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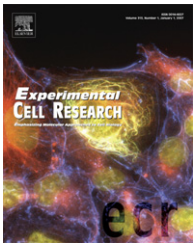

Research Article

# Altered poly(ADP-ribose) metabolism impairs cellular responses to genotoxic stress in a hypomorphic mutant of poly(ADP-ribose) glycohydrolase

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ABSTRACT

Genotoxic stress activates nuclear poly(ADP-ribose) (PAR) metabolism leading to PAR synthesis catalyzed by DNA damage activated poly(ADP-ribose) polymerases (PARPs) and rapid PAR turnover by action of nuclear poly(ADP-ribose) glycohydrolase (PARG). The involvement of PARP-1 and PARP-2 in responses to DNA damage has been well studied but the involvement of nuclear PARG is less well understood. To gain insights into the function of nuclear PARG in DNA damage responses, we have quantitatively studied PAR metabolism in cells derived from a hypomorphic mutant mouse model in which exons 2 and 3 of the PARG gene have been deleted (PARG-Δ2,3 cells), resulting in a nuclear PARG containing a catalytic domain but lacking the N-terminal region (A domain) of the protein. Following DNA damage induced by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), we found that the activity of both PARG and PARPs in intact cells is increased in PARG-Δ2,3 cells. The increased PARG activity leads to decreased PARP-1 automodification with resulting increased PARP activity. The degree of PARG activation is greater than PARP, resulting in decreased PAR accumulation. Following MNNG treatment, PARG-Δ2,3 cells show reduced formation of XRCC1 foci, delayed H2AX phosphorylation, decreased DNA break intermediates during repair, and increased cell death. Our results show that a precise coordination of PARPs and PARG activities is important for normal cellular responses to DNA damage and that this coordination is defective in the absence of the PARG A domain.



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## Introduction

Polymers of ADP-ribose (PAR) are synthesized from $NAD^+$ by the action of poly(ADP-ribose) polymerases (PARPs) [1] and PAR hydrolysis is catalyzed by poly(ADP-ribose) glycohydrolases [2,3]. Multiple genes encoding PARPs have been identified [1] and these enzymes have been implicated in many different cellular functions including DNA repair, RNA transcription, centrosome function, and mitotic spindle assembly [4,5]. In contrast, a single gene that encodes PARG activity has been identified in mammals [6], although the gene is expressed to generate multiple PARG isoforms specifically targeted to different cell compartments [7]. Another protein with PARG activity *in vitro* has been reported [8] but its role, if any, in PAR metabolism is unknown.

The best understood aspect of PAR metabolism is that which occurs in response to genotoxic stress, where PARP-1 and PARP-2 activation by DNA strand breaks formed directly by the DNA damaging agent or indirectly by DNA repair nucleases leads to rapid nuclear PAR synthesis that also is accompanied by rapid PAR turnover catalyzed by PARG [9–11]. At high levels of DNA damage, the coordinated activities of PARP-1/PARP-2 and PARG can selectively deplete the cellular NAD pool [12] with possible consequences on cellular energy metabolism and other reactions that use NAD as a substrate [4]. PAR metabolism plays an important role in cell fate determination following genotoxic stress, promoting cell survival following limited DNA injury [5] and increasing cell death following more extensive injury [13,14]. PARP-1, the first identified and best characterized member of the PARP family, accounts for the majority of PAR synthesis following genotoxic stress and also serves as a major acceptor protein for PAR via automodification [15,16]. PARP-2, which accounts for PAR synthesis following genotoxic stress in PARP-1 knockout cells [17], also is involved in maintenance of genomic integrity and simultaneous disruption of PARP-1 and PARP-2 genes results in embryonic lethality [18]. Knowledge of the structural biology of PARP-1/PARP-2 [1] and the availability of potent and selective PARP inhibitors [19] and gene knockout animals [20,21] has been instrumental in revealing the roles of the DNA damage responsive PARPs in responses to genotoxic stress. This has led to the clinical development of PARP inhibitors as therapeutic agents used in combination with DNA alkylating agents [22] and as potential stand alone agents for treatment of BRCA related tumors [23,24].

Although the kinetics of PAR metabolism following DNA damage reveal that PAR synthesis and turnover are closely linked, the role of PARG in cellular responses to genotoxic stress is poorly understood. A domain structure of the PARG protein has been proposed [25] but its structural biology is still not well defined. Potent and selective PARG inhibitors are not generally available and disruption of the PARG gene that results in total loss of cellular PARG activity leads to early embryonic lethality of homozygous mutant mice [26]. Recently, a mouse model that achieves a selective, partial PARG gene deletion that results in viable animals has been described [27], offering a tool for study of PARG function. The gene disruption deletes exons 2 and 3 of the PARG gene, eliminating several PARG isoforms including the normal

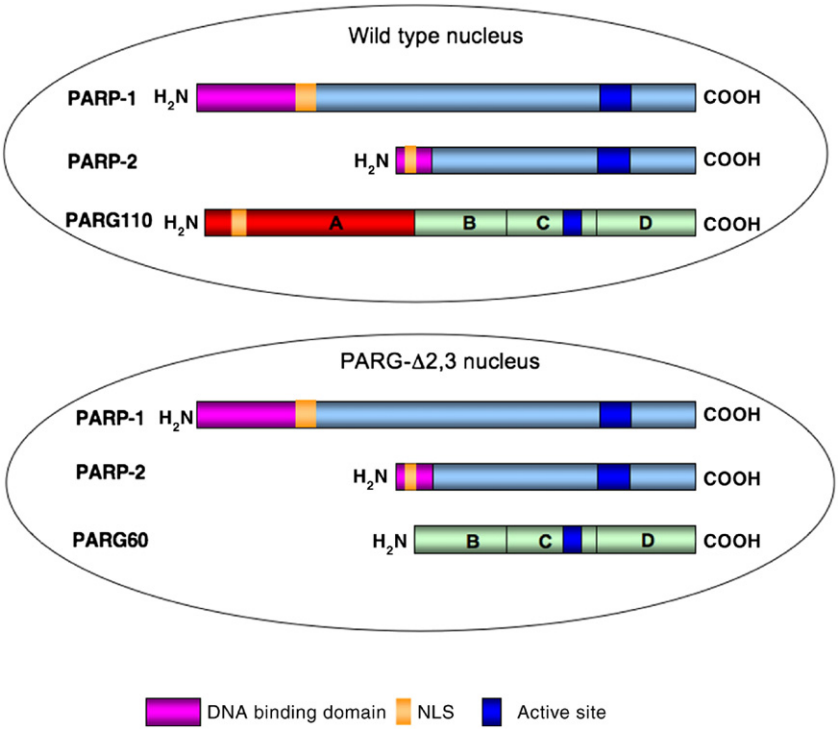


**Fig. 1 – Comparison of DNA damage responsive PARPs and PARG in wild type and PARG-Δ2,3 cell nuclei. The top diagram shows proteins involved in PAR metabolism following DNA damage present in the nucleus of wild type cells and the lower diagram shows the proteins present in PARG-Δ2,3 cells [27]. Nuclear localization signal is abbreviated as NLS. The domains A to D of PARG are described in [25].**