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Short Communication

DNA repair by polymerase δ in *Saccharomyces cerevisiae* is not controlled by the proliferating cell nuclear antigen-like Rad17/Mec3/Ddc1 complex

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ABSTRACT. DNA damage activates several mechanisms such as DNA repair and cell cycle checkpoints. The *Saccharomyces cerevisiae* heterotrimeric checkpoint clamp consisting of the Rad17, Mec3 and Ddc1 subunits is an early response factor to DNA damage and activates checkpoints. This complex is structurally similar to the proliferating cell nuclear antigen (PCNA), which serves as a sliding clamp platform for DNA replication. Growing evidence suggests that PCNA-like complexes play a major role in DNA repair as they have been shown to interact with and stimulate several proteins, including specialized DNA polymerases. With the aim of extending our knowledge concerning the link between checkpoint activation and DNA repair, we tested the possibility of a functional interaction between the Rad17/Mec3/Ddc1 complex and the replicative DNA

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polymerases α, δ and ε. The analysis of sensitivity response of single and double mutants to UVC and 8-MOP + UVA-induced DNA damage suggests that the PCNA-like component Mec3p of *S. cerevisiae* neither relies on nor competes with the third subunit of DNA polymerase δ, Pol32p, for lesion removal. No enhanced sensitivity was observed when inactivating components of DNA polymerases α and ε in the absence of Mec3p. The hypersensitivity of *pol32*Δ to photoactivated 8-MOP suggests that the replicative DNA polymerase δ also participates in the repair of mono- and bi-functional DNA adducts. Repair of UVC and 8-MOP + UVA-induced DNA damage via polymerase δ thus occurs independent of the Rad17/Mec3/Ddc1 checkpoint clamp.

Key words: Checkpoint; DNA replication; DNA repair; Proliferating cell nuclear antigen; Rad17/Mec3/Ddc1; *Saccharomyces cerevisiae*

Cells ensure the stability of their genomes by activating a range of cellular responses following DNA damage, including DNA repair, apoptosis, damage tolerance mechanisms, and complex signaling networks that arrest the cell cycle at appropriate points. These so-called checkpoints allow recovery of the integrity of DNA before re-entering the cell cycle (Hartwell and Weinert, 1989). Checkpoint activation requires the action of DNA damage sensors and transducers. While two distinct complexes independently bind to sites of DNA damage in *Saccharomyces cerevisiae*, the presence of both is required for proper checkpoint function. The first complex, the Mec1/Ddc2 heterodimeric protein kinase, functions in DNA damage recognition and signal transduction. The second set of proteins is homologous to the replication clamp PCNA (proliferating cell nuclear antigen) and its clamp loader, replication factor C (RFC) (reviewed in Harrison and Haber, 2006). The functional homolog of PCNA (PCNA-like) is a heterotrimeric clamp consisting of the *S. cerevisiae* Ddc1, Rad17 and Mec3 protein subunits, which are orthologous to the human and *S. pombe* Rad9, Rad1 and Hus1 subunits, respectively, the 9-1-1 complex. Protein threading algorithms have predicted a PCNA-like fold for these subunits, while biochemical studies show their heterotrimeric structure (Sun et al., 1996; Venclovas and Thelen, 2000). The five-subunit Rad24-RFC clamp loader, consisting of Rad24 (Rad17 in human and *S. pombe*) and the four small subunits of RFC (Rfc2 to Rfc5), is a specific loader of the checkpoint clamp (reviewed in Majka and Burgers, 2004). Once loaded, the clamp has the ability to slide across double-stranded DNA, similar to PCNA, and search for DNA damage (Majka and Burgers, 2003, 2004).

Although the link between checkpoint engagement and recruitment of repair machinery to DNA lesions is far from being understood, recent studies have shown the interaction or co-localization of PCNA-like complexes with several DNA repair factors, including the Rad14 nucleotide excision repair protein of *S. cerevisiae* (Giannattasio et al., 2004), the human FEN1 nuclease (Wang et al., 2004), DNA ligase I (Smirnova et al., 2005), the MYH glycosylase of *S. pombe* (Chang and Lu, 2005), and the apurinic/apyrimidinic endonuclease 1 (Ape1) of *S. cerevisiae* (Gembka et al., 2007).

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Central to these processes are DNA polymerases (Pols) that have also been found to interact with PCNA-like sliding clamps. It is known that the human 9-1-1 complex interacts with Pol β, pointing to a function in base excision repair (Toueille et al., 2004; Gembka et al., 2007). In *S. pombe*, the 9-1-1 complex was found to co-immunoprecipitate with Pol κ which is important for translesion synthesis (Ohashi et al., 2000). The potential role of PCNA-like complex in regulating the access of translesion polymerases to DNA was confirmed by Sabbioneda et al. (2005), who showed that the Rad17/Mec3/Ddc1 complex interacts with the Rev7 subunit of Pol ζ and regulates Pol ζ-dependent spontaneous mutagenesis. In order to address further the link between checkpoint proteins and DNA repair, we aimed to investigate whether the yeast PCNA-like sliding clamp could interact with DNA polymerases other than those engaged in translesion synthesis, particularly replicative DNA polymerases. Therefore, we analyzed the response to DNA damage (UVC and photoactivated 8-methoxypsoralen [8-MOP + UVA]) of yeast double mutants that lack the yeast PCNA-like component Mec3p and either Pol32p (third subunit of Pol δ), Dpb3p (subunit C of Pol ε) or Ctf4p (Pol α-binding protein) (for reviews, see Hubscher et al., 2002).

The genotypes of strains used in this study are listed in Table 1. Strains JC010, JC011 and JC012 were derived from Y16841, Y15550 and Y15726, respectively, by one-step gene replacement, using an *mec3::HIS3* disruption cassette. The deletion cassette was amplified by PCR with the primers MECHISF (5'*CAATGGTTGCGGCTACAAATATAGGCGAGTTATACTTGCC*CTGTGCGGTATTTCACACCG3') and MECHISR (5'*AGCCCTTCGATCTTGCTATATAATATATGATTTGTCCTCT*AGATTGTACTGAGAGTGC AC3'), where the first 40 bases (italicized) are homologous to *MEC3* sequence just inside of the start and stop sites, respectively, of the *MEC3*-coding region and where the remaining bases are homologous to 20 bases of the 5' and 3' ends of *HIS3* in the plasmid pRS313 (Sikorski and Hieter, 1989) used as template. When gene deletions were to be created, the high efficiency lithium acetate method was used for transformation (Gietz and Woods, 2002). Accuracy of all gene replacements was verified by PCR analysis using specific primers for the *MEC3* gene [MEC3F (5'-TCAGCATTTTTATGTGCAACTAGTTT-3') and MEC3R (5'-GTAGCAAAGAAATGTACCGCTGTAG-3')]. Yeast strains were routinely grown and stored on YPD medium (1% yeast extract, 2% peptone, 2% glucose). For mutagen treatments, stationary phase cells were washed and resuspended in 0.9% NaCl to a titer of 10^8/mL. Treatment with photoactivated 8-MOP was according to Henriques and Moustacchi (1980), and sensitivity to $UV_{254\ nm}$ (UVC) was assayed by irradiating (Stratalinker, Stratagene) cells plated on solid medium. Plates were incubated for 3-4 days at 30°C. Survival data represent the average of at least three experiments.

Table 1. Yeast strains used in the present study.

Strain	Genotype	Source
Y10'000	*MATα his3Δ1leu2Δ0 lys2Δ0 ura3Δ0*	EUROSCARF
Y15198	*MATα his3Δ1leu2Δ0 lys2Δ0 ura3Δ0 mec3::kanMX4*	EUROSCARF
Y16841	*MATα his3Δ1leu2Δ0 lys2Δ0 ura3Δ0 pol32::kanMX4*	EUROSCARF
Y15550	*MATα his3Δ1leu2Δ0 lys2Δ0 ura3Δ0 ctf4::kanMX4*	EUROSCARF
Y15726	*MATα his3Δ1leu2Δ0 lys2Δ0 ura3Δ0 dpb3::kanMX4*	EUROSCARF
JC010	*MATα his3Δ1leu2Δ0 lys2Δ0 ura3Δ0 pol32::kanMX4mec3::HIS3*	Present study
JC011	*MATα his3Δ1leu2Δ0 lys2Δ0 ura3Δ0 ctf4::kanMX4 mec3::HIS3*	Present study
JC012	*MATα his3Δ1leu2Δ0 lys2Δ0 ura3Δ0 dpb3::kanMX4 mec3::HIS3*	Present study

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Yeast strains were exposed to mutagen treatments and cell inactivation was analyzed. Figure 1 shows that the *dpb3*Δ *and ctf4*Δ single mutants have WT-like sensitivity to treatment with these agents, i.e., they are not involved in repair of DNA lesions. In contrast, *pol32*Δ is clearly hypersensitive as it is inactivated like an *mec3*Δ mutant (Cardone et al., 2006). The *mec3*Δ*pol32*Δ double mutant displayed the highest sensitivity of the three double mutants after both UVC and 8-MOP + UVA treatments. Since the sensitivity shown by *mec3*Δ*pol32*Δ is about the sum of the sensitivities of the single mutants, we can assume an additive interaction between *MEC3* and *POL32*.

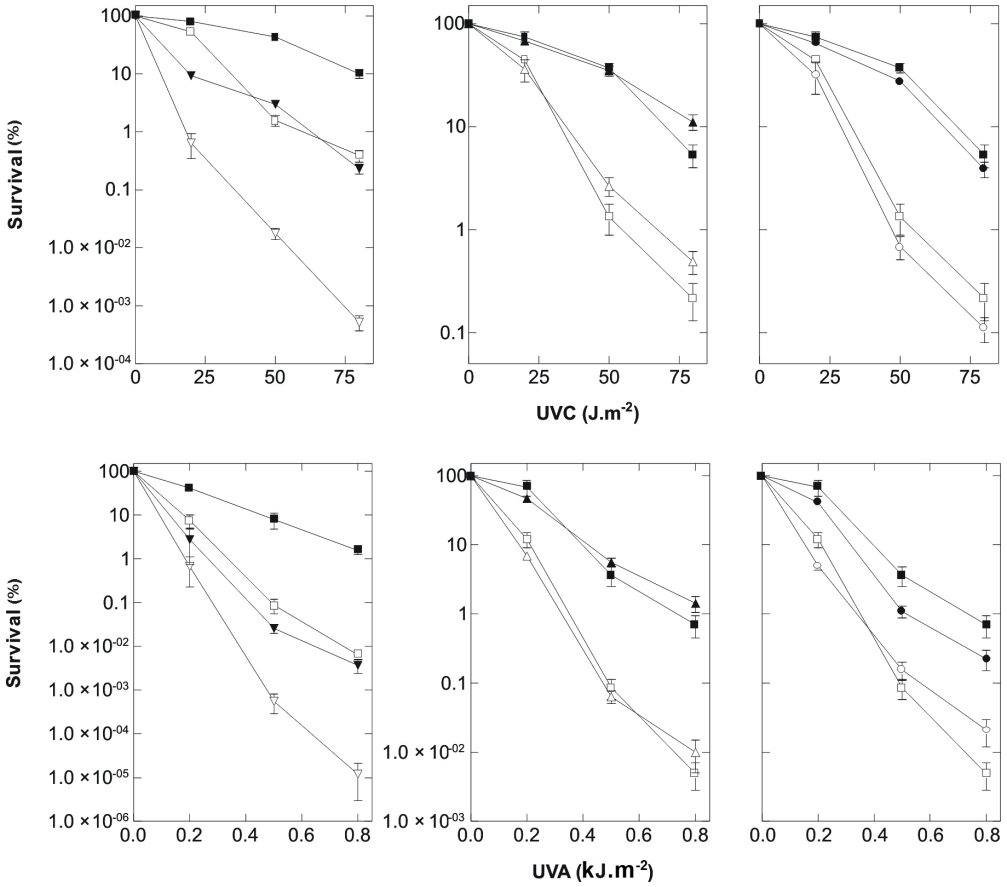

Figure 1. Sensitivity of DNA polymerase mutants in an *mec3*Δ background. UVC (upper panel); 8-MOP + UVA (lower panel). WT (filled squares); *mec3*Δ (open squares); *ctf4*Δ (filled circles); *ctf4*Δ *mec3*Δ (open circles); *dpb3* (filled triangles); *dpb3*Δ *mec3*Δ (open triangles); *pol32*Δ (open inverted triangles); *pol32*Δ *mec3*Δ (filled inverted triangles). Where no error bar is seen, it is smaller than the symbol.

The identification of the biochemical function of the subunits of PCNA-like complex and their roles in checkpoint activation have led to a model in which genotoxins create DNA structures that attract the loading of PCNA-like complexes via the RFC clamp-loading complex (Majka and Burgers, 2003, 2004). Once bound to DNA, the PCNA-like complex serves

as a sliding clamp that functions as a central regulator of checkpoint activation and DNA repair by tethering specific proteins to the sites of DNA damage. While experimental data point to its role in low-fidelity DNA polymerase-utilizing translesion synthesis (Ohashi et al., 2000; Toueille et al., 2004; Sabbioneda et al., 2005), little effort has been made in searching for interactions of PCNA-like complexes with replicative DNA polymerases.

In this study, we were able to show that the *S. cerevisiae* Rad17/Mec3/Ddc1 complex is not necessary for the repair function of the replicative DNA polymerase Pol δ. By analyzing the relative sensitivities of single and double mutants lacking Mec3p and components of the DNA polymerases α, δ and ε (Ctf4, Pol32 and Dpb3, respectively) to DNA damaging agents, we found an additive interaction between *MEC3* and *POL32*, for both UVC and 8-MOP + UVA treatments. This phenotype was exclusive to Pol δ, since the inactivation of Ctf4 and Dpb3 components did not lead to any sensitivity increase over the WT, and since the double mutants only showed an apparent epistatic interaction. Further characterization of conditional mutants of other polymerase subunits remains to be done.

DNA polymerase δ, one of four eukaryotic B family polymerases, is required for replicating the nuclear genome and also for DNA synthesis during recombination, nucleotide excision repair, base excision repair and also in mismatch repair (reviewed in Hubscher et al., 2002), and the UVC and 8-MOP + UVA sensitivity phenotype of *pol32*Δ (Figure 1) confirms this. So far, only translesion polymerases were found to participate in cellular recovery from 8-MOP + UVA-induced DNA lesions (Dronkert and Kanaar, 2001; Lehoczký et al., 2007). Removal of interstrand cross-link proceeds via DNA double-strand breaks that are thought to be repaired either by homologous recombination or non-homologous-end joining (McHugh et al., 2000; Barber et al., 2005). The high sensitivity of *pol32*Δ to 8-MOP + UVA suggests a role of Pol δ in the gap filling prior to the re-ligation of DNA ends. These findings, together with our results, suggest that Pol32p might be playing a role in non-homologous-end joining.

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