DNA Repair 35 (2015) 106–115

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

Poly(ADP-ribose)-binding promotes Exo1 damage recruitment and suppresses its nuclease activities

Abigael Cheruiyot[a,1], Sharad C. Paudyal[a,1], In-Kwon Kim[b,1], Melanie Sparks[a], Tom Ellenberger[b], Helen Piwnica-Worms[c], Zhongsheng You[a,*]

[a] Department of Cell Biology & Physiology, Washington University School of Medicine, St. Louis, MO 63110, United States
[b] Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, United States
[c] Department of Cancer Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77230, United States

ARTICLE INFO

Article history:
Received 26 August 2015
Received in revised form 9 September 2015
Accepted 11 September 2015
Available online 30 September 2015

Keywords:
Exo1
Poly(ADP-ribosyl)ation
14-3-3
PCNA
DNA end resection
DNA replication

ABSTRACT

Exonuclease 1 (Exo1) has important roles in DNA metabolic transactions that are essential for genome maintenance, telomere regulation and cancer suppression. However, the mechanisms for regulating Exo1 activity in these processes remain incompletely understood. Here, we report that Exo1 activity is regulated by a direct interaction with poly(ADP-ribose) (PAR), a prominent posttranslational modification at the sites of DNA damage. This PAR-binding activity promotes the early recruitment of Exo1 to sites of DNA damage, where it is retained through an interaction with PCNA, which interacts with the C-terminus of Exo1. The effects of both PAR and PCNA on Exo1 damage association are antagonized by the 14-3-3 adaptor proteins, which interact with the central domain of Exo1. Although PAR binding inhibits both the exonuclease activity and the 5′ flap endonuclease activity of purified Exo1, the pharmacological blockade of PAR synthesis does not overtly affect DNA double-strand break end resection in a cell free *Xenopus* egg extract. Thus, the counteracting effects of PAR on Exo1 recruitment and enzymatic activity may enable appropriate resection of DNA ends while preventing unscheduled or improper processing of DNA breaks in cells.

1. Introduction

First identified in *Schizosaccharomyces pombe,* Exo1 is an evolutionarily conserved nuclease that participates in DNA replication and repair pathways, including DNA double-strand break repair (DSBR), mismatch repair (MMR) and error-free post-replicative repair by template switching [1–3]. Exo1 exhibits both 5′–3′ exonuclease activity and 5′ flap endonuclease activity [4]. The exonuclease activity of Exo1 is crucial for the resection of DNA double-strand break (DSB) ends and the removal of mispaired nucleotides to enable the rejoining of DNA ends [1,5,6]. During MMR, Exo1 excises DNA from the nick generated by Mlh1-Pms1 in the 5′–3′ direction to create a gap for subsequent repair steps [3,5]. The resection of DSBs generates long ssDNA tails that initiate DNA repair by homologous recombination (HR) and activate the ATR-dependent cell cycle checkpoint [7,8]. The process of DNA resection is thought to be initiated by endonucleolytic cleavage near the breakpoint that is mediated by the Mre11-Rad50-NBS1 (MRN) complex (Mre11-Rad50-Xrs2 in budding yeast) together with the CtIP (Sae2 in budding yeast) protein. Following this initial endocleavage, the resulting "clean" 5′ ends are further resected by Exo1 and Dna2, which act redundantly to generate long 3′ ssDNA overhangs (*e.g.* 2–4 kb in budding yeast) required for HR and the ATR checkpoint [9,10]. The resection of dsDNA ends to create ssDNA overhangs also inhibits DSB repair by nonohomologous end-joining (NHEJ) and attenuates the ATM-dependent checkpoint pathway [11,12]. Exo1's 5′ flap endonuclease activity is thought to resolve DNA intermediates formed during replication and recombination. Genetic studies in yeast suggest that the flap endonuclease activity of Exo1 plays a redundant role with Fen1 (Rad27 in budding yeast) in Okazaki fragment maturation. Consistent with this notion, overexpression of yeast EXO1 or human Exo1 proteins functionally complements the replication defects of a rad27 mutant [13].

The regulation of Exo1 activity ensures efficient break processing while avoiding unscheduled or uncontrolled DNA digestion that could lead to cell death or genomic instability. Indeed, Exo1 function is regulated in some manner by a number of proteins that function in DSB resection, including MRN, CtIP, Ku, RPA, SOSS1,

* Corresponding author at: Washington University School of Medicine, Campus Box 8228, 660 S. Euclid Ave., St. Louis 63110, United States. Fax: +1 314 362 7463.
E-mail address: zyou@wustl.edu (Z. You).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.dnarep.2015.09.021
1568-7864/

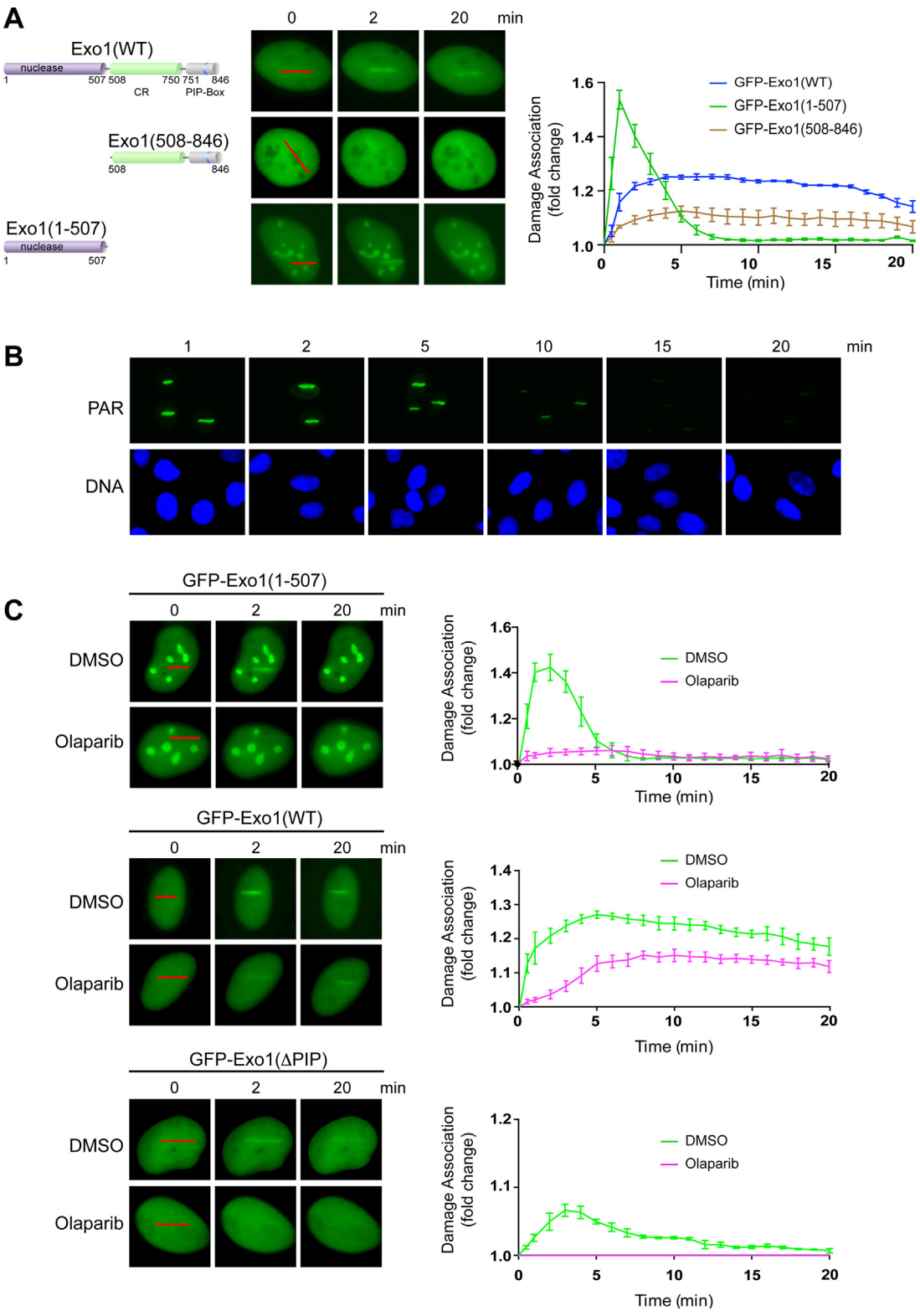

Fig. 1. The N-terminal domain of Exo1 promotes its initial damage recruitment in a PARylation-dependent manner.
(A) Left panel: Diagram of functional domains in wild type Exo1 and truncation mutants (not to scale). Middle panel: Representative images for the damage association of GFP-Exo1, GFP-Exo1(508–846) and GFP-Exo1(1–507) shown in the left panel. Red lines indicate the sites of laser irradiation in cells. Right panel: Quantified results for the damage association of GFP-Exo1 and its mutants during the first 20 min after laser irradiation. Each data point is the average of independent measurements of 5 cells. Error bars represent standard deviation.
(B) Immunofluorescence staining of PAR at sites of DNA damage at various times after laser irradiation.
(C) Left panels: Representative images for the damage association of GFP-Exo1(1–507), GFP-Exo1(WT) and GFP-Exo1(ΔPIP) in cells treated with DMSO or Olaparib. Red lines indicate the sites of laser irradiation in cells. Right Panel: Quantified results for the damage association GFP-Exo1(1–507), GFP-Exo1(WT) and GFP-Exo1(ΔPIP) in cells treated with DMSO or Olaparib during the first 20 min after laser irradiation. Each data point is the average of independent measurements of 5 cells. Error bars represent standard deviation.