Current Biology *16*, 586–590, March 21, 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.01.063

Report

ATR Homolog Mec1 Controls Association of DNA Polymerase ζ-Rev1 Complex with Regions near a Double-Strand Break

Yukinori Hirano[1] and Katsunori Sugimoto[1,*]
[1] Department of Cell Biology and Molecular Medicine
University of Medicine and Dentistry of New Jersey
New Jersey Medical School
Newark, New Jersey 07103

Summary

DNA polymerase zeta (Polζ) and Rev1 contribute to the bypassing of DNA lesions, termed translesion DNA synthesis (TLS) [1–3]. Polζ consists of two subunits, one encoded by *REV3* (the catalytic subunit) and the other encoded by *REV7*. Rev1 acts as a deoxycytidyl transferase, inserting dCMP opposite lesions. Polζ and Rev1 have been shown to operate in the same TLS pathway in the budding yeast *Saccharomyces cerevisiae* [2, 3]. Here, we show that budding yeast Polζ and Rev1 form a complex and associate together with double-strand breaks (DSBs). As a component of the Polζ-Rev1 complex, Rev1 plays a noncatalytic role in the association with DSBs. In budding yeast, the ATR-homolog Mec1 plays a central role in the DNA-damage checkpoint response [4, 5]. We further show that Mec1-dependent phosphorylation promotes the Polζ-Rev1 association with DSBs. Rev1 association with DSBs requires neither the function of the Rad24 checkpoint-clamp loader [5] nor the Rad6-Rad18-mediated ubiquitination of PCNA [3]. Our results reveal a novel role of Mec1 in the localization of the Polζ-Rev1 complex to DNA lesions and highlight a linkage of TLS polymerases to the checkpoint response.

Results and Discussion

Complex Formation of Polζ and Rev1

We examined the physical interaction among the Polζ components (Rev3 and Rev7) and Rev1. Coimmunoprecipitation experiments were performed with extracts from cells arrested at G2/M with nocodazole. This approach would eliminate detecting potential interactions that depend on DNA replication. We first determined physical interaction between Rev1 and Rev3. Extracts from cells expressing HA- or myc-tagged Rev1 and Rev3 were subjected to immunoprecipitation with anti-HA antibodies, and immunoprecipitates were analyzed by immunoblotting with anti-HA or anti-myc antibodies. The Rev1-Rev3 interaction was detected only in cells expressing both tagged Rev1 and Rev3 (Figures 1A and 1B). When we examined the effect of *rev7Δ* mutation on the Rev1-Rev3 interaction, no Rev1-Rev3 interaction was observed (Figures 1A and 1B), suggesting that Rev1 interacts with the Polζ (Rev3-Rev7) complex rather than with Rev3 alone. We next investigated the Rev1-Rev7 interaction (Figures 1C and 1D). Extracts prepared from cells expressing tagged Rev1 and Rev7 were analyzed as above. Again, the Rev1-Rev7 interaction was observed in cells expressing both tagged Rev1 and Rev7, but undetectable in *rev3Δ* mutants (Figures 1C and 1D), suggesting that Rev1 interacts with the Polζ (Rev3-Rev7) complex. We confirmed that Rev3 and Rev7 compose Polζ independently of Rev1 (Figure 1E). These results indicate that Rev1 interacts physically with Polζ (the Rev3-Rev7 complex).

If Polζ and Rev1 form a complex, they should act in the same DNA repair pathway. We examined the effects of *rev1Δ*, *rev3Δ*, and *rev7Δ* mutations on cell survival after exposure to ultraviolet (UV) light and methyl methanesulfonate (MMS) (Figure 1F and data not shown). Cells carrying a *rev1Δ*, *rev3Δ*, or *rev7Δ* single mutation exhibited very similar sensitivity to UV light and MMS. No additive phenotype was observed in *rev1Δ rev3Δ rev7Δ* triple mutants. These results are consistent with the previous finding that Polζ and Rev1 are similarly involved in damage-induced mutagenesis [2], and they support the model in which Polζ and Rev1 form a complex that functions in the same DNA repair pathway.

Localization of the Polζ-Rev1 Complex to DSBs

Polζ contributes to error-prone DNA synthesis during homologous recombination (HR) of double-strand breaks (DSBs), although it is dispensable for the HR repair [6, 7]. Using an experimental system in which the HO endonuclease induces a single DSB at the *ADH4* locus [8] (Figure 2A), we therefore investigated whether the Polζ-Rev1 complex associates with regions near DSBs. DSBs can be repaired either by Rad52-dependent HR or Ku-dependent nonhomologous end-joining (NHEJ) [9]. We first asked whether Rev1 or Rev3 acts during DSB repair (Figure 2B). Cells carrying a *rev1Δ*, *rev3Δ*, *rad52Δ*, or Ku (*hdf1Δ*) mutation were transformed with the GAL-HO plasmid that contains the *HO* coding sequence fused to the *GAL10* promoter [8]. Cells were grown initially in sucrose to maintain the *GAL* promoter noninduced. Aliquots of the culture were then plated out on medium containing galactose to activate *HO* expression or medium containing glucose to repress expression. We estimated cell viability after *HO* expression by scoring colony formation (Figure 2B). Wild-type cells lost viability after *HO* expression, and only 20% of cells remained viable. Introduction of *rad52Δ* mutation decreased viability to 0.2%, suggesting that some of the DSBs at the *ADH4* locus are repaired by Rad52-dependent mechanisms. In contrast, *rev1Δ* or *rev3Δ* mutants retained viability similar to that of wild-type cells. As proposed previously [7], Polζ and Rev1 might contribute to bypass lesions of the single-strand DNA that is generated as a recombination intermediate, although they are dispensable for HR. Rev3 plays a more important role in DNA repair when recombination repair is compromised [7]. Consistently, *rad52Δ rev1Δ*, *rad52Δ rev3Δ*, or *rad52Δ rev1Δ rev3Δ* mutation caused a 5-fold reduction in survival compared to *rad52Δ* single mutation.

*Correspondence: sugimoka@umdnj.edu

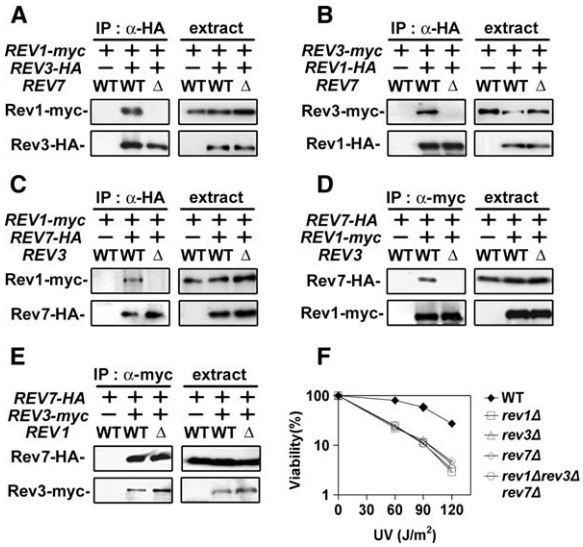

Figure 1. Interaction among Rev1, Rev3 and Rev7

(A and B) Rev1-Rev3 interaction in wild-type (WT) or *rev7⊿* mutant (Δ) cells. Cells carrying untagged (−) or tagged (+) genes were arrested with nocodazole at G2/M. Aliquots of the cells were collected, and the cell extracts were immunoprecipitated with anti-HA antibodies. Immunoprecipitates (IP) and whole extracts (extract) were subjected to immunoblotting analysis with anti-HA and anti-myc antibodies. Strains used are *REV1-myc* (KSC2032), *REV1-myc REV3-HA* (KSC2033), *REV1-myc REV3-HA rev7⊿* (KSC2034), *REV3-myc* (KSC2035), *REV3-myc REV1-HA* (KSC2036), and *REV3-myc REV1-HA rev7⊿* (KSC2037).

(C and D) Rev1-Rev7 interaction in wild-type (WT) or *rev3⊿* mutant (Δ) cells. Cell extracts were immunoprecipitated with anti-HA or anti-myc antibodies and then examined as in (A) or (B). Strains used are *REV1-myc* (KSC2032), *REV7-HA* (KSC2040), *REV1-myc REV7-HA* (KSC2038), and *REV1-myc REV7-HA rev3⊿* (KSC2039).

(E) Rev3-Rev7 interaction in wild-type (WT) or *rev1⊿* mutant (Δ) cells. Cell extracts were immunoprecipitated with anti-myc antibodies and then examined as in (A) or (B). Strains used are *REV7-HA* (KSC2040), *REV7-HA REV3-myc* (KSC2041), and *REV7-HA REV3-myc rev1⊿* (KSC2042).

(F) Sensitivity to UV light. Wild-type (KSC1516), *rev1⊿* (KSC2028), *rev3⊿* (KSC2029), *rev7⊿* (KSC2030), and *rev1⊿ rev3⊿ rev7⊿* (KSC2031) mutant cells were grown to log-phase and irradiated with UV light. Viability was determined after UV irradiation at the indicated dosages.

In budding yeast, Ku-dependent NHEJ becomes apparent in the absence of Rad52 functions [10]. In fact, the survival of *rad52⊿ hdf1⊿* double mutants was 20-fold lower than that of *rad52⊿* single mutants. Interestingly, neither *rev1⊿* nor *rev3⊿* mutation increased sensitivity in *rad52⊿ hdf1⊿* mutants. Polζ and Rev1 might contribute to DNA synthesis in a Ku-dependent pathway as well.

We then investigated whether the Polζ-Rev1 complex localizes to regions near the HO-induced DSB at the *ADH4* locus by chromatin immunoprecipitation (ChIP) assay [8]. ChIP assays were performed with G2/M-arrested cells to avoid detecting potential interactions mediated by chromosome replication. We first examined the association of Rev1 with the DSB (Figure 2C). Cells expressing Rev1-HA were transformed with the GAL-HO plasmid. Transformants were grown initially in sucrose and incubated with nocodazole to arrest at G2/M. After arrest, galactose was added to induce *HO* expression. Cells were collected at various times, and extracts prepared after formaldehyde cross-linking were subjected to immunoprecipitation with anti-HA antibodies. Coprecipitated DNA was amplified by polymerase chain reaction (PCR) by using the HO1 primer set for regions near the HO cleavage site on the *ADH4* locus on chromosome VII and the primer set for the *SMC2* locus, which contains no HO cleavage site on chromosome VI (Figure 2A). PCR amplification with the HO1 primer set was detected in *REV1-HA* cells carrying the GAL-HO plasmid after incubation with galactose (Figure 2C). In contrast, there was no increase in the PCR product amplified from the *SMC2* locus after incubation with galactose (Figure 2C). No PCR amplification was observed in untagged cells or cells lacking an HO cleavage site at the *ADH4* locus (data not shown). These results indicate that Rev1 associates with sites near the HO-induced DSB in G2/M-arrested cells. Similarly, Rev7 associates with regions near the DSB (Figure 2D).

To explore the significance of the Polζ-Rev1 complex formation, we examined the effects of *rev1⊿* or *rev3⊿* mutation on Rev7 association with the HO-induced DSB. No Rev7 association with DSBs was detected in *rev1⊿* or *rev3⊿* mutants (Figure 2D). This association defect was not due to decreased expression or mislocalization of Rev7. As shown above, neither *rev1⊿* nor *rev3⊿* mutation impaired the Rev7 expression (Figure 1). Moreover, Rev7 was found to predominantly localize to nuclei, and its localization was unaffected by *rev1⊿* or *rev3⊿* mutation (data not shown). We also examined the effects of *rev3⊿* or *rev7⊿* mutation on Rev1 association with DSBs. Rev1 association with DSBs was undetectable in *rev3⊿* or *rev7⊿* mutants (Figure 2E), although its expression or cellular localization was similar (Figure 1; data not shown). Thus, Polζ and Rev1 associate together with sites of DNA damage.

Noncatalytic Role of Rev1 in Localization of the Polζ-Rev1 Complex to DNA Lesions

Rev1-1 mutant protein is defective for induced mutagenesis in vivo, although the mutant protein retains a substantial amount of the transferase activity in vitro [11]. Interestingly, the *rev1-1* mutation is within the BRCT motif at its N terminus [2, 11], a domain that is implicated in physical interactions between DNA repair proteins [12]. We first postulated that the BRCT domain might mediate the interaction between Rev1 and Polζ. However, coimmunoprecipitation experiments revealed that the *rev1-1* mutation did not impair the Rev1-Rev3 or Rev1-Rev7 interaction (Figures 3A and 3B), indicating that the Rev1-1 mutant protein efficiently forms a complex with Polζ. We next examined the effect of *rev1-1* mutation on the association of Rev1 with the HO-induced DSBs. Rev1 association was not detected in cells containing the *rev1-1* mutation (Figure 3C). The *rev1-1* mutation did not affect nuclear localization or expression levels of Rev1 protein (data not shown). Likewise, Rev7 association with DSBs was undetectable in *rev1-1* mutants (Figure S1 in the Supplemental Data available online). Similar to *rev1⊿* mutation, *rev1-1* mutation increased the viability loss in *rad52⊿* cells after DSB induction (Figure S2). Thus, the Rev1 BRCT domain mediates the association of the Polζ-Rev1 complex with DNA lesions. The *rev1*$^{Ala467\text{-}Ala468}$ mutation does not significantly affect mutagenesis in vivo, although it abolishes the transferase activity in vitro [13]. Consistently, the

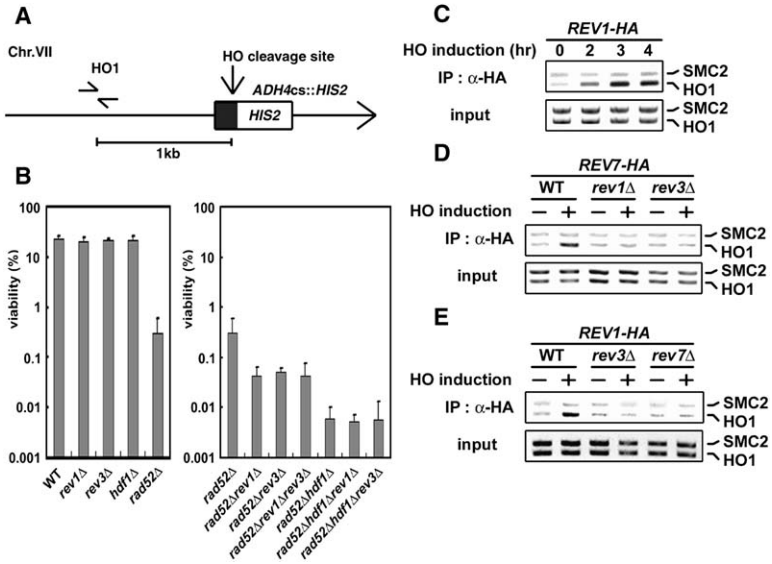

Figure 2. Association of the Polζ-Rev1 Complex with HO-Induced DSBs

(A) Schematic of the HO cleavage site at *ADH4* locus (ADH4cs). An HO cleavage site, marked with *HIS2*, was introduced at the *ADH4* locus on chromosome VII. The HO1 primer pair amplifies the region 1 kb apart from the HO cleavage site. An arrow represents the telomere.

(B) Viability after DSB induction. Cells were transformed with the GAL-HO plasmid. Transformed cells were grown in sucrose and then plated out on either glucose or galactose medium selectable for the plasmid. Viability was estimated from colony-formation ability on galactose medium. Strains used are wild-type (KSC1516), *rev1Δ* (KSC2028), *rev3Δ* (KSC2029), *hdf1Δ* (KSC1521), *rad52Δ* (KSC2043), *rad52Δ rev1Δ* (KSC2044), *rad52Δ rev3Δ* (KSC2045) *rad52Δ rev1Δ rev3Δ* (KSC2046), *rad52Δ hdf1Δ* (KSC2047), *rad52Δ hdf1Δ rev1Δ* (KSC2048), and *rad52Δ hdf1Δ rev3Δ* (KSC2049). The bars represent standard errors.

(C) Association of Rev1 with HO-induced DSBs. Cells expressing Rev1-HA (KSC2050) were transformed with the GAL-HO plasmid. Transformed cells were grown in sucrose and then incubated with nocodazole. After arrest at G2/M, the culture was incubated with galactose to induce *HO* expression, whereas part of the culture was maintained in sucrose to repress *HO* expression. Aliquots of cells were collected at the indicated times after *HO* expression and subjected to ChIP assay. PCR was done with the HO1 primer set for the HO cleavage site at the *ADH4* locus and the SMC2 primer set for the control locus. PCR products from the respective input extracts are shown below.

(D) Association of Rev7 with HO-induced DSBs in wild-type, *rev1Δ*, and *rev3Δ* mutant cells. Wild-type (KSC2040), *rev1Δ* (KSC2053), and *rev3Δ* (KSC2054) mutant cells expressing Rev7-HA were analyzed by ChIP assay as in (C). The cultures were incubated for 4 hr with galactose (+) to induce *HO* expression or maintained in sucrose (−) to repress *HO* expression.

(E) Association of Rev1 with HO-induced DSBs in wild-type, *rev3Δ*, and *rev7Δ* mutants. Wild-type (KSC2050), *rev3Δ* (KSC2051), and *rev7Δ* (KSC2052) mutant cells expressing Rev1-HA were analyzed by ChIP assay as in (D).

Rev1$^{Ala467\text{-}Ala468}$ mutant protein was found to associate with the HO-induced DSB as efficiently as the wild-type Rev1 protein (Figure 3D). Together, these results suggest that Rev1 plays a noncatalytic role in the localization of the Polζ-Rev1 complex to DNA lesions.

Rev1 Association with DSBs in PCNA-Ubiquitination-Defective Cells

The Rad6-Rad18 ubiquitin-conjugating enzyme complex promotes Polζ-dependent translesion DNA synthesis by ubiquitinating lysine-164 of PCNA (Pol30) [14, 15]. We also asked whether ubiquitination via the Rad6-Rad18 pathway regulates association of the Polζ-Rev1 complex with sites of DNA damage. However, neither *pol30-K164R* nor *rad18Δ* mutation decreased the Rev1 association with DSBs (Figure 4A and Figure S3A). Thus, the monoubiquitination of PCNA does not play a critical role in the recruitment of the Polζ-Rev1 complex to DSB sites. Rev1 associated with DSBs in *rad52Δ*, *hdf1Δ* single, or *rad52Δ hdf1Δ* double mutants as efficiently as in wild-type cells (Figure S3B), suggesting that the Polζ-Rev1 association is not coupled to DSB processing in HR and NHEJ.

Requirement of Mec1 Kinase Activity for Association of the Polζ-Rev1 Complex with DNA Lesions

The RFC-related checkpoint-clamp loader recruits the PCNA-related checkpoint clamp to sites of DNA damage [16]. In budding yeast, *RAD24* encodes a large subunit of the checkpoint-clamp loader, and Ddc1, Mec3, and Rad17 constitute the checkpoint-clamp [5]. Because of the structural relatedness, the checkpoint-clamp has been proposed to act as an alternative clamp for DNA polymerases [16]. However, Rev1 was found to associate with HO-induced DSBs in *rad24Δ* and *ddc1Δ* mutants as efficiently as in wild-type cells (Figure 4B and Figure S3C). These results suggest that the Rad24 checkpoint-clamp loader and Ddc1 checkpoint-clamp are not essential for the Polζ-Rev1 association. Recent studies showed that Rev7 binds to damaged chromosomes in a Ddc1-dependent manner during S phase [17]. However, it is not clear how the Ddc1 clamp controls the Rev7 binding, because the Rad24 function is not essential for Polζ-dependent mutagenesis [17].

In budding yeast, the ATR homolog Mec1 plays a central role in the activation of checkpoint responses [4, 5]. We next examined whether Rev1 association with DSBs requires Mec1 functions (Figure 4C). Interestingly, no Rev1 association was detected in cells carrying a *mec1Δ* mutation or expressing a kinase-negative version of Mec1 (Mec1-KN) [18]. Deletion mutation in *MEC1* did not affect the expression level of Rev1, Rev3, and Rev7 or their complex formation (data not shown). Thus, Mec1 kinase activity is critical for the association of the Polζ-Rev1 complex with DSBs. HO-induced DSBs do not activate the Mec1 pathway at G1 [19, 20]. Correspondingly, Rev1 association with HO-induced DSBs was not detected in G1-arrested cells (data not shown).

Mec1 phosphorylates the Rad9 checkpoint protein [21, 22] and promotes the association of Rad9 with sites of DNA damage [23]. We therefore investigated the effect of the *rad9Δ* mutation on Rev1 association with HO-induced DSBs. However, the *rad9Δ* mutation did not decrease the Rev1 association (Figure 4D). In budding yeast, the histone H2A proteins, Hta1 and Hta2, carry the serine-glutamine (SQ) motif at the C terminus.

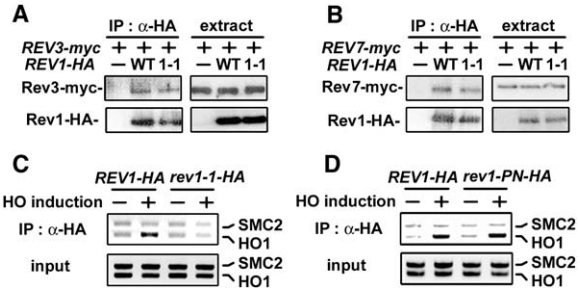

Figure 3. Noncatalytic Role of Rev1 in Localization to Sites of DNA Damage

(A and B) Physical interaction of Rev1-1 mutant protein with Rev3 and Rev7. Cells expressing Rev3-myc (A) or Rev7-myc (B) and untagged Rev1 (−), Rev1-HA (WT), or Rev1-1-HA (1-1) were subjected to immunoprecipitation with anti-HA antibodies and subsequent immunoblotting analysis with anti-HA and anti-myc antibodies. Strains used are *REV3-myc* (KSC2035), *REV3-myc REV1-HA* (KSC2036), *REV3-myc rev1-1-HA* (KSC2055), *REV7-myc* (KSC2056), *REV7-myc REV1-HA* (KSC2057), and *REV7-myc rev1-1-HA* (KSC2058).

(C) Association of Rev1-1 mutant protein with DSBs. Cells expressing Rev1-HA (KSC2050) or Rev1-1-HA (KSC2059) were analyzed by ChIP assay as in Figure 2C. After G2/M arrest, the cultures were incubated for 4 hr with galactose (+) or maintained in sucrose (−).

(D) Association of Rev1 catalytic mutant protein with DSBs. Cells expressing Rev1-HA (KSC2050) or Rev1 $^{Ala467\text{-}Ala468}$-HA (Rev1-PN-HA) (KSC2060) were analyzed by ChIP assay as in (C).

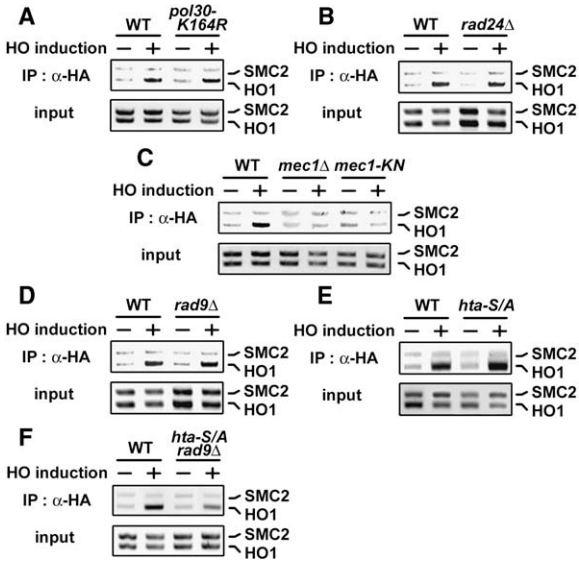

Figure 4. Control of Rev1 Association with DSBs

(A) Effect of *pol30-K164R* mutation.

(B) Effect of *rad24Δ* mutation.

(C) Effects of *mec1Δ* or *mec1-KN* mutation.

(D) Effect of *rad9Δ* mutation. (E) Effect of *hta-S/A* mutation.

(F) Effect of *hta-S/A rad9Δ* mutation. Cells expressing Rev1-HA were analyzed by ChIP assay as in Figure 2C. After G2/M arrest, the cultures were incubated for 4 hr with galactose (+) or maintained in sucrose (−). The strains in (C) contained an *sml1Δ* mutation to rescue the viability loss associated with *mec1Δ* mutation. Strains used are as follows: (A) wild-type (KSC2050) and *pol30-K164R* (KSC2065); (B) wild-type (KSC2050) and *rad24Δ* (KSC2066); (C) wild-type (KSC2067), *mec1Δ* (KSC2068), and *mec1-KN* (KSC2069); (D) wild-type (KSC2050) and *rad9Δ* (KSC2070); (E) wild-type (KSC2050) and *hta-S/A* (KSC2088); and (F) wild-type (KSC2050) and *hta-S/A rad9Δ* (KSC2089).

Mec1 phosphorylates the serine residue of the H2A proteins at DNA lesions and promotes the recruitment of cohesion, histone modifiers, and chromatin-remodeling complexes to DNA lesions [20, 24, 25]. We next monitored the Rev1 association in cells carrying the H2A mutations, *hta1-S129A* and *hta2-S129A*, which substitute serine with alanine at the SQ motif (Figure 4E). The *hta1-S129A hta2-S129A* double (*hta-S/A*) mutation did not affect the Rev1 association with DSBs. We further examined the combined effect of the *hta-S/A* and *rad9Δ* mutations (Figure 4F and Figure S4). Although still detectable, the Rev1 association with DSBs was significantly decreased in cells carrying the *hta-S/A* and *rad9Δ* mutations. The *hta-S/A rad9Δ* mutation did not affect the Mec1 association with DSBs (Figure S5). These results support the model in which Mec1 phosphorylates multiple proteins and promotes association of the Polζ-Rev1 complex with DNA lesions.

In this study, we showed that Polζ and Rev1 form a complex, and they localize together to sites of DNA damage through a Mec1-dependent mechanism. Their complex formation explains the previous genetic observation that Polζ and Rev1 act in the same pathway [2]. The Polζ-Rev1 complex could benefit the sequential action of these two catalytic activities on damaged templates [2, 3] like the Polα-primase complex coordinates primer synthesis with primer extension at the early step of DNA replication [26]. Our findings, however, do not exclude the possibility that Rev1 forms a separate complex with proteins other than Polζ. Recent evidence indicates that mammalian Rev1 interacts with the TLS polymerase Polη, Poli, and Polκ as well [27–29].

Mec1 responds to various types of DNA damage, and it phosphorylates many proteins including Rad9 and histone H2A [21, 22, 24]. Correspondingly, Rev1 association with DNA lesions becomes defective in cells lacking Rad9 proteins and H2A phosphorylation. However, this association defect is partial, suggesting that other Mec1-dependent phosphorylation events control the Polζ-Rev1 association. The Rev1 association is mediated through the Rev1 BRCT domain, which acts as a phosphopeptide binding module [30]. One possible mechanism for the Polζ-Rev1 localization could be that Mec1 phosphorylates multiple proteins at sites of DNA damage, and the phosphorylated residues directly interact with the Rev1 BRCT domain. The BRCT domain has been implicated in protein-protein interactions [12]. Alternatively, the BRCT domain might interact with protein(s) that target those phosphorylated residues. The Polζ-Rev1 association with DNA lesions might be a critical prerequisite for its action, because the *rev1-1* mutation at the BRCT domain behaves like a null mutation [11]. At the moment, however, we cannot rule out the possibility that Rev1-independent (or BRCT-independent) mechanisms control the association of the Polζ-Rev1 complex, because Rev1 fails to associate with DSBs in the absence of Rev3 or Rev7. The Rev1 association is not detected at G1-arrested cells, where DSBs are specifically repaired by NHEJ [10]. The Polζ-Rev1 association with DSBs might facilitate DNA synthesis during HR rather than NHEJ.

We found that PCNA ubiquitination is dispensable for association of the Polζ-Rev1 complex with sites of DNA

damage. Several studies have proposed the model in which ubiquitination disrupts the interaction of PCNA with replicative DNA polymerases, allowing the access of TLS polymerases to the primer terminus [14, 15]. After localizing to sites of DNA damage, the Polζ-Rev1 complex might be loaded onto the primer ends where replicative polymerases are not associated. The Polζ-Rev1 complex thus could contribute to repair synthesis near sites of DNA damage. Recent studies show that another TLS polymerase Polη plays a dual role in TLS and HR [31, 32]. Involvement in multiple repair pathways might be a common feature for TLS polymerases.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, five figures, and one table and are available with this article online at: http://www.current-biology.com/cgi/content/full/16/6/586/DC1/.

Acknowledgments

We thank J. Kang and L. Ripley for critical reading; C. Lawrence for materials; and D. Nakada, C. Newlon, and L. Ripley for helpful discussion. This work was supported by grants from National Institutes of Health (NIH GM073876) and the University of Medicine and Dentistry of New Jersey (UMDNJ) foundation.

Received: October 24, 2005
Revised: January 23, 2006
Accepted: January 31, 2006
Published: March 20, 2006

References

1. Friedberg, E.C., Wagner, R., and Radman, M. (2002). Specialized DNA polymerases, cellular survival, and the genesis of mutations. Science *296*, 1627–1630.
2. Lawrence, C.W. (2002). Cellular roles of DNA polymerase zeta and Rev1 protein. DNA Repair (Amst.) *1*, 425–435.
3. Prakash, S., Johnson, R.E., and Prakash, L. (2005). Eukaryotic translesion synthesis DNA polymerases: Specificity of structure and function. Annu. Rev. Biochem. *74*, 317–353.
4. Elledge, S.J. (1996). Cell cycle checkpoints: Preventing an identity crisis. Science *274*, 1664–1672.
5. Zhou, B.B., and Elledge, S.J. (2000). The DNA damage response: Putting checkpoints in perspective. Nature *408*, 433–439.
6. Holbeck, S.L., and Strathern, J.N. (1997). A role for REV3 in mutagenesis during double-strand break repair in Saccharomyces cerevisiae. Genetics *147*, 1017–1024.
7. Rattray, A.J., Shafer, B.K., McGill, C.B., and Strathern, J.N. (2002). The roles of REV3 and RAD57 in double-strand-break-repair-induced mutagenesis of Saccharomyces cerevisiae. Genetics *162*, 1063–1077.
8. Nakada, D., Matsumoto, K., and Sugimoto, K. (2003). ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. Genes Dev. *17*, 1957–1962.
9. Haber, J.E. (2000). Partners and pathways repairing a double-strand break. Trends Genet. *16*, 259–264.
10. Daley, J.M., Palmbos, P.L., Wu, D., and Wilson, T.E. (2005). Nonhomologous end joining in yeast. Annu. Rev. Genet. *39*, 431–451.
11. Nelson, J.R., Gibbs, P.E., Nowicka, A.M., Hinkle, D.C., and Lawrence, C.W. (2000). Evidence for a second function for Saccharomyces cerevisiae Rev1p. Mol. Microbiol. *37*, 549–554.
12. Callebaut, I., and Mornon, J.P. (1997). From BRCA1 to RAP1: A widespread BRCT module closely associated with DNA repair. FEBS Lett. *400*, 25–30.
13. Haracska, L., Unk, I., Johnson, R.E., Johansson, E., Burgers, P.M., Prakash, S., and Prakash, L. (2001). Roles of yeast DNA polymerases delta and zeta and of Rev1 in the bypass of abasic sites. Genes Dev. *15*, 945–954.
14. Stelter, P., and Ulrich, H.D. (2003). Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. Nature *425*, 188–191.
15. Haracska, L., Torres-Ramos, C.A., Johnson, R.E., Prakash, S., and Prakash, L. (2004). Opposing effects of ubiquitin conjugation and SUMO modification of PCNA on replicational bypass of DNA lesions in Saccharomyces cerevisiae. Mol. Cell. Biol. *24*, 4267–4274.
16. Parrilla-Castellar, E.R., Arlander, S.J., and Karnitz, L. (2004). Dial 9–1-1 for DNA damage: The Rad9-Hus1-Rad1 (9–1-1) clamp complex. DNA Repair (Amst.) *3*, 1009–1014.
17. Sabbioneda, S., Minesinger, B.K., Giannattasio, M., Plevani, P., Muzi-Falconi, M., and Jinks-Robertson, S. (2005). The 9–1-1 checkpoint clamp physically interacts with Polzeta and is partially required for spontaneous Polzeta-dependent mutagenesis in Saccharomyces cerevisiae. J. Biol. Chem. *280*, 38657–38665.
18. Wakayama, T., Kondo, T., Ando, S., Matsumoto, K., and Sugimoto, K. (2001). Pie1, a protein interacting with Mec1, controls cell growth and checkpoint responses in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *21*, 755–764.
19. Ira, G., Pellicioli, A., Balijja, A., Wang, X., Fiorani, S., Carotenuto, W., Liberi, G., Bressan, D., Wan, L., Hollingsworth, N.M., et al. (2004). DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. Nature *431*, 1011–1017.
20. Shroff, R., Arbel-Eden, A., Pilch, D., Ira, G., Bonner, W.M., Petrini, J.H., Haber, J.E., and Lichten, M. (2004). Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Curr. Biol. *14*, 1703–1711.
21. Emili, A. (1998). MEC1-dependent phosphorylation of Rad9p in response to DNA damage. Mol. Cell *2*, 183–189.
22. Vialard, J.E., Gilbert, C.S., Green, C.M., and Lowndes, N.F. (1998). The budding yeast Rad9 checkpoint protein is subjected to Mec1/Tel1-dependent hyperphosphorylation and interacts with Rad53 after DNA damage. EMBO J. *17*, 5679–5688.
23. Naiki, T., Wakayama, T., Nakada, D., Matsumoto, K., and Sugimoto, K. (2004). Association of Rad9 with double-strand breaks through a Mec1-dependent mechanism. Mol. Cell. Biol. *24*, 3277–3285.
24. Downs, J.A., Lowndes, N.F., and Jackson, S.P. (2000). A role for Saccharomyces cerevisiae histone H2A in DNA repair. Nature *408*, 1001–1004.
25. Redon, C., Pilch, D.R., Rogakou, E.P., Orr, A.H., Lowndes, N.F., and Bonner, W.M. (2003). Yeast histone 2A serine 129 is essential for the efficient repair of checkpoint-blind DNA damage. EMBO Rep. *4*, 678–684.
26. Waga, S., and Stillman, B. (1998). The DNA replication fork in eukaryotic cells. Annu. Rev. Biochem. *67*, 721–751.
27. Guo, C., Fischhaber, P.L., Luk-Paszyc, M.J., Masuda, Y., Zhou, J., Kamiya, K., Kisker, C., and Friedberg, E.C. (2003). Mouse Rev1 protein interacts with multiple DNA polymerases involved in translesion DNA synthesis. EMBO J. *22*, 6621–6630.
28. Ohashi, E., Murakumo, Y., Kanjo, N., Akagi, J., Masutani, C., Hanaoka, H., and Ohmori, H. (2004). Interaction of hREV1 with three human Y-family DNA polymerases. Genes Cells *9*, 525–531.
29. Tissier, A., Kannouche, P., Reck, M.P., Lehmann, A.R., Fuchs, R.P., and Cordonnier, A. (2004). Co-localization in replication foci and interaction of human Y-family members, DNA polymerase pol eta and REVl protein. DNA Repair (Amst.) *3*, 1503–1514.
30. Yu, X., Chini, C.C., He, M., Mer, G., and Chen, J. (2003). The BRCT domain is a phospho-protein binding domain. Science *302*, 639–642.
31. Kawamoto, T., Araki, K., Sonoda, E., Yamashita, Y.M., Harada, K., Kikuchi, K., Masutani, C., Hanaoka, F., Nozaki, K., Hashimoto, N., et al. (2005). Dual roles for DNA polymerase eta in homologous DNA recombination and translesion DNA synthesis. Mol. Cell *20*, 793–799.
32. McLlwraith, M.J., Vaisman, A., Liu, Y., Fanning, E., Woodgate, R., and West, S.C. (2005). Human DNA polymerase eta promotes DNA synthesis from strand invasion intermediates of homologous recombination. Mol. Cell *20*, 783–792.