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Brief Report

Crosstalk between replicative and translesional DNA polymerases: PDIP38 interacts directly with Polη

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ABSTRACT

Replicative DNA polymerases duplicate genomes in a very efficient and accurate mode. However their progression can be blocked by DNA lesions since they are unable to accommodate bulky damaged bases in their active site. In response to replication blockage, monoubiquitination of PCNA promotes the switch between replicative and specialized polymerases proficient to overcome the obstacle. In this study, we characterize novel connections between proteins involved in replication and TransLesion Synthesis (TLS). We demonstrate that PDIP38 (Polδ interacting protein of 38 kDa) directly interacts with the TLS polymerase Polη. Interestingly, the region of Polη interacting with PDIP38 is found to be located within the ubiquitin-binding zinc finger domain (UBZ) of Polη. We show that the depletion of PDIP38 increases the number of cells with Polη foci in the absence of DNA damage and diminishes cell survival after UV irradiation. In addition, PDIP38 is able to interact directly not only with Polη but also with the specialized polymerases Rev1 and Polζ (via Rev7). We thus suggest that PDIP38 serves as a mediator protein helping TLS Pols to transiently replace replicative polymerases at damaged sites.

1. Introduction

In human cells, replicative DNA polymerases α, δ and ε work in concert to duplicate the bulk of the genome. The DNA polymerases exist as multi-protein assemblies, which show complex patterns of interaction, not only among their subunits, but also with other components of the replication and repair machineries. Several components of the DNA replication apparatus also interact with elements of the networks that regulate cell cycle progression and checkpoint control (reviewed in [1]).

Under normal conditions, replicative DNA polymerases synthesize DNA with a high degree of efficiency. However, many lesions can be produced by DNA damaging agents and as they significantly distort the geometry of the DNA they can potentially block the progression of the replication machinery. To overcome these obstacles, cells have evolved TransLesion DNA Synthesis (TLS) pathway. TLS allows the bypass of damaged nucleotides by a set of specialized DNA polymerases that can accommodate various bulky lesions in their active sites and can insert correct or incorrect nucleotides opposite lesions. Five eukaryotic proteins are able to carry out TLS of damaged DNA *in vitro*: DNA polymerase ζ (Polζ, composed of Rev3 and Rev7 subunits) and the 4 Y-family members, DNA polymerase η (Polη), DNA polymerase ι (Polι), DNA polymerase κ (Polκ) and Rev1 (dCMP transferase) (reviewed in [2]). Lesion bypass is an essential cellular process. Indeed, inactivating mutations in the POLH gene, coding for Polη, lead to the skin cancer-prone disease Xeroderma Pigmentosum variant type (XP-V) [3,4] and confer UV sensitivity to cells in the presence of caffeine [5].

One important question is to know how TLS polymerases gain access to the damaged site in order to transiently replace replicative DNA polymerases.

The access of TLS Pols to the replication complex, as visualized by their localizations in replication foci relies on specific motifs in TLS Pols: PIP box (PCNA-interacting peptide), UBZ (ubiquitin-binding zinc finger domain), UBM (ubiquitin-binding motif), and/or BRCT (BRCA1 C-terminal domain) [6–12].

So far, PCNA is the sole link described between replicative and TLS DNA polymerases. The activity of Polδ is stimulated by PCNA, the processivity clamp allowing it to replicate long stretches of DNA. PCNA interacts with two of the four subunits of Polδ, i.e. p125 and p66 (reviewed in [13]). PCNA interacts both physically and functionally with human Polη, ι and κ and mouse Rev1 [10,11,14–19]. In response to replication fork arrest, PCNA

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Table 1
Primers used for the generation of the point mutations.

Mutation	Sequence of the forward primer	Sequence of the reverse primer
$V_{644}A$	5′-GATATGCCAGAACACATGGACTAT-3′	5′-CCATGCCGGTACCAGGGAGCCACA-3′
$W_{645}A$	5′-GATATGCCAGAACACATGGACTAT-3′	5′-CGCTACCGGTACCAGGGAGCCACA-3′
$D_{646}A$	5′-GCTATGCCAGAACACATGGACTAT-3′	5′-CCATACCGGTACCAGGGAGCCACA-3′
$D_{646}K$	5′-AAAATGCCAGAACACATGGACTAT-3′	5′-CCATACCGGTACCAGGGAGCCACA-3′
$D_{652}A$	5′-CCAGAACACATGGCATATCATTTTGCA-3′	5′-CATATCCCATACCGGTACCAGGGA-3′
$H_{650}A/H_{654}A$	5′-TTTTGCATTGGAGTTGCAGAAATCC-3′	5′-GCATAGTCCATGGCTTCTGGCAT-3′

is monoubiquitinated by the Rad6–Rad18 protein complex [15,20]. The current model proposes that PCNA ubiquitination facilitates the access of TLS polymerases to the site of damage through the ubiquitin-binding domains (UBD) present in the Y-family polymerases [19,21,22]. However, it has been shown that while ubiquitination of PCNA increases the residency time of Polη within foci, it is not a prerequisite for its accumulation in foci, suggesting that multiple signals control the dynamics of Polη [23]. As a matter of fact, TLS polymerases can be activated by two separate modes in DT40 cells [12]; (1) TLS Pols operate at stalled replication forks "on the fly" to maintain fork progression following DNA damage in a Polη and Rev1 dependent mechanism; (2) TLS is employed for filling postreplicative gaps generated after repriming of DNA synthesis downstream of the lesion. While PCNA monoubiquitination is dispensable to maintain "on the fly" fork progression on damaged DNA, it is required for postreplicative TLS.

To address the question of the regulation of the switch between replicative and TLS DNA Pols, we aimed to determine and characterize novel connections between proteins involved in these two processes. Here, we reveal new interactions among TLS Pols and the PDIP38 protein (Polδ interacting protein of 38 kDa).

PDIP38 was initially identified as a partner of p50, an essential subunit of Polδ. Subsequently PDIP38 was found to interact with PCNA [24]. Using immuno-affinity chromatography, as well as non-denaturing gel electrophoresis, Liu et al. demonstrated that PDIP38 is associated with the Polδ complex [24]. Recently, it has been shown that PDIP38 shuttles between the cytoplasm and the nucleus in a cell cycle dependent manner. PDIP38 is present in the nucleus of a subpopulation of proliferating cells, but completely absent in the nucleus of quiescent cells [25]. PDIP38 is also involved in cell-cell contact, in spindle organization and in chromosome segregation [26].

In this study, we demonstrate that PDIP38 interacts directly with the UBZ domain of Polη and modulates the focal localization of Polη. Indeed PDIP38 depletion increases the number of cell with Polη foci in the absence of DNA damage. We also show that PDIP38 is involved in cell survival after UV damage. Furthermore, PDIP38 also interacts with other specialized polymerases such as Rev1 and Polζ (Rev7 subunit). Our data establish PDIP38 as a novel link between replicative and specialized DNA polymerases and we propose that this protein could help TLS Pols to transiently replace replicative polymerases at damaged sites.

2. Materials and methods

2.1. Cell lines

The SV40-transformed normal human fibroblasts MRC5V1 (called MRC5 in this paper) and the SV40-transformed XPV human fibroblasts (XP30RO) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Eurobio) and 50 μg/ml of gentamicin (Sigma).

2.2. Yeast two-hybrid assay

Two-hybrid methodology and Polη screen were described elsewhere [27]. Mutations in the coding sequence of Polη were generated by site-directed mutagenesis. The sequences of the primers used for the generation of the point mutations are presented in Table 1.

2.3. Pull-down assay

PDIP38 was cloned in pET52b+ vector (Invitrogen) and produced in *Escherichia coli* as a 6-His-Cter tagged and Strep Nter tagged PDIP38. An internal T7 promoter was used to express Polη as a Nter-Myc tagged Polη in the pGBKT7 vector. These vectors were introduced into a recipient Rosetta strain and expression of the myc-Polη or His-PDIP38 was triggered by addition of IPTG (1 mM final) to a 50 ml culture grown in independent experiment. Ni purified his-PDIP38 were mixed with whole cell extracts from the strain producing the myc or the myc-Polη, applied to an anti-myc column (Pierce) and analyzed by western blot using anti-His antibodies.

2.4. SiRNA transfection and proliferation assay

Synthetic siRNA pools (Dharmacon) were transfected using Interferin (PolyPlus) as described by the manufacturer. The next day, cells were plated at 1×10^5 per well of a six-well plate and incubated for 24 h. Cells were then exposed to increasing doses of UVC without any medium. After treatment, cells were incubated for 72 h in complete MEM medium containing 0.4 mM caffeine (Sigma) and counted by Trypan blue staining using a Neubauer haemocytometer.

2.5. Fluorescence analysis

1×10^6 MRC5 fibroblasts cells were plated per 150-mm-diameter dish and incubated overnight. Synthetic siRNA was transfected using Lipofectamin 2000 (Invitrogen) as described by the manufacturer and 4 h later, peGFP-Polη was transfected using JetPEI (Polyplus) according to the manufacturer's protocol. 24 h later cells were plated at 3×10^5 per well onto six-well plates containing microscope slides. After 24 h, cells were irradiated at 14 J/m^2 and incubated for a further 15 h in the presence of caffeine (0.4 mM – Sigma). Cells were fixed for 20 min at RT in 4% formaldehyde in PBS and mounted onto slides using Fluorescent mounting medium (DakoCytomation). A minimum of 150 nuclei was analyzed per experiment, for each cell line and treatment. Cells forming GFP-Polη in foci as described in [28] were scored using a fluorescence microscope (Olympus, FluoView FV1000).

2.6. Flow cytometry analysis

Cells were plated at a density of 2×10^5 cells per 100-mm-diameter dish and allowed 12 h for attachment. Cells were re-fed with complete culture medium containing 2 mM Thymidine (Sigma). After 16 h of incubation, cells were washed twice in PBS, and released from the block at the G1/S border by culture in fresh culture medium. Nine hours later, culture medium containing aphidicolin at the final concentration of 2 μg/ml was added to the dishes for 14 h to synchronize the cells at the G1/S border [29]. The cells were released from the block by rinsing twice