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Poly- and mono(ADP-ribosyl)ation of DNA strand breaks by PARP2 and PARP3 enzymes

G. Zarkovic[1], E.A. Belousova[2], I. Talhaoui[1], C. Saint-Pierre[3], M.M. Kutuzov[2], B.T. Matkarimov[4], D. Gasparutto[3], O.I. Lavrik[2], A.A. Ishchenko[1]*

[1] *Groupe «Réparation de l'ADN», Equipe Labellisée par la Ligue Nationale Contre le Cancer, CNRS UMR8200, Univ. Paris-Sud, Université Paris-Saclay, Villejuif, France*

[2] *Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia*

[3] *Université Grenoble Alpes, INAC/SPrAM UMR5819 CEA CNRS UGA, Grenoble, France*

[4] *National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan*

** e-mail: Alexander.ISHCHENKO@gustaveroussy.fr*

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DNA dependent poly(ADP-ribose) polymerases (PARP1-3) play important roles in the repair of DNA strand breaks and are known to be activated in the presence of DNA strand breaks to catalyze poly- or mono(ADP-ribosyl)ation (PARylation or MARylation, respectively) of themselves or other nuclear acceptor proteins. Each PARP recognizes distinct sets of DNA structures with breaks, suggesting that PARP1, 2 and 3 have non-overlapping functions in DNA repair. At present, the detailed molecular mechanisms of PARP-dependent DNA breaks repair remain unknown. Previously, we showed that mammalian PARP1 and PARP2 can catalyze covalent addition of ADP-ribose units not only to proteins but also to DNA strand breaks containing terminal phosphates or a 2′-OH group, thus producing a covalent PAR–DNA adduct in vitro. The PARP-catalyzed DNA PARylation is a reversible process because PAR can be entirely degraded by poly(ADP-ribose) glycohydrolase (PARG). Here, we examined DNA ADP-ribosylation activity and the DNA substrate preference of PARP3 as compared with structurally similar PARP2. PARP3 can effectively produce MAR–DNA adducts covalently linked to terminal phosphates at DSB and SSB termini of short and long DNA molecules, exhibiting similar substrate specificity with PARP2. Notably, ADP-ribosylation of 5′-terminal thiophosphates at DSB termini by PARPs generates MAR–DNA adducts resistant to PARG hydrolysis. We found that depending on configuration of DNA strand breaks, the DNA termini can become preferred acceptor sites for ADP-ribosylation as compared to proteins. According to the data obtained, we propose a putative mechanistic model of DNA strand break-oriented DNA ADP-ribosylation by PARP3 or PARP2. Our findings reveal effective PARP3- or PARP2-catalyzed ADP-ribosylation of ~3-kb DNA plasmid-based substrates and DNA PARylation activity in nuclear extracts from HeLa cells. Finally, immunoblotting of purified genomic DNA from PARG-depleted HeLa cells after genotoxic treatment provides indirect evidence of the presence of PAR–DNA adducts in live cells. These results suggest that certain types of complex DNA breaks can be effectively ADP-ribosylated by PARPs in cellular response to DNA damage.

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