Role of non-classical polymerases and SUMO PCNA dependent mechanisms in the maintenance of genome stability

Ph.D Thesis

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Introduction

Cellular DNA of living organisms is constantly exposed to genotoxic agents (both exogenous and endogenous) resulting in damage to DNA. Exogenous sources include ultraviolet radiation (UV), ionizing radiation, and chemical agents resulting in both single and double stranded breaks, as well as many types of base lesions. These DNA breaks are a major block to DNA replication and, if left unrepaired, can ultimately lead to genomic rearrangement or cell death. Endogenous sources are the predominate means by which DNA is damaged in the cell under normal conditions. Endogenously generated lesions are mainly produced through hydrolytic and oxidative reactions, which are the consequences of the cellular environment and byproducts of cellular processes. With so many types of DNA lesions occurring at such a high frequency, the cell has developed multiple biological responses to DNA damage. These processes can be divided into two main categories: DNA repair and DNA damage tolerance. Even with multiple pathways to repair DNA damage there will persist some amount of damage that must be tolerated during DNA replication. This process of coping with DNA damage is referred to as the DNA damage tolerance pathway.

The predominant mechanism of DNA damage tolerance is translesion synthesis. Translesion synthesis is the replicative bypass of DNA damage by non-classical DNA polymerase. This process involves the direct incorporation of nucleotides across from a DNA lesion, which blocks DNA replication by classical polymerases that are unable to accommodate the lesion in their active site. This process is error-prone because the polymerases responsible for translesion synthesis have a reduced fidelity of nucleotide incorporation, a property that allows them to accommodate the structural distortions caused by various types of DNA lesions. The non-classical polymerases involved in translesion synthesis in eukaryotes are polymerase η, polymerase ι, polymerase ζ, polymerase κ, and the Rev1 protein. To employ these non-classical polymerases, the stalled classical polymerase at the site of DNA damage must be exchanged for a non-classical polymerase. The non-classical polymerase will then bypass the damage and a second exchange will occur between the non-classical and the classical polymerase. This switching event is believed to be mediated by replication accessory factors at the

replication fork such as PCNA, key regulator of translesion synthesis. All Y-family polymerase homologs are members of the Rad6 epistasis group.

The role of PCNA in TLS is governed by post-translational modifications that occur to it in response to an arrested fork. These include sumoylation, monoubiquitylation, and polyubiquitylation. Rad6 protein functions as an E2 ubiquitin-conjugating enzyme, which interacts with the E3 ligase Rad18. The concerted actions of Rad6 and Rad18 are required for the monoubiquitination of Lys164 of PCNA in response to stalled replication caused by DNA damage. PCNA is monoubiquitylated at a highly conserved lysine residue, K164, by the Rad6/Rad18 complex. Ubc13/Mms2 in cooperation with Rad5 can then extend this modification to a polyubiquitin chain of an unconventional, nonproteolytic K63 linkage. The SUMOylation of PCNA and its functions are known mainly from the studies coming from *Saccharomyces cerevisae* and function of the sumoylation at Lys-164 is to recruit the Srs2 helicase that will strip the recombinase Rad51 off the DNA. This helps to prevent any unwanted homologous recombination during S phase. Although PCNA SUMOylation has been reported in higher eukaryotes such as in Xenopus and chicken cells, the SUMO dependent regulation of recombination has been assumed to be important only in particular eukaryotic cells with a naturally high rate of recombination such as yeast. Only recently in a parallel study, SUMOylation of human PCNA has also been found and shown that it preferentially interacts with a PCNA interacting protein (PARI). PARI was suggested to suppress inappropriate recombination events at the replication fork; however, the direct role of SUMO modification of human PCNA has not been studied.

Main objectives of the Thesis

Important objective of this thesis was to better understand the role of DNA damage tolerance mechanisms in maintaining genome stability and preventing cancer-prone human disorders. Specifically our studies were aimed at gaining insights into functions of the translesion synthesis polymerases Polη, Rev1 and Rev3; establishing SUMO-PCNA dependent mechanisms in human cells. Towards the above mentioned aims we addressed the following questions:

- PCNA is known to play a critical role in polymerase exchange process at the site of a DNA lesion, so we asked what is the role of UBZ and PIP domains of Polymerase η in gaining access to the replication foci with PCNA?

- Translesion synthesis polymerases are proposed to play a central role in the bypass of damaged DNA template, therefore we asked if Rev1 and Rev3 deficiency in cells effect the progression of replication of UV damaged DNA?

- Inappropriate recombination can have deleterious effects on the genome. SUMO-PCNA in yeast is known to regulate recombination. It is not known in detail how human cells deal with collapsed replication forks and inappropriate recombination events? Are SUMO-PCNA dependent mechanisms conserved in human cells?

Experimental Approach

In vivo localization studies
DNA fiber assay
Protein purification
Immunoprecipitations

Summary of findings

Some of the important findings obtained during the current thesis work are summarized as follows:

- The binding to PCNA via its PIP domain is a prerequisite for Polη's ability to function in TLS in human cells and that the direct binding of the Ub moiety on PCNA via its UBZ domain is not required.

- Rev1 plays a coordinating role in two modes of DNA damage bypass, i.e., an early and a late pathway. These studies reflect a role of the BRCT domain of Rev1 in mutagenic translesion synthesis. Rev1, but not its BRCT domain, is required for post-replication repair by gap filling, which dominates at a later stage.

- Rev3 is essential for a late mode of DNA damage tolerance whereas immediate translesion synthesis proceeds independently of Rev3.

- Human PCNA is predominantly modified by SUMO1 both *in vivo* and *in vitro* and regulates recombination frequency in vivo demonstrating the conservation and significance of the SUMO modification of PCNA in human cells.

List of Publications

Himabindu Gali, Szilvia Juhasz, Monika Morocz, Ildiko Hajdu, Karoly Fatyol, Valeria Szukacsov, Peter Burkovics and Lajos Haracska. (2012) Role of SUMO modification of human PCNA at stalled replication fork. Nucleic Acids Research (Manuscript in press)

Jacob G. Jansen, Anastasia Tsaalbi-Shtylik, Giel Hendriks, Johan Verspuya, **Himabindu Gali**, Lajos Haracska, Niels de Wind. (2009) Mammalian polymerase ζ is essential for post-replication repair of UV-induced DNA lesions. DNA Repair.; 8(12):1444-51.

Jacob G. Jansen, Anastasia Tsaalbi-Shtylik, Giel Hendriks, **Himabindu Gali**, Ayal Hendel, Fredrik Johansson, Klaus Erixon, Zvi Livneh, Leon H. F. Mullenders, Lajos Haracska, Niels de Wind. (2009) Separate Domains of Rev1 Mediate Two Modes of DNA Damage Bypass in Mammalian Cells. Mol Cell Biol.(11):3113-23.

Narottam Acharya, Jung-Hoon Yoon, **Himabindu Gali**, Ildiko Unk, Lajos Haracska, Robert E. Johnson, Jerard Hurwitz, Louise Prakash, Satya Prakash. (2009) Reply to Sabbioneda et al.: Role of ubiquitin-binding motif of human DNA polymerase eta in translesion synthesis. Proc Natl Acad Sci U S A.106:(8) p.E21.

Narottam Acharya, Jung-Hoon Yoon, **Himabindu Gali**, Ildiko Unk, Lajos Haracska, Robert E. Johnson, Jerard Hurwitz, Louise Prakash, Satya Prakash. (2008) Roles of PCNA-binding and ubiquitin-binding domains in human DNA polymerase eta in translesion DNA synthesis. Proc Natl Acad Sci U S A.; 105(46):17724-9.