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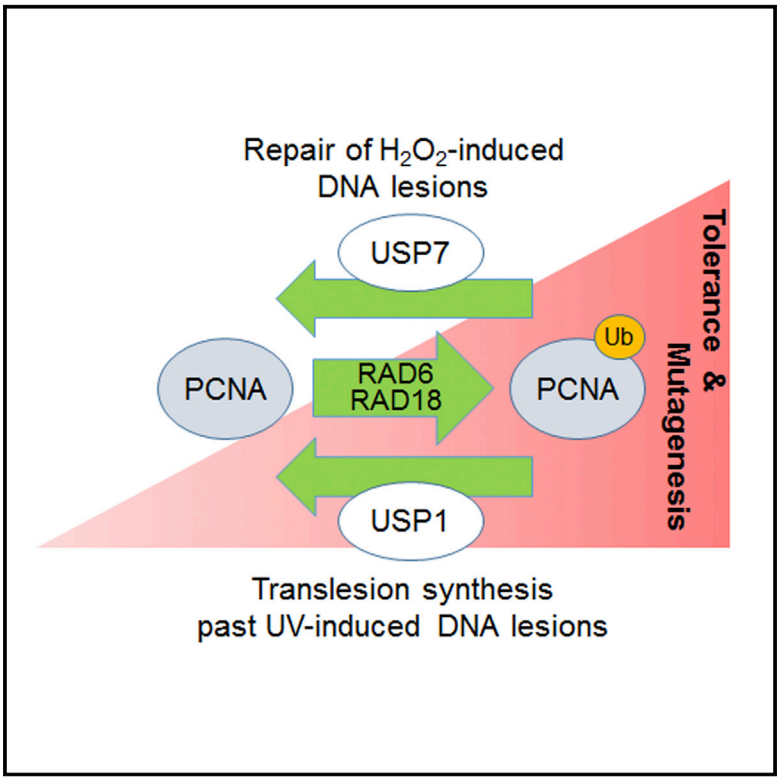
USP7 Is a Suppressor of PCNA Ubiquitination and Oxidative-Stress-Induced Mutagenesis in Human Cells

Graphical Abstract



Authors

Shu-ichiro Kashiwaba, Rie Kanao, Yuji Masuda, Rika Kusumoto-Matsuo, Fumio Hanaoka, Chikahide Masutani

Correspondence

masutani@riem.nagoya-u.ac.jp

In Brief

PCNA mono-ubiquitination regulation is crucial for a balance of DNA damage tolerance and mutagenesis. Kashiwaba et al. reveal that USP7 deubiquitinates mono-ubiquitinated PCNA. Different from USP1, which suppresses DNA-replication-coupled PCNA ubiquitination and mutagenesis after UV irradiation, USP7 suppresses oxidative-stress-induced PCNA ubiquitination and mutagenesis independently of the cell cycle.

Highlights

- USP7 deubiquitinates mono-ubiquitinated PCNA in vitro
- USP7 suppresses UV- and oxidative-stress-induced PCNA mono-ubiquitination in cells
- USP1 acts in S-phase, whereas USP7 acts throughout interphase
- USP1 and USP7 suppress UV- and H_2O_2-induced mutagenesis, respectively

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USP7 Is a Suppressor of PCNA Ubiquitination and Oxidative-Stress-Induced Mutagenesis in Human Cells

Shu-ichiro Kashiwaba,[1,4,5] Rie Kanao,[1,5] Yuji Masuda,[1,2,5] Rika Kusumoto-Matsuo,[1] Fumio Hanaoka,[3] and Chikahide Masutani[1,*]

[1]Department of Genome Dynamics, Research Institute of Environmental Medicine, Nagoya University, Nagoya 464-8601, Japan
[2]Department of Toxicogenomics, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
[3]Faculty of Science, Gakushuin University, Tokyo 171-8588, Japan
[4]Present address: Faculty of Industrial Science and Technology, Tokyo University of Science, Tokyo 125-8585, Japan
[5]Co-first author
*Correspondence: masutani@riem.nagoya-u.ac.jp
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SUMMARY

Mono-ubiquitinated PCNA activates error-prone DNA polymerases; therefore, strict regulation of PCNA mono-ubiquitination is crucial in avoiding undesired mutagenesis. In this study, we used an in vitro assay system to identify USP7 as a deubiquitinating enzyme of mono-ubiquitinated PCNA. Suppression of USP1, a previously identified PCNA deubiquitinase, or USP7 increased UV- and H_2O_2-induced PCNA mono-ubiquitination in a distinct and additive manner, suggesting that USP1 and USP7 make different contributions to PCNA deubiquitination in human cells. Cell-cycle-synchronization analyses revealed that USP7 suppression increased H_2O_2-induced PCNA ubiquitination throughout interphase, whereas USP1 suppression specifically increased ubiquitination in S-phase cells. UV-induced mutagenesis was elevated in USP1-suppressed cells, whereas H_2O_2-induced mutagenesis was elevated in USP7-suppressed cells. These results suggest that USP1 suppresses UV-induced mutations produced in a manner involving DNA replication, whereas USP7 suppresses H_2O_2-induced mutagenesis involving cell-cycle-independent processes such as DNA repair.

INTRODUCTION

Post-translational mono-ubiquitination of PCNA (proliferating cell nuclear antigen) is an important event in the regulation of translesion DNA synthesis (TLS) pathways (Hoege et al., 2002). Human DNA polymerase η (Polη), mutations that are responsible for the cancer-prone syndrome *Xeroderma pigmentosum* variant (Johnson et al., 1999; Masutani et al., 1999), and the other Y-family DNA polymerases—polymerase ι (Polι), polymerase κ (Polκ), and REV1—have PCNA- and ubiquitin-interacting domains and are recruited to stalled replication forks (Bienko et al., 2005; Garg and Burgers, 2005; Guo et al., 2006; Jones et al., 2012; Kannouche et al., 2004; Qin et al., 2013). These DNA polymerases are intrinsically mutagenic; therefore, mono-ubiquitination of PCNA must be strictly tuned in order to regulate these error-prone polymerases and maintain genome integrity. In human cells, the E2-ubiquitin-conjugating enzyme RAD6 and the E3 ubiquitin ligase RAD18 play crucial roles in PCNA ubiquitination in response to DNA damage induced by UV irradiation or agents including reactive oxygen species (Kannouche et al., 2004; Niimi et al., 2008; Zlatanou et al., 2011).

Deubiquitinating enzymes (DUBs) also play crucial roles in regulating the ubiquitination levels of cellular proteins. Downregulation of USP1 increases the basal level of PCNA mono-ubiquitination, which is produced during DNA replication and after UV irradiation. Notably, USP1 is autocleaved and subsequently degraded by the proteasomal pathway several hours after UV irradiation at a rate that is inversely proportional to the increase in mono-ubiquitinated PCNA (Huang et al., 2006). By contrast, oxidative-stress-induced PCNA mono-ubiquitination is regulated independently of USP1 (Zlatanou et al., 2011). Furthermore, mono-ubiquitination of PCNA in response to DNA damage does not occur only in S-phase and facilitates the gap-filling step of DNA repair (Ogi et al., 2010; Yang et al., 2013). USP1 is suppressed by proteasomal degradation mediated by APC/C^{Cdh1} in G0/G1 phase (Cotto-Rios et al., 2011); therefore, other DUBs might be responsible for deubiquitinating the ubiquitinated PCNA outside of S-phase.

USP7 (or HAUSP) is a regulator of the p53-Mdm2 ubiquitination pathway (Brooks and Gu, 2006). In addition, a number of recent reports suggested that USP7 contributes to DNA damage responses. Specifically, USP7 plays crucial roles in transcription-coupled nucleotide excision repair by interacting with UVSSA (Schwertman et al., 2012; Zhang et al., 2012), and it is also involved in global genome nucleotide excision repair by deubiquitinating XPC protein (He et al., 2014). In addition, USP7 is involved in the efficient repair of oxidative DNA lesions