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RTT109 CHROMATIN MODIFIER REGULATES MUTAGENIC DNA DAMAGE BYPASS

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(Manuscript in preparation)

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ABSTRACT

Mutations have been a driving force in the evolution of every organism. Although they can be beneficial, in most of the cases they lead to protein dysfunction, which in turn can impair important cellular pathways and cause diseases such as cancer. Mutations arise primarily from processing of DNA lesions that are induced by environmental threats or by endogenous cellular metabolism. Although cells have evolved mechanisms for the repair of DNA damage, they can also tolerate (unrepaired) DNA lesions that may pose blocks to replication forks. To date, template switching and translesion synthesis (TLS) are the best-characterized DNA damage tolerance pathways. The first process is error-free while the latter can be mutagenic. How DNA damage tolerance is regulated in the context of chromatin is largely unclear. Here, we report on a new role for the histone acetyltransferase Rtt109 in TLS. Our results suggest that Rttt109 affects DNA damage bypass mediated by different TLS polymerases.

INTRODUCTION

To cope with the deleterious effects of DNA damage, cells have evolved a variety of repair processes. However despite their efficacy, the replication machinery may encounter unrepaired DNA lesions that can block progression of replication forks. To prevent prolonged replication fork arrest and subsequent collapse of the complex molecular machinery that carries out DNA replication namely the replisome, cells possess error-free and error-prone mechanisms collectively referred to as DNA damage tolerance or post-replication repair (PRR) pathways. The coordination of these pathways during DNA replication is mediated by two ubiquitin-conjugating and ligating complexes Rad6-Rad18 and Rad5-Mms2-Ubc13. When replication forks are blocked by DNA lesions, PCNA is mono-ubiquitylated by the Rad6-Rad18 complex. Mono-ubiquitylation of PCNA promotes error-free and error-prone bypass through a mechanism called translesion synthesis (TLS), while its polyubiquitilation by the Mms2-Ubc13 complex favors the error-free bypass pathway of template switching. During TLS, the replicative polymerase Polδ and specialized TLS polymerases such as Polη (Rad30), Rev1 and Polζ are able to incorporate nucleotides at the 3' of the lesion and as such allow the replication to restart downstream of the lesion [1]. Template switching is a mechanism that uses the sister chromatid as a template to bypass the lesion in a recombination-like event and likely involves key homologous recombination factors, including Rad52 [2]. Polη's (encoded by RAD30) major function is to ensure error-free bypass of cys-syn cyclobutane pyrimidine dimers (CPDs), which are lesions typically caused by ultraviolet (UV) light [3]. However, some reports also suggest that Polη is involved in mutagenic bypass events depending on the type of DNA lesion [4, 5]. On the other hand, Rev1 and the Polζ complex composed of Rev7 and the catalytic subunit Rev3, are responsible for the vast majority of the mutagenic bypass events [6]. Biochemical and genetic evidence suggests that Rev1 stimulates Polζ-mediated extension from a mismatch or bypass of a lesion [7]. At last, in yeast, Pol3 and Pol32 (two subunits of the replicative polymerase Polδ) have also been implicated in damage-induced mutagenesis [8, 9]. Importantly, the two Polδ subunits Pol31 and Pol32 were recently found in a stoichiometric four-subunit complex containing the Polζ subunits Rev3 and Rev7, providing a biochemical explanation for how Polδ could affect DNA damage-induced mutagenesis [10].

In eukaryotic cells, DNA is wrapped around histone proteins resulting in densely packed chromatin, which limits the access of repair enzymes to DNA lesions. How chromatin is converted into a dynamic molecule that allows access and repair of DNA lesion has become a growing field of investigation. Histone modifications including phosphorylation, methylation, ubiquitylation and acetylation, as well as ATP-dependent chromatin remodeling (a process that changes nucleosome composition or positioning) affect the detection and repair of DNA damage. The role of histone modifiers and chromatin remodelers in the cellular response to DSB has been intensively investigated [11-13]. However, how they regulate DNA damage tolerance is largely unknown. The histone methyltransferase Dot1 and the chromatin remodeler Ino80 have been suggested to play a role in DNA damage tolerance, which indicates that chromatin modulation may be a critical step during this process [14, 15]. Here, we show that Rtt109, by promoting acetylation of histone H3 at lysine 56, affects the mutagenic bypass of UV- and MMS-induced DNA lesions through TLS, providing further insight into the mechanisms that regulate DNA damage tolerance in the context of chromatin.

RESULTS

Epistatic interactions between RTT109 and the Polζ and Polδ complexes.

We have made use of an advanced technology for analysis of epistasis between pairs of genes in yeast (EMAP), to investigate the changes in genetic interactions induced by three different DNA damaging compounds, namely methyl methanesulfonate (MMS), camptothecin (CPT) and zeocin (ZEO). To focus our study, we crossed 55 query DNA damage response (DDR) genes with an array of 2022 genes involved in the DDR, as well as in other cellular processes, such as cell cycle regulation, chromatin organization, replication, transcription, and protein transport (see chapter 1). We measured growth rates 48h after the obtained double mutant strains were exposed or not to the different DNA damaging agents. Finally, genetic interaction scores were obtained by normalization and statistical analysis of colony size (a measure of cell growth).

The resulting genetic interaction map revealed a strong MMS-induced response of the post-replication repair pathways comprising the Mms2-Ubc13 ubiquitin ligase known to channel bypass of replication fork-blocking lesions through the error-free template switching pathway (reviewed by [1]), suggesting that our genetic interaction map can highlight interactions relevant for DNA damage tolerance. To further interpret these drug-induced interactions, we combined our genetic networks with available protein-protein interaction data and generated a map that shows the rewiring of connections between functional modules in response to MMS, ZEO and CPT (Chapter 1, Figure 5). The module map not only shows that the Mms2-Ubc13 complex is a major hub in response to MMS, but also that the TLS polymerase Polζ engages multiple interactions under that same condition (Figure 1A). In agreement with two previous studies reporting that mutants of the 9-1-1 checkpoint complex have decreased efficiency in TLS-induced mutagenesis, we found that the 9-1-1 complex and TLS polymerase Polζ interact in our network (Figure 1A) [16, 17]. Interestingly, the module map also highlighted MMS-dependent interactions involving the histone acetyltransferase Rtt109, including a positive interaction with POL32, one of the subunit of the replicative polymerase Polδ, consistent with work showing that Rtt109 affects replisome stability in response to replication fork-blocking lesions (Figure 1B) [18]. We also observed unanticipated epistatic relationships between RTT109 and the TLS polymerase genes REV1, REV3 and REV7; the latter two encoding for Polζ subunits (Figure 1B), were validated in spot dilution assays (Figure 1C). We also observed epistasis between the Rev1-Polζ complex and Rtt109 in response to DNA damage induced by UV light or camptothecin (CPT) (Figure 1C). Polζ-dependent TLS enables cells to replicate through DNA lesions, thereby preventing collapse of replication forks [19]. Moreover, Polζ, in conjunction with Polδ, is responsible for as much as 85% of the bypass events at abasic sites [20], mainly in an error-prone fashion [19]. Consequently, the epistatic links seen between Polζ, Polδ and Rtt109 suggest a role for the histone acetyltransferase in TLS.

To specifically study the role of Rtt109 in TLS, we employed UV light in concert with nucleotide excision repair (NER)-deficient rad14 strains (to prevent repair of DNA photolesions through NER) to favor lesion bypass by TLS. We also monitored their sensitivity to MMS-induced damage. Surprisingly and in contrast to the observed epistatic interaction between REV3 and RTT109 in NER-proficient

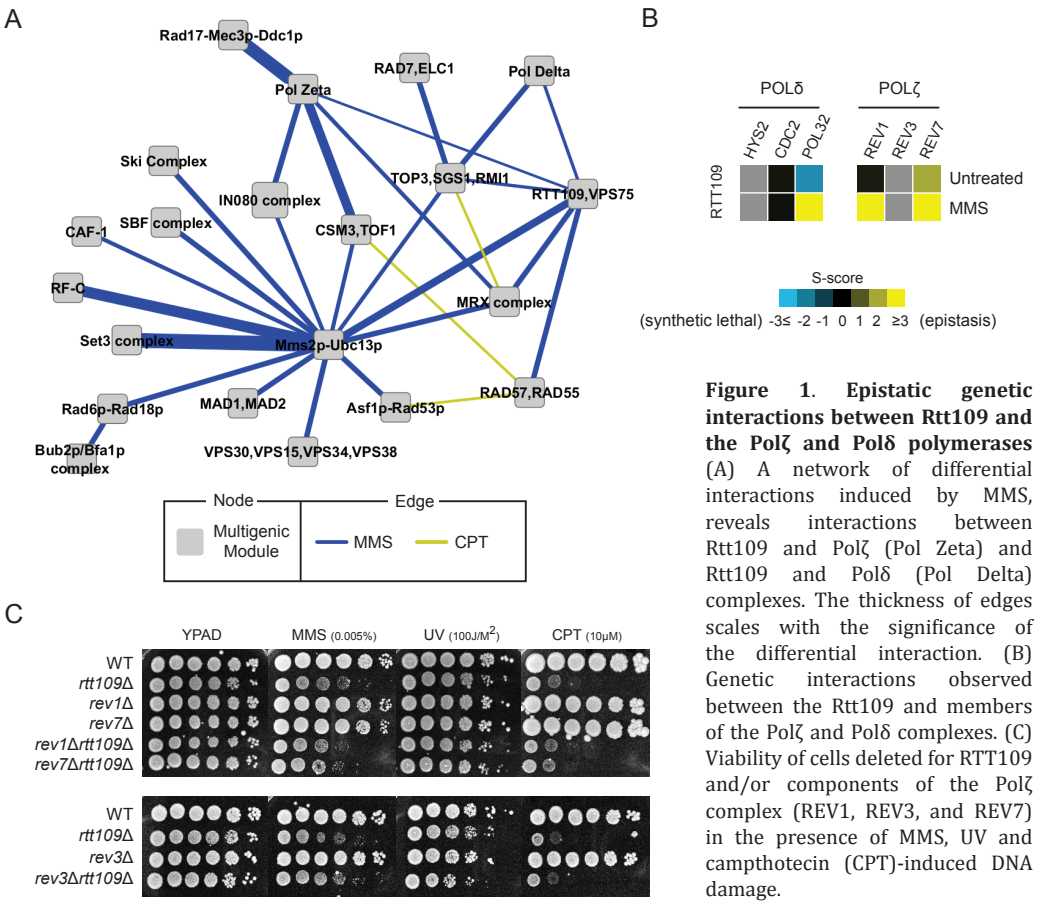

Figure 1. Epistatic genetic interactions between Rtt109 and the Polζ and Polδ polymerases (A) A network of differential interactions induced by MMS, reveals interactions between Rtt109 and Polζ (Pol Zeta) and Rtt109 and Polδ (Pol Delta) complexes. The thickness of edges scales with the significance of the differential interaction. (B) Genetic interactions observed between the Rtt109 and members of the Polζ and Polδ complexes. (C) Viability of cells deleted for RTT109 and/or components of the Polζ complex (REV1, REV3, and REV7) in the presence of MMS, UV and campthotecin (CPT)-induced DNA damage.

strains, it appeared that the NER deficient *rev3*Δ*rtt109*Δ strain was more sensitive to MMS than either single mutant (Figure 2A). This indicates that in the absence of NER Rtt109 and Rev3 play redundant roles in cell survival after MMS-induced DNA damage. To examine whether this phenotype depends on H3K56 acetylation by Rtt109, we assessed the MMS sensitivity of cells expressing a non-acetylatable histone H3 in which lysine 56 was replaced by an arginine (H3K56R). Similarly to the *rev3*Δ*rtt109*Δ strain, the *rev3*ΔH3K56R strain was more sensitive to MMS than either single mutant strain (Figure 2B).

Next, to test the possible role of acetylation of H3K56 by Rtt109 in other type of DNA damage that can be bypassed by TLS, the two strains defectives for that process, *rtt109*Δ and H3K56R, were assessed for their UV sensitivity. We also measured the UV sensitivity of cells deleted for ASF1, which encodes a H3/H4 histone chaperone that stimulates Rtt109's histone acetyltransferase activity by forming a heterodimeric complex with Rtt109 [21]. Again, the *rev3*Δ*rtt109*Δ, the *rev3*ΔH3K56R as well as the *rev3*Δ*asf1*Δ mutants were more sensitive to UV compared with either single mutant (Figure 2C-E). Altogether these results strongly suggest that Rtt109-dependent acetylation of H3K56 is a pathway that acts redundantly to Rev3 in mediating MMS and UV survival when NER activity is perturbed.

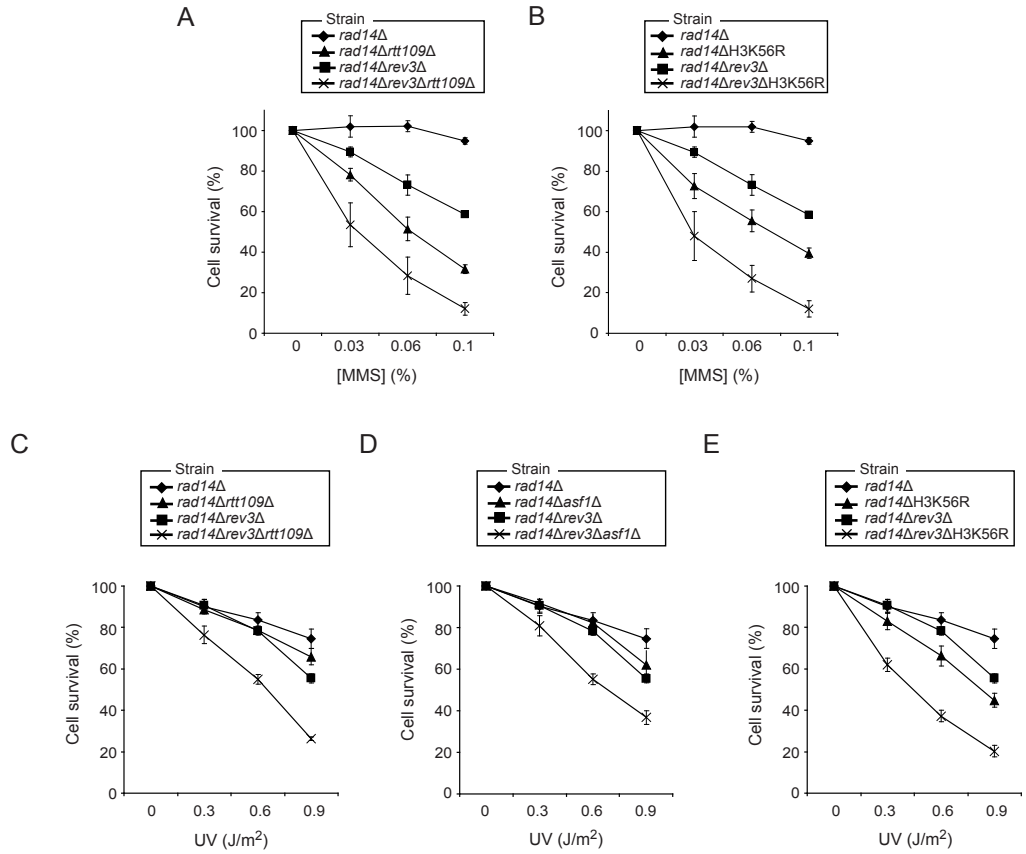

Figure 2. **Effect of Rtt109-mediated H3K56 acetylation on cell survival after MMS and UV exposure** (A) MMS survival was examined in wild-type (WT) *rev3*Δ, *rtt109*Δ, *rev3*Δ*rtt109*Δ cells, and (B) in WT, *rev3*Δ, H3K56R non-acetylatable H3 mutant, *rev3*ΔH3K56R. (C) UV survival was examined in WT, *rev3*Δ, *rtt109*Δ, *rev3*Δ*rtt109*Δ cells, (D) in WT, *rev3*Δ, *asf1*Δ, *rev3*Δ*asf1*Δ cells and (E) in WT, *rev3*Δ, H3K56R, *rev3*ΔH3K56R cells. Cells were exposed for 20 minutes to the indicated doses of MMS before dilution and spreading on plates without or with canavanine (60mg/l). In the UV experiments, cells were first diluted and spread on plates before being exposed to the indicated UV doses. WT and all mutant strains were derived from a NER-deficient *rad14*Δ background. The data represent averages of three independent experiments and error bars represent the mean +/- standard deviations.

Rtt109 is involved in MMS and UV-induced mutagenesis

To investigate the possible role of Rtt109 in the mutagenic bypass of MMS and UV-induced damage, we utilized a CAN1 forward mutation assay, which reports any mutation that disrupts Can1 function resulting in a canavanine-resistance (can1r) phenotype. Cells with proficient TLS activity will accrue mutations at this locus at a much higher rate enabling them to survive selection on media containing canavanine. As expected and in accordance with a role for Polζ in mutagenic bypass, *rev3*Δ cells (which were also deleted for RAD14 and therefore NER-deficient) were unable to form can1r colonies after transient exposure to MMS (Figure 3A). Interestingly, we found that MMS-exposed *rtt109*Δ cells were 2-fold less effective in accumulating canr colonies compared to the NER deficient *rad14*Δ

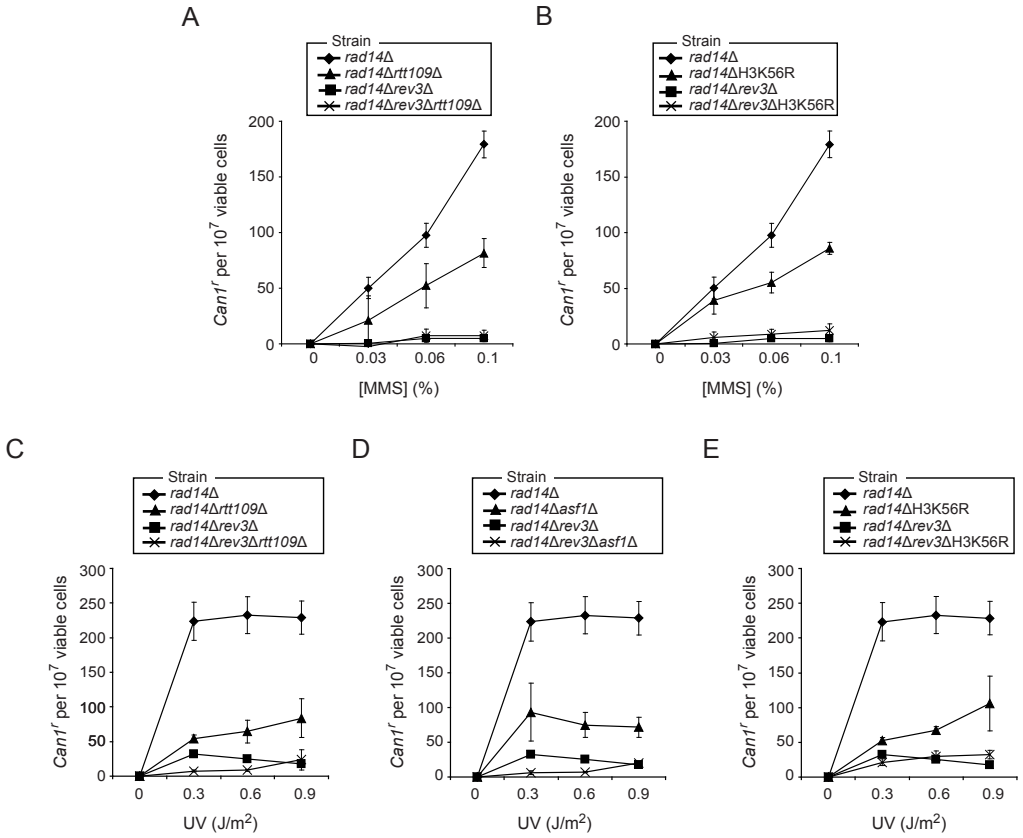

Figure 3. **H3K56 acetylation by Rtt109 affects the proficiency of UV-induced mutagenesis** (A) MMS-induced can1r mutation frequencies were examined in wild-type (WT), *rev3*Δ, *rtt109*Δ, *rev3*Δ*rtt109*Δ cells and (B) in WT, *rev3*Δ, H3K56R (the non-acetylatable H3K56 mutant) and *rev3*ΔH3K56R mutant. (C) UV-induced can1r mutation frequencies were examined in wild-type (WT), *rev3*Δ, *rtt109*Δ, *rev3*Δ*rtt109*Δ cells, (D) in WT, *rev3*Δ, *asf1*Δ, *rev3*Δ*asf1*Δ cells and (E) in WT, *rev3*Δ, H3K56R, *rev3*ΔH3K56R cells. Cells were prepared as described in Figure 2. All strains are derived from a NER-deficient *rad14*Δ background. The data represent averages of three independent experiments and error bars represent the mean +/- standard deviations.

mutant (for reasons of clarity we will refer to this background as "wild-type" in the rest of this chapter) (Figure 3A). Similar to the *rtt109*Δ mutant, the H3K56R mutant showed a 2-fold decrease in can1r colonies formation after MMS treatment (Figure 3B). Altogether, these results imply that Rtt109 may promote mutagenic bypass of MMS-induced damage. It is important to note that the rates of spontaneous mutation were neither affected by deletion of RTT109 nor in cells expressing the H3K56 mutant version of H3 (Figure S1).

Polζ is responsible for the bypass of multiple types of DNA damage of which UV lesions have been best studied [22, 23]. Thus, we turned our analysis to evaluate the role of Rtt109 in the bypass of UV-induced damages. By using the afore mentioned CAN1 forward mutation assay we tested wild-type, *rev3*Δ and *rtt109*Δ cells after exposure to various doses of UV.

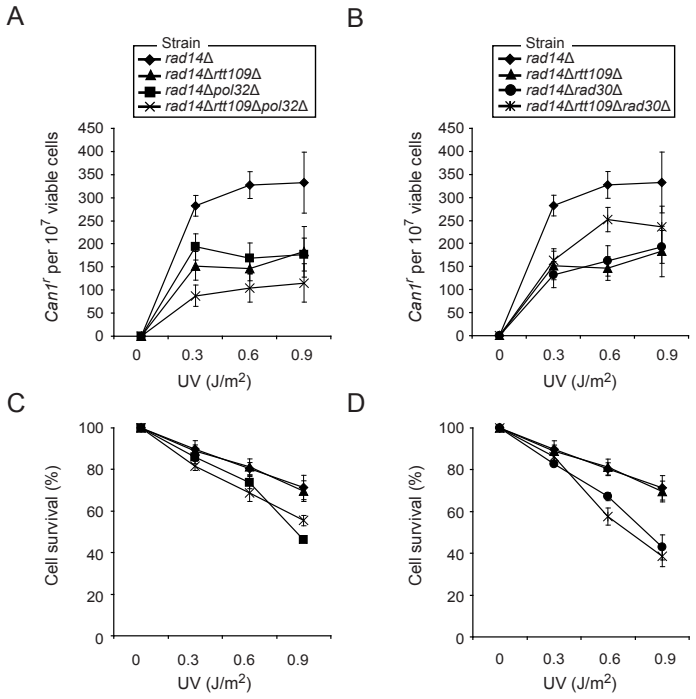

Figure 4. Epistasis analysis of Rtt109, Polδ and Polη in their proficiency of UV-induced mutagenesis (A) UV-induced can1r mutation frequencies were examined in wild-type (WT), *pol32*Δ, *rtt109*Δ, *rtt109*Δ*pol32*Δ cells, (B) in WT, *rad30*Δ, *rtt109*Δ, *rtt109*Δ*rad30*Δ cells. (C) UV survival was examined in *pol32*Δ, *rtt109*Δ, *rtt109*Δ*pol32*Δ cells and (D) in WT, *rad30*Δ, *rtt109*Δ, *rtt109*Δ*rad30*Δ cells. Cells were diluted and plated on plates without or with canavanine (60mg/l) and exposed to the indicated UV doses. All strains are derived from a NER-deficient *rad14*Δ background. The data represent averages of three independent experiments and error bars represent the mean +/- standard deviations.

In agreement with previous work, we found that in the absence of REV3, cells almost abrogate UV damage-induced mutagenesis (Figure 3A; [17]). Strikingly, *rtt109*Δ strains displayed a nearly 5-fold decrease in the number of canr colonies compared to wild-type cells. To validate that this phenotype is a consequence of impaired Rtt109 activity, we measured the UV-induced mutagenesis rates in cells that were either deleted for ASF1 or expressed the H3K56R mutant allele of histone H3. These two mutants produced nearly the same number of canr colonies as the rtt109Δ strain did (Figure 3C-E). These results imply that Rtt109 is partially involved in the Rev3-dependent mutagenic bypass of DNA lesions (accounting for at least 50 % of the MMS- and UV-induced mutation; Figures 3 and 4A-B).

Rtt109 is epistatic with Polη but acts synergistically with Polδ in UV-induced mutagenesis.

We next asked to what extent Rtt109 and TLS polymerases cooperate to promote efficient lesion bypass. To this end, we monitored mutagenesis in Polη-deficient *rad30*Δ and Polδ-deficient *pol32*Δ strains that were either proficient or deficient for Rtt019 (*rtt109*Δ*rad30*Δ and

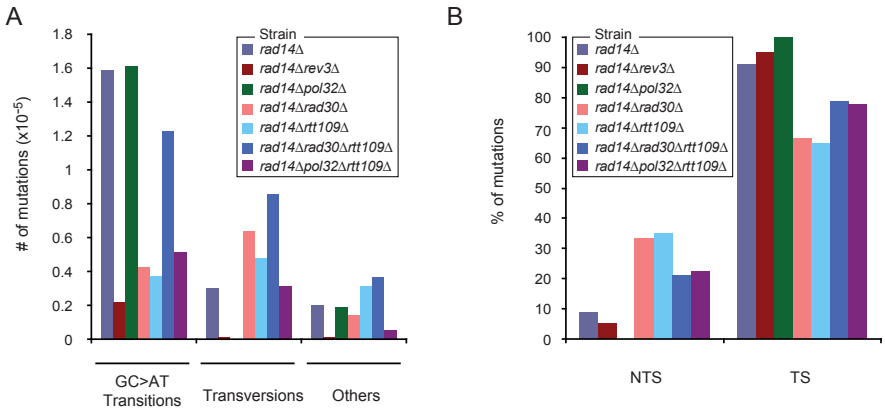

Figure 5. Effect of Rtt109, Polζ, Polδ and Polη on UV-induced mutagenesis of CAN1 (A) UV-induced frequencies of GC to AT transitions, transversions and other mutations in WT, *rev3*Δ, *pol32*Δ, *rad30*Δ, *rtt109*Δ, *rtt109*Δ*rad30*Δ and *rtt109*Δ*pol32*Δ cells. (B) Strand distribution of the UV-induced mutations in WT, *rev3*Δ, *pol32*Δ, *rad30*Δ, *rtt109*Δ, *rtt109*Δ*rad30*Δ and *rtt109*Δ*pol32*Δ. The non-transcribed strand and the transcribed strand are noted NTS and TS, respectively. All strains are derived from a NER-deficient *rad14*Δ background.

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*rtt109*Δ*pol32*Δ). Consistent with a study from Giot et al., we found that *pol32*Δ cells display a 2-fold decrease in mutation rate when compared to wild-type (Figure 4A; [8]), an effect similarly to that observed in *rtt109*Δ cells. Surprisingly, *rtt109*Δ*pol32*Δ mutants formed 1.7-fold less canr colonies than either single mutant (Figure 4A), whereas the UV sensitivity of this double mutant was comparable to that of the most sensitive single mutant (*pol32*Δ; Figure 4C). This suggests that Rtt109 and Pol32 work in two different UV-induced mutagenic pathways. On the other hand, the mutation rates in *rad30*Δ mutants were reduced as much as those observed in *rtt109*Δ cells (~2-fold decrease compared to wild-type; Figure 4B). Interestingly, this decrease was slightly alleviated by deletion of RTT109 in the *rad30*Δ mutant (Figure 4B), while the UV sensitivity of the *rtt109*Δ*rad30*Δ double mutant was still comparable to that of the most sensitive single mutant (*rad30*Δ; Figure 4D). This suggests that Polη and Rtt109 co-operate during DNA damage bypass, but that loss of both these factors may alleviate their (combined) suppressive effect on an error-prone, mutagenic pathway that likely involves Rev3.

Rtt109 loss shows a pattern of mutations that lack the typical GC to AT transition induced by the bypass of UV lesions.

Understanding mechanisms of bypass by TLS have been profited from exploring the patterns of mutations induced by polymerases across UV lesions [22, 23]. Thus, we sought to further investigate the role of Rtt109 in TLS-mediated mutagenesis by comparing the UV-induced spectra of mutations of *rtt109*Δ, *rev3*Δ, *rad30*Δ and *pol32*Δ mutants under NER deficient conditions.

In agreement with previous reports, we observed that the prominent mutations induced after UV exposure were transitions and transversions [24]. Moreover, we found that GC to AT transitions represented 70 to 90% of all transitions [25]. These mutations are thought to arise from insertion of A's by Polη opposite cytosines that are

unstable in CPDs and rapidly deaminate into uracil [26, 27]. In accordance with this, we see a 4-fold decrease of this type of transition in *rad30*Δ cells. The remaining GC to AT transitions are likely caused by Polζ [25]. Loss of REV3 on the other hand nearly abolished mutagenesis and only a low level of GC to AT transitions possibly induced by Polη remained (Figure 5A). Interestingly, in *rtt109*Δ cells the frequency of GC to AT transitions was dramatically reduced, suggesting that Rtt109, like Rev3 and Rad30, promotes GC to AT transitions. Finally, in *pol32*Δ cells, the number of GC to AT transitions was similar to that of wild-type cells, indicating that Pol32 does not affect GC to AT transitions.

Together the data suggest that Rtt109 promotes UV-induced GC to AT transitions most likely depending on Rev3 and Rad30 activities. To test the latter, we examined these mutations in the *rtt109*Δ*rad30*Δ double mutant. Surprisingly, the rate at which GC to AT transitions appeared was 3-fold increased when compared to either of the single mutants and nearly returned to wild-type levels (Figure 5A). In contrast, loss of Pol32 did not affect the transition rates observed in the *rtt109*Δ mutant in agreement with our previous notion that Pol32 does not promote GC to AT transitions. Collectively, these results suggest that the Rtt109-dependent GC to AT transitions are most likely generated by Rev3, but not Polη (Figure 5A).

The rate at which transversions occurred was much lower than that observed for transitions (wild-type; Figure 5A). Transversions were primarily induced by Rev3 and Pol32 activities as indicated by the lack of these mutations in *rev3*Δ and *pol32*Δ mutants (Figure 5A). Remarkably, elevated levels of transversions were observed in both *rad30*Δ and *rtt109*Δ mutants when compared to wild-type (Figure 5A). These levels even further increased in the combined absence of Rad30 and Rtt109 (*rad30*Δ*rtt109*Δ mutant; Figure 5A), which suggests that Rtt109 may co-operate with Rad30 to suppress transversion formation by Rev3 and Pol32. In line with this we found that loss of Pol32 in the *rtt109*Δ mutants partially reduced the transversion rates (Figure 5A).

UV light induces helix-distorting lesions that can perturb cellular processes including DNA replication and transcription. Fortunately, eukaryotic cells are equipped with a transcription-coupled NER (TC-NER) mechanism that efficiently clears DNA damage from the transcribed strand (TS) [28]. Here we used NER deficient *rad14*Δ strains which explains why UV-induced mutations were found primarily in the TS (90% in the TS, Figure 5B). When REV3 or POL32 was deleted in this background, mutations still accrued in the TS (95% and 100% respectively). Surprisingly, however, deletion of RTT109 or RAD30 decreased the levels of mutations in the TS, yet resulted in a bigger proportion of mutations in the non-transcribed strand (NTS) (35% versus 10% and 5% in wild-type and *rev3*Δ cells respectively, Figure 5B). These effects were partially reversed by the loss of Rad30 or Pol32. In conclusion, our work suggests that while Rtt109 may suppress the formation of transversions by Rev3 and Pol32, it may favor the formation of GC to AT transitions in the TS, which are most likely induced by Rad30 and Rev3. Thus, Rtt109 seems to operate at the crossroads of Rad30 and Rev3-dependent DNA damage bypass routes.

DISCUSSION

In this study we found that acetylation of histone H3 on lysine K56 by Rtt109 plays a role in TLS-induced mutagenesis. Using

the CAN1 forward mutation assay, we first showed that Rtt109 loss in a NER deficient background reduces the frequency of MMS- and UV-induced mutations, which suggests that Rtt109 promotes DNA damage-induced mutagenesis (Figure 3A and 3C). Secondly, we found that *rtt109*Δ cells were inefficient at inducing the typical UV-signature of mutations i.e GC to AT transitions. It has recently been shown that while Rad30 is required, Polζ is essential for generating this GC to AT signature [25]. Accordingly, we found that Rtt109 may affect both the activities of Rad30 and Polζ. The mechanism by which the histone acetyltransferase Rtt109 promotes UV-induced GC to AT mutations is still unclear. Recently, a study by Hendriks et al. in mammalian cells proposed that transcription (particular under repair deficient conditions) enhances the formation of uracil by deamination of CPD-containing cytosines [29]. In yeast, a similar phenomenon referred to as transcription-associated mutagenesis or TAM was also described to be enhanced in presence of DNA damage [30, 31]. Interestingly, Rtt109 by acetylating H3K56 has been associated with transcriptional activation and RNA PolII elongation [32, 33]. More recently, H3K56ac by Rtt109 was found to facilitate transcription of heterochromatic regions [34]. This suggests that loss of Rtt109 perturbs transcription and thereby may reduce transcription-associated cytosine-deamination mediated by DNA damage and decrease the relative proportion of mutations induced by lesions in the TS. We also showed that MMS-induced mutagenesis, likely caused by the bypass of abasic sites, is impaired in *rtt109*Δ cells (Figure 3A). A report proposed that the stalling of RNA PolII at abasic sites caused by MMS treatment increased the mutation rate [35]. Thus, the role of Rtt109 in promoting transcription could similarly explain the MMS-induced mutagenesis defect seen in *rtt109*Δ cells. However, neither the loss of Rtt109, nor that of Rad30 affects the transcription levels of CAN1 in unperturbed cells (Figure S2). Although this suggests that Rtt109 may not indirectly affect transcription-associated mutagenesis in this manner, it still needs to be examined whether Rtt109 loss affects transcription of CAN1 in UV- or MMS-exposed cells. Importantly, the frequency of GC to AT transitions observed in *rtt109*Δ*rad30*Δ cells is nearly the same as in wild-type cells, which attests that together Polδ (or more precisely the two Pol31 and Pol32 subunits found in a complex with Polζ) and Polζ are able to replace Polη's role in the mutagenic bypass of CPDs. This could be due to the recovery of a wild-type level of transcription in the *rtt109*Δ*rad30*Δ mutant. Or in the contrary, this could come from massive deamination of cytosines caused by persistent transcription stalling (that would enhance the rate of uracil to be replicated). These two options need to be further investigated. Another validation would be to show how reversion of CPDs by photoreactivation affects mutagenesis in *rtt109*Δ cells. At last, analyzing the epistasis between Rtt109 and the uracil-glycosylase Ung1 which is required for the repair of spontaneous deaminated-cytosines would give further insight into the contribution of Rtt109 in this mechanism.

Emerging evidence emphasizes the importance of histone modifications in controlling TLS in the context of chromatin. For instance, two recent studies show that loss of Dot1, the H3K79 methyltransferase, alleviates the sensitivity of the *rtt107*Δ, *rad52*Δ, *rad14*Δ, *apn1*Δ repair mutants to the DNA alkylating agent MMS [14, 36]. In both reports, the authors explain the alleviating phenotype by an increased TLS efficiency in *dot1*Δ and propose that H3K79 methylation by Dot1 downregulates TLS.

We could envisage that H3K56 acetylation by Rtt109 prevents the action of Dot1 thereby alleviating the Dot1 inhibiting effect on TLS. It becomes apparent that these histone modifications are important to ease the access to DNA in a chromatin context and to facilitate the recruitment of factors involved in DNA metabolism such as transcription and DNA replication that can impact on DNA integrity.

MATERIAL & METHODS

Sensitivity assays on plates

Overnight cultures were diluted 1:20, grown for 3 hours at 30 °C and diluted to $1x10^7$ cells/ml. Fivefold dilution series were spotted on plates containing 10μM CPT or 0.005% MMS or spotted on plates and then exposed to a UV dose of $100J/m^2$. Growth was scored after 3 days at 30 °C.

Mutagenesis assays

Mutagenesis assays were performed as described previously (Johnson, 1998). Briefly, serial dilutions of cells were spread on YPAD to determine cell viability and on SC-Arg plates containing canavanine (60mg/l) to monitor the efficiency of can1r colony formation. In the MMS experiment, cells were exposed for 20 minutes to the indicated MMS concentrations and washed before they were diluted and plated.

Can^r mutation spectra

Canr mutation spectra were determined by PCR amplification and sequence analysis of the CAN1 gene of independent canr colonies generated during the mutagenesis experiments. Genomic DNA was isolated and the CAN1 gene was amplified by PCR using the forward primer oHA-1071 (5'-CTTCAGACTTCTTAACTCC-3') and the reverse primer oHA-1072 (5'-GAGGGTGAGAATGCGAAAT-3'). Purified PCR products were then sequenced with primers oHA-1072, oHA-1073 (5'-GGCATATTCTGTCACGCAG-3'), oHA-1159 (5'-GGAACAAGTTCATTATTGTG-3'). DNA sequencing was performed according to the standard Sanger sequencing method using the 96-capillary 3730XL system from Applied Biosystems. The sequences were analyzed with ContigExpress software (Vector NTI).

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SUPPLEMENTAL DATA

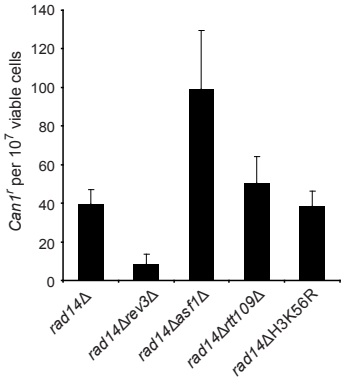

Figure S1: The spontaneous mutations are not affected by Rtt109 deletion. Spontaneous can1r mutation frequencies were examined in wild-type, *rev3*Δ, *asf1*Δ, *rtt109*Δ and H3K56-expressing cells. Cells were diluted and plated on plates without or with canavanine (60mg/l). All strains are derived from a NER-deficient *rad14*Δ background. The data represent averages of three independent experiments and error bars represent the mean +/- standard deviations.

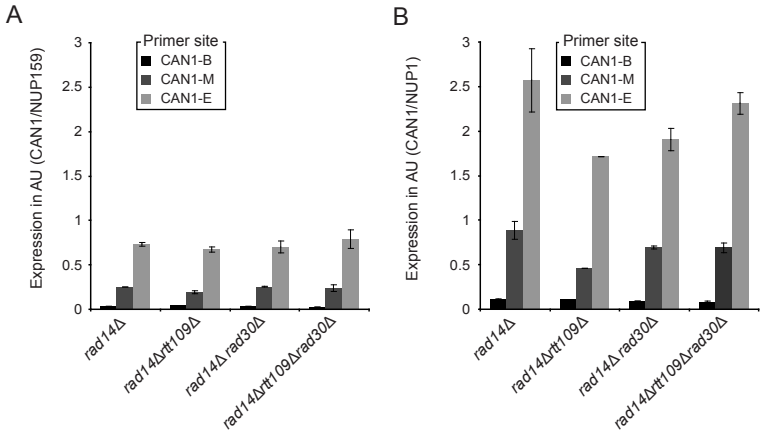

Figure S2: The transcription level of the CAN1 gene is not affected by deletions of Rtt109 and Rad30 under unchallenged conditions (A) Bar-plot showing the transcription levels of the beginning (CAN1-B), the middle of the gene (CAN1-M) and the end of the CAN1 gene (CAN1-E) in wild-type, *rtt109*Δ, *rad30*Δ and *rad30*Δ*rtt109*Δ unchallenged cells. The transcription level analysis was done according to the procedure described in Taddei et al. (2006). The levels were normalized on the NUP159 gene. (B) as in (A) except that the transcription levels were normalized on the NUP1 gene. All strains are derived from a NER-deficient *rad14*Δ background. The data represent averages of two independent RT-PCR runs and error bars represent the mean +/- standard deviations.