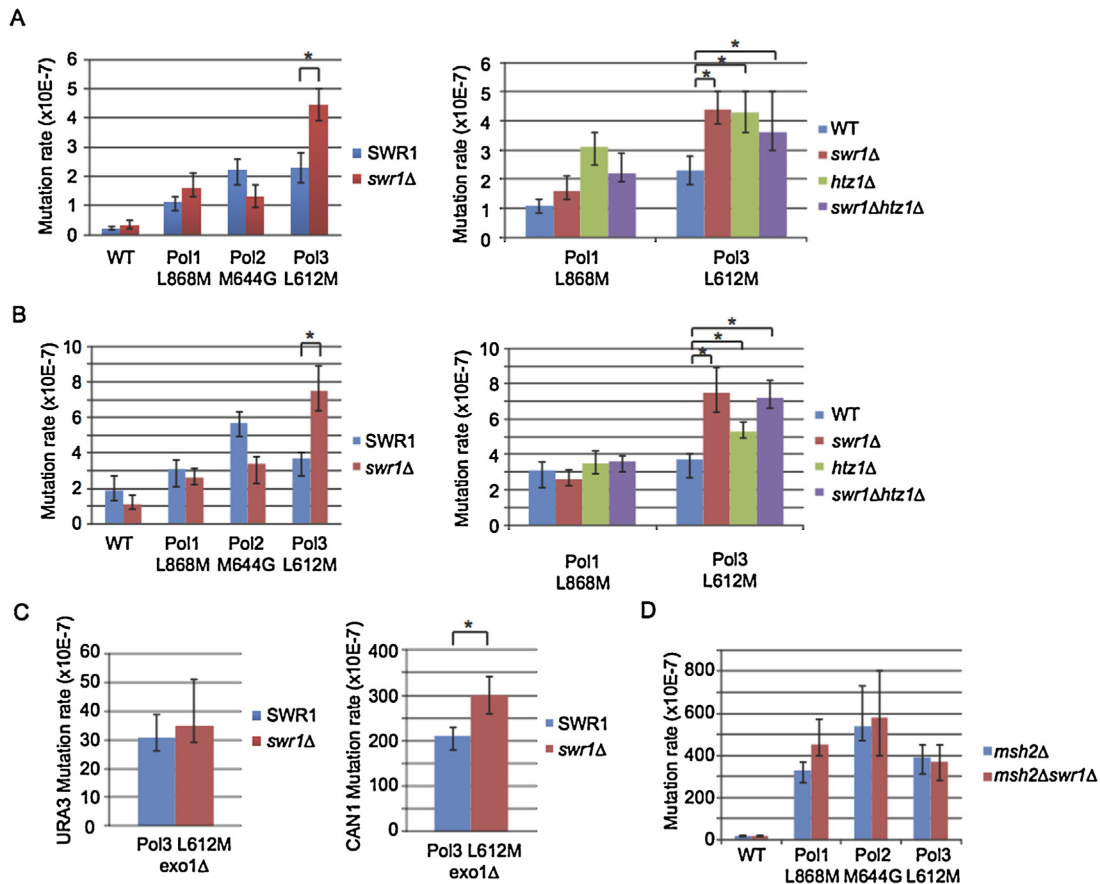

Fig. 1. H2A.Z deposition by SWR-C reduces the mutation rate due to *pol3-L612M*. Spontaneous mutation rates to 5-FOA resistance (A) and canavanine resistance (B) with 95% confidence intervals are shown. Statistically significant differences ($p < 0.0001$) in mutation rate are indicated by an asterisk. 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 *exo1Δ* 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 *msh2Δ* background. Mismatch repair is required for the mutagenic effect of *SWR1* deletion.

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