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A non-catalytic function of Rev1 in translesion DNA synthesis and mutagenesis is mediated by its stable interaction with Rad5

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ABSTRACT

DNA damage tolerance consisting of template switching and translesion synthesis is a major cellular mechanism in response to unrepaired DNA lesions during replication. The Rev1 pathway constitutes the major mechanism of translesion synthesis and base damage-induced mutagenesis in model cell systems. Rev1 is a dCMP transferase, but additionally plays non-catalytic functions in translesion synthesis. Using the yeast model system, we attempted to gain further insights into the non-catalytic functions of Rev1. Rev1 stably interacts with Rad5 (a central component of the template switching pathway) *via* the C-terminal region of Rev1 and the N-terminal region of Rad5. Supporting functional significance of this interaction, both the Rev1 pathway and Rad5 are required for translesion synthesis and mutagenesis of 1,N^6-ethenoadenine. Furthermore, disrupting the Rev1–Rad5 interaction by mutating Rev1 did not affect its dCMP transferase, but led to inactivation of the Rev1 non-catalytic function in translesion synthesis of UV-induced DNA damage. Deletion analysis revealed that the C-terminal 21-amino acid sequence of Rev1 is uniquely required for its interaction with Rad5 and is essential for its non-catalytic function. Deletion analysis additionally implicated a C-terminal region of Rev1 in its negative regulation. These results show that a non-catalytic function of Rev1 in translesion synthesis and mutagenesis is mediated by its interaction with Rad5.

1. Introduction

Unrepaired DNA lesions often persist in the genome during replication. Since nearly all DNA lesions block replicative polymerases, replication cannot be completed by the replication apparatus when DNA templates are damaged. Completion of replication in the presence of DNA damage requires damage tolerance, which consists of template switching and translesion DNA synthesis. While template switching is error-free, translesion synthesis is often error-prone.

Damage tolerance is signaled by stalled replicative DNA polymerase at the lesion site, leading to mono-ubiquitination of PCNA by the Rad6–Rad18 ubiquitin-conjugating/ligation complex [1]. Mono-ubiquitinated PCNA leads to the recruitment of translesion synthesis polymerases such as the Y family polymerases and DNA polymerase ζ (Polζ), replacing the replicative polymerase [2]. Then, translesion synthesis occurs by polymerase-catalyzed nucleotide insertion opposite the lesion followed by extension synthesis from opposite the lesion [3]. When an incorrect base is inserted opposite the lesion, translesion synthesis results in mutation. In the yeast model system, error-prone translesion synthesis constitutes the major mechanism of base damage-induced mutagenesis [4–14]. Accumulating evidence also supports the notion that error-prone translesion synthesis is a major mechanism of base damage-induced mutagenesis in higher eukaryotes including mammals [15–19].

Mono-ubiquitinated PCNA can be further ubiquitinated by the ubiquitin-conjugating/ligation complex Rad5–Ubc13–Mms2. The poly-ubiquitinated PCNA channels the damage tolerance to the template switching pathway, preventing potential damage-induced mutagenesis by the translesion synthesis pathway. Rad5 is a member of the SWI/SNF super family. Three catalytic activities are associated with this protein, ATPase, helicase, and ubiquitin ligase [20–22]. The catalytic activities are required for its function in template switching [23]. Recruitment of template switching proteins and commitment to this pathway is thought to be initiated by the interaction between Rad18 and Rad5 [24]. Therefore, Rad5 is a central component and indispensible for template switching. For a long time, Rad5 was thought to function specifically in the template switching pathway. Recent studies [23] confirmed the 1978

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report of Lawrence and Christensen [25] that the yeast *rad5* mutant cells are deficient in UV-induced reversion mutations. That is, the Rad5 protein is involved in error-prone translesion synthesis of UV damage at the tested reversion loci, which is independent of its ATPase and ubiquitin ligase domains [23]. However, the precise mechanism by which Rad5 affects translesion synthesis of UV DNA damage is not known.

Rev1 is a member of the Y family of DNA polymerases. However, it is unique in that it is a template-dependant dCMP transferase, rather than a typical DNA polymerase [26–28]. *In vitro*, Rev1 is capable of catalyzing C insertion opposite multiple types of DNA lesions including AP sites and 1,N^6-ethenoadenine [26,28]. During catalysis, Rev1 uses the Arg324 of the protein, instead of the DNA template base, as the template for choosing dCTP as the incoming base [29]. Rev1 plays ubiquitous non-catalytic functions in translesion synthesis [6,30]. Additionally, its dCMP transferase has been definitively shown to function during translesion synthesis and mutagenesis of 1,N^6-ethenoadenine [30], and implicated in the bypass of AP sites [7,31]. Thus, Rev1 also functions catalytically in a lesion-specific manner, like other Y family DNA polymerases [28,30]. The Rev1 non-catalytic functions are not well understood. One possibility is that Rev1 acts to recruit other Y family DNA polymerases to lesion sites through protein–protein interactions [32]. Another possibility involves Rev1 interaction with mono-ubiquitinated PCNA [33]. Thus, the non-catalytic functions of Rev1 are complex and likely reflect its multiple mechanistic roles in the translesion synthesis pathway.

Using the yeast model system, we attempted to gain further insights into the non-catalytic functions of Rev1 in translesion synthesis. In this report, we identified regions responsible for the Rev1–Rad5 interaction and examined its functional significance. Supporting functional importance of this interaction, Rad5 is required for translesion synthesis and mutagenesis of 1,N^6-ethenoadenine. Furthermore, the Rev1 non-catalytic function in translesion synthesis is inactivated by disrupting the Rev1–Rad5 interaction. Our results led to the conclusion that a non-catalytic function of Rev1 in translesion synthesis and mutagenesis is mediated by its stable interaction with Rad5, and revealed a mechanism by which Rad5 functions non-catalytically in translesion synthesis, distinct from its catalytic function in template switching of DNA damage tolerance.

2. Materials and methods

2.1. Materials

The EZ yeast ColonyScreen Kit was obtained from Enzmax (Lexington, KY). The 22-mer damaged oligonucleotide, 5′-GTAAGCTAG-ATCCTCTAGAGCG-3′, contained a site-specific 1,N^6-ethenoadenine at the underlined A. This oligonucleotide and the 51-mer uracil-containing scaffold, 5′-CTGUGCCCUCCAUGCGCUCUGGAGGAU-CUAGCTUACGAAAAAUCAGTCAAG-3′, were used for construction of site-specifically damaged plasmid DNA. The 36-mer templates, 5′-GAAGGGATCCTTAAGACTXTAACCGGTCTTCGCGCG-3′, contained a site-specific tetrahydrofuran (AP site analog) (designated as X). This oligonucleotide was used for Rev1 dCMP transferase assay *in vitro*. Oligonucleotides were synthesized *via* automated DNA phosphoramidite methods by Integrated DNA Technologies (Coralville, IA). Enhanced chemiluminescence (ECL) kit was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). Mouse monoclonal antibodies against His-tag and 3-phosphoglycerate kinase (Pgk) were purchased from Qiagen (Valencia, CA) and Invitrogen (Carlsbad, CA), respectively. Wild-type and mutant yeast Rev1 proteins were purified by Enzymax according to our previously described method [34].

2.2. Yeast strains

Yeast strains used were the wild-type BY4741 (*MATa his3 leu2 met15 ura3*) and its isogenic mutants BY4741Δrad5 (*rad5* deletion), BY4741Δrad18 (*rad18* deletion), BY4741Δrad30 (*rad30* deletion), BY4741Δrev3 (*rev3* deletion), BY4741Δrev3Δrad30 (*rev3* and *rad30* double deletion); the wild-type CL1265-7C (*MATα arg4-17 leu2-3,112 his3Δ trp1 ura3-52*) and its isogenic strains CL1265-7CΔrev1/pEAT (*rev1* deletion), CL1265-7CΔrev1/REV1, CL1265-7CΔrev1/REV1ΔC105, CL1265-7CΔrev1/REV1ΔC21 (*rev1* deletion mutant containing the empty vector, wild-type *REV1* gene, mutant *rev1ΔC105*, and mutant *rec1ΔC21* genes, respectively, on the plasmid vector pEAT). BY4741 was purchased from ATCC (Manassas, VA). BY4741Δrad30, BY4741Δrad5, and BY4741Δrad18 were purchased from Research Genetics (Huntsville, AL). BY4741Δrev3 and BY4741Δrev3Δrad30 were constructed previously [7,35]. CL1265-7C was provided by Christopher Lawrence of the University of Rochester [12]. CL1265-7CΔrev1 was constructed previously [14]. CL1265-7CΔrev1/pEAT, CL1265-7CΔrev1/REV1, CL1265-7CΔrev1/REV1ΔC105, and CL1265-7CΔrev1/REV1ΔC21 were obtained by transforming the expression plasmid vector pEAT alone, the constructs pEAT-REV1, pEAT-REV1ΔC105, and pEAT-REV1ΔC21, respectively, into the CL1265-7CΔrev1 strain. The expression vector pEAT contained the 2 μm origin for multi-copy plasmid replication in yeast, the *TRP1* gene for plasmid selection, and the *ADH1* promoter for constitutive expression of the *REV1* or *REV1* mutant genes. Expression plasmids were constructed by Enzymax.

2.3. Yeast two-hybrid assays

Yeast two-hybrid assays were performed essentially as reported [36]. Vectors used were pAS2 and pACT2 containing the *GAL4* DNA binding domain and the *GAL4* activation domain, respectively. Yeast Y190 cells were transformed with two plasmids: pAS2Rad5 + pACT2; pAS2 + pACT2Rad5; pAS2 + pACT2Rev1$_{301–985}$; pAS2 Rev1$_{301–985}$ + pACT2; pAS2Rad5 + pACT2Rev1$_{301–985}$; or pAS2 Rev1$_{301–985}$ + pACT2Rad5. Transformed cells were grown on minimum plates for 4–6 days at 30 °C. Colonies from each plate were transferred onto a nitrocellulose filter and permeabilized by freezing the filters in liquid nitrogen for 10 s followed by thawing at room temperature. The filters with the colony side up were placed on Whatman #1 paper presoaked with Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, and 1 mM $MgSO_4$) containing 38.6 mM β-mercaptoethanol and 0.5 mg/ml X-gal. Following incubation at 30 °C, cellular β-galactosidase activity indicative of protein–protein interaction was detected by the appearance of blue color in colonies derived from the colorless X-gal. Colorless colonies are indicative of no interaction.

2.4. Protein identification by mass spectrometry

The N-terminal His_6-tagged yeast Rev1 protein was expressed in yeast cells from a plasmid vector. The tagged Rev1 protein was purified sequentially through Ni-Sepharose, FPLC Mono S, and FPLC Superdex 200 (gel filtration column). Rev1 was identified at each step of the purification by Western blot analysis using a mouse monoclonal antibody specific for the His_6-tag. Fraction 16 of the Superdex 200 column was separated by electrophoresis on a 10% SDS-polyacrylamide gel. The gel was fixed for 20 min in 20 ml of a solution containing 50% methanol and 50% acetic acid. The gel was washed for 15 min in 20 ml of 50% methanol, followed by washing for several h in 30 ml of ddH_2O. After treating for 1 min in 20 ml of 0.02% $Na_2S_2O_3$, the gel was washed twice for 1 min each in 30 ml of ddH_2O, followed by incubation in 0.1% $AgNO_3$ for 20 min at 4 °C and washing twice for 1 min each in 30 ml of