

# Interaction of PARP2 with DNA structures mimicking DNA repair intermediates

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*Poly(ADP-ribosylation) is a posttranslational protein modification significant for the genomic stability and cell survival in response to DNA damage. Poly(ADP-ribosylation) is catalyzed by poly(ADP-ribose)polymerases (PARPs). Whereas the role of PARP1 in response to DNA damage has been widely illustrated, the contribution of another DNA-dependent PARP, PARP2, has not been studied so far. Aim. To find out specific DNA targets of PARP2. Methods. The EMSA and the PARP activity tests were used. Results. We evaluated  $K_d$  values of PARP2-DNA complexes for several DNA structures mimicking intermediates of different DNA metabolizing processes and tested these DNA as «activators» of PARP1 and PARP2 in poly(ADP-ribose) synthesis. Conclusions. Like PARP1, PARP2 does not show correlation between the activation efficiency and  $K_d$  values for DNA. PARP2 was activated most effectively in the presence of over5DNA.*

*Keywords: PARP1, PARP2, poly(ADP-ribosylation), DNA binding.*

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**Introduction.** Poly(ADP-ribosylation) is catalyzed by poly(ADP-ribose)polymerases (PARPs), which use  $NAD^+$  as substrate, synthesize polymer of (ADP)-ribose (PAR) covalently attached to nuclear proteins including PARPs themselves. PARPs now constitute a large family of 17 proteins displaying a conserved catalytic domain, in which PARP1 and PARP2 are so far the sole enzymes whose catalytic activity is stimulated by some types of DNA damages.

PARP2 is the closest homolog of PARP1. Whereas the contribution of PARP1 in the response to DNA damages has been widely illustrated, the role of PARP2 has not been studied so far. Although PARP1 and PARP2 functions could overlap, *in vivo* experiments with *Parp1*<sup>-/-</sup> and *Parp2*<sup>-/-</sup> mice demonstrated that lack of each of them

could not be compensated for another one. As a whole, PARP2 is much less active than PARP1, despite high homology of their catalytic domains. The analysis of the crystal structures of their catalytic domains revealed a particular structural feature of PARP2 [1] that could account for the PARP2 specificity in the recognition of protein targets to be poly(ADP-ribosyl)ated. Moreover, the DNA binding domain (DBD) of hPARP2 has an unknown structure and differs from the structure of PARP1 DBD that appears to reflect the differences in the DNA structure recognition by each enzyme. Therefore, study on the PARP2 interaction with DNA intermediates of different DNA-dependent processes is required to reveal specific DNA targets and to determine contribution of PARP2 to DNA repair.

**Materials and methods.** Human PARP1 was expressed in *Escherichia coli* and purified as described

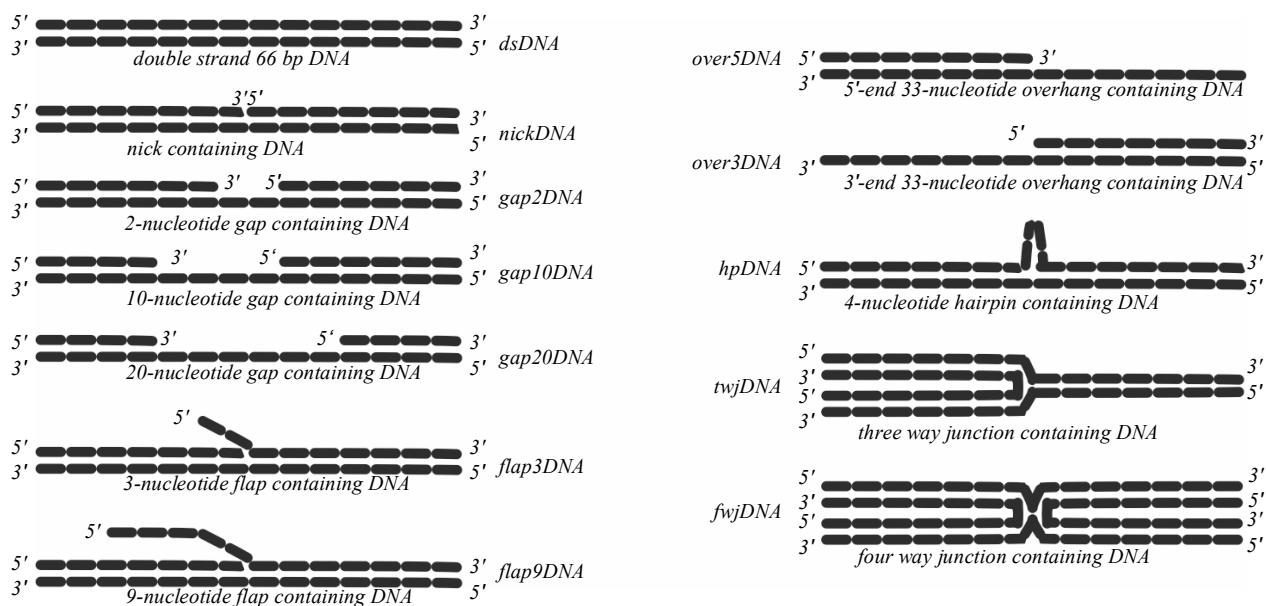


Fig. 1. Structures of used DNA duplexes

[2]. Murine PARP2 was expressed in insect cells and purified according to [3].

**Evaluation of  $K_d$  of PARP2-DNA complexes.** PARP2 (200 nM or 1  $\mu$ M in the case of dsDNA) was incubated for 15 min at 0 °C with DNA (5'-labeled with Alexa 647) (4.7–455 nM) in the 10  $\mu$ l reaction mixture containing 50 mM Tris HCl (pH 8.0), 40 mM NaCl, 0.5 mg/ml of BSA, 10 mM EDTA and 1 mM DTT. The samples were electrophoresed at 4 °C through 10 % native polyacrylamide gels for 3 h at 75 V to separate free and protein-bound DNA. Thereafter, the gels were dried and scanned on «Odissey» (Li-Cor). The  $K_d$  values were evaluated using «GraphPad Prism» software.

**PARP activity assay.** To calculate initial velocity values the kinetics of poly(ADP-ribose) synthesis were analyzed using radioactively labeled  $\text{NAD}^+$ . The amount of PAR was valued by incorporation of radioactively labeled ADP. PARP2 (PARP1) in concentration of 100 nM (50 nM) was incubated with 400  $\mu$ M  $\text{NAD}^+$  and 100 nM DNA in a total volume of 30  $\mu$ l containing 50 mM Tris HCl (pH 8.0), 40 mM NaCl, 0.1 mg/ml of BSA, 6 mM  $\text{MgCl}_2$ , 1 mM DTT at 37 °C. The 5  $\mu$ l aliquots were taken at 1, 2, 5, 8 and 11 min and placed on Whatman paper pre-impregnated with trichloroacetic acid (TCA) to stop the PAR synthesis. The PAR attached to proteins was precipitated on filters in the presen-

ce of TCA. Unreacted  $\text{NAD}^+$  was removed from the filters by washing with 5 % TCA.

**Results and discussion.** In this work we examined a few DNA structures (Fig. 1) mimicking intermediates of different DNA metabolizing processes in order to identify the structure specifically recognized by PARP2.

Taking into account the principle difference in the structure of the DBDs of PARP1 and PARP2 one could expect that each of these PARPs recognizes specific structural elements in DNA. To this end, the  $K_d$  values of PARP2-DNA complexes were evaluated. In contrast to PARP1, which displays rather high affinity ( $K_{d \text{ app}} = 116$  pM) to blunt end DNA (dsDNA) [4], PARP2 shows lower affinity to dsDNA ( $K_d \sim 200$  nM). Thus, DNA-duplexes with blunt ends and containing specific structural elements may be used to study an interaction of these DNA with PARP2 without a confounding contribution of blunt ends to the affinity. We found that PARP2 efficiently binds gap20DNA and flap9DNA ( $K_d \sim 6$  and 10 nM, respectively). These DNA structures can potentially be formed during the homologous recombination process, LP-BER or in DNA replication. For other DNA used the  $K_d$  values varied in the range of 16–110 nM. As a whole, among the DNA structures studied, excluding blunt end DNA, the affinities of DNAs to PARP2 differ less than one order of magnitude. In

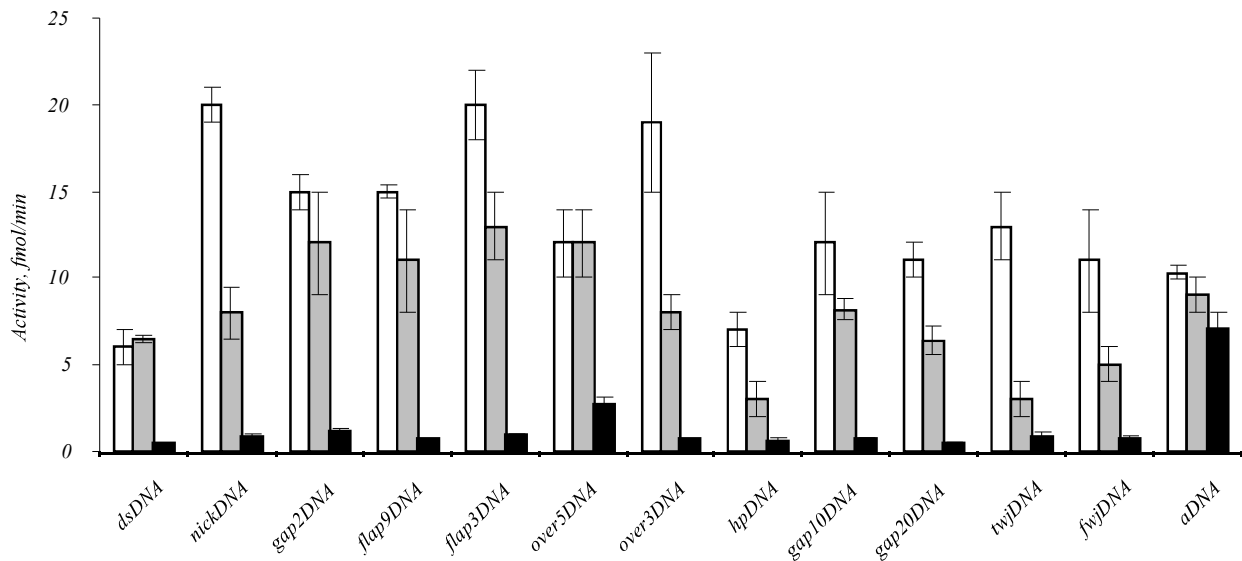


Fig. 2. Comparison of PARP1 and PARP2 activities in the presence of different DNA. Blank bars – PARP1; grey bars – PARP1/PARP2; black bars – PARP2

spite of extensive study of the PARP1 interaction with structural peculiarities in DNA (nicks, bubbles, hairpins and so on) only limited data on quantitative characteristics of binding are available [4]. Interestingly, that affinity of PARP1 to DNA with 3' or 5' overhangs of few nucleotides ( $K_{d\text{ app}}$  are in the range of 2.6–5.0 nM) differs considerably from that for blunt end DNA ( $K_{d\text{ app}} = 116\text{ pM}$ ) [4].

Another interesting issue is that in spite of lower affinity of PARP1 to nicks than to blunt ends ( $K_{d\text{ app}} = 467$  and 116 pM, respectively) nicks are rather good activator for the enzyme ( $V_{\text{max}}$  for nicked DNA is 4-fold higher). Thus, activator characteristic of DNA is dependent on both the binding efficiency and catalysis rate.

To determine the contribution of PARP1 and PARP2 to the total poly(ADP-ribose) synthesis, the activation of both enzymes, when present separately or together, by each of DNAs was estimated (Fig. 2).

In whole, the activity of PARP2 was much lower than PARP1 activity that is in accordance with the literature data that about 90 % of total nuclear PAR synthesis in response to DNA damages is performed by PARP1 [3]. However, on activated DNA (aDNA), i. e. high molecular weight DNA treated with DNase I, the rates of autopoly(ADP-ribosylation) for PARP2 and PARP1 were comparable.

Interestingly, that the overall PAR synthesis rate catalyzed by PARP1 and PARP2, when present toge-

ther, was lower than in the case of PARP1 alone (with a few exceptions). Thus, we can hypothesize a competition between PARP1 and PARP2 for DNA *in vitro*. Absence of the precise data on PARP1 and PARP2 amounts in cells does not allow us to make a conclusion about the role of this competition *in vivo*. It should be noted that the ratios of the rates of PAR synthesis catalyzed by PARP1 on activated DNA, over5-, over3-, ds- and nickDNA obtained here and in [5] are in full agreement.

Like PARP1, PARP2 does not show correlation between activation efficiency and  $K_d$  values for DNA. PARP2 was activated the most efficiently in the presence of over5DNA but it displayed higher affinity to gap20- and flap9DNAs. These data show that over5DNA is the most specific activator for PARP2.

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Взаємодія PARP2 зі структурами ДНК, імітуючими  
інтермедіати процесів репарації ДНК

Резюме

Полі(ADP-рибозил)ювання – це тип посттрансляційної модифікації білків, який є важливим для забезпечення стабільності гено-

му та виживання клітин у відповідь на пошкодження ДНК. Полі(ADP-рибозил)ювання каталізується полі(ADP-рибоза)полімеразами (PARP). У той час як роль PARP1 у клітинній відповіді на пошкодження ДНК детально досліджено, внесок іншої ДНК-залежної полі(ADP-рибоза)полімерази – PARP2 – вивчено поки що слабо. **Мета.** Виявити специфічні ДНК-мішені PARP2. **Методи.** Метод «затримки в гелі» (EMSA) і тест активності PARP. **Результати.** Проведено оціночні виміри значень  $K_d$  комплексів PARP2–ДНК для деяких структур ДНК, імітуючих інтермедіати різних процесів метаболізму ДНК, а також ці ДНК проаналізовано як «активатори» PARP1 і PARP2 у синтезі полі(ADP-рибозу). **Висновки.** Як і для PARP1, для PARP2 не спостерігається кореляції між ефективністю активації та значенням  $K_d$  для різних ДНК. Найефективніше PARP2 активується за присутності over5DNA.

Ключові слова: PARP1, PARP2, полі(ADP-рибозил)ювання, зв'язування ДНК.

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Взаимодействие PARP2 со структурами ДНК, имитирующими  
интермедиа́ты процессов репарации ДНК

#### Резюме

Полі(ADP-рибозил)ювання – це тип посттрансляційної модифікації білків, який важливий для забезпечення стабільності геному та клітинної виживаємості в відповідь на пошкодження ДНК. Полі(ADP-рибозил)ювання каталізується полі(ADP-рибоза)полімеразами (PARP). В той час як роль PARP1 в клітинній відповіді на пошкодження ДНК детально досліджена, внесок іншої ДНК-залежної полі(ADP-рибоза)полімерази – PARP2 – вивчено поки що слабо. **Ціль.** Обнайти специфічні ДНК-мішені PARP2. **Методи.** Метод «задержки в гелі» (EMSA) і тест активності PARP. **Результати.** Проведені оціночні виміри значень  $K_d$  комплексів PARP2–ДНК для деяких структур ДНК, імітуючих інтермедіати різних процесів метаболізму ДНК, а також ці ДНК проаналізовані як «активатори» PARP1 і PARP2 в синтезі полі(ADP-рибозу).

**Выводы.** Как и для PARP1, для PARP2 не наблюдается корреляции между эффективностью активации и значением  $K_d$  для различных ДНК. Наиболее эффективно PARP2 активируется в присутствии over5DNA.

Ключевые слова: PARP1, PARP2, поли(ADP-рибозил)ювание, связывание ДНК.

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