DOI 10.18699/SBB-2022-589

PARP2 and BER in the nucleosomal context

Kutuzov M.*, Belousova E., Kurgina T., Ukraintsev A., Vasil'eva I., Khodyreva S., Lavrik O.

Institute of Chemical Biology and Fundamental Medicine, SB RAS, Novosibirsk, Russia
** kutuzov.mm@mail.ru*

Key words: PARP2, PARP1, nucleosome, base excision repair, poly(ADP-ribosyl)ation, APE1, DNA polymerase β, DNA ligase IIIα, XRCC1

Motivation and Aim: The most often existing damage types such as oxidative DNA lesions or spontaneously appeared AP sites are repaired via base excision repair, or BER. This system includes the following main stages: DNA damage recognition, excision of the damaged base, incision of the sugar-phosphate backbone, gap processing (including the incorporation of dNMP) and DNA ligation, which catalysed by specific glycosylases, AP endonuclease 1, DNA polymerase β (Polβ) and DNA ligase IIIα (LigIIIα) in complex with XRCC1 respectively.

In eukaryotic cell the metabolism of poly(ADP-ribose) (PAR) in the nucleus is carried out by nuclear PARPs – PARP1 and PARP2. PARP1 is the most abundant and active PARP; it's strongly activates in response to DNA damage and synthesises most of PAR after genotoxic or oxidative stress. Mice devoid of PARP1 or PARP2 are viable but exhibit sensitivity to genotoxic agents. Nonetheless, double deletion of PARP1 and PARP2 causes early embryonic lethality in mice, indicating that each of these two proteins is involve in DNA repair and can partly compensate for the absence of the other one. As for biochemical data, the protein-protein interaction pattern of PARP1 has been well estimated quantitatively. In particular, it efficient interacts with main repair enzymes, including BER system, and with histone and non-histone chromatin proteins and XRCC1 by itself or through the PAR molecule.

As for PARP2, its role in the repair processes is described to a much lesser extent and the interactions of PARP2 with the BER proteins have not been estimated in details. So, the final function of PARP2 in this process is not clear. Additionally, it is interesting how BER is regulated by the nuclear PARPs and how the roles are distributed between different PARPs.

The most part of these data were obtained using naked DNA substrates, while human genome is presented by the chromatin with nucleosomes as a lowest level of DNA compaction. Our work aimed to obtain a biochemical data set concerning the influence of PARP2 and PARylation in comparison with PARP1 at different stages of BER during the repair process in the context of NCP. Based on all of the data, we proposed a model of BER regulation by PAR in the nucleosomal context.

Methods and Algorithms: To determine the contribution of the interaction of PARPs with DNA or histone components of an NCP in the BER process, we evaluated the affinity of the proteins for native, AP-NCP or gap-NCP by fluorescence measurements and EMSA. Additionally, we evaluated the activation efficacy of both proteins under PARylation. Then, we analyzed the activity of main BER enzymes – APE1, Polβ, LigIIIα in complex with XRCC1, in the presence of PARPs and under PARylation in the nucleosomal contex.

Results: It was found that whereas PARP1 suppresses the activity of APE1, Polβ and to a smaller extent LigIIIα, PARP2 exerts a significant effect on the activity of LigIIIα only. The decreases in the activities of APE1 and Polβ are reversed in the presence of the PARylation catalyzed by PARP1 and to a lesser extent by PARP2. Meanwhile, the complementation with XRCC1 protects the dNMP transferase activity of Polβ from the inhibition by PARP1 and PARP2. Moreover, this complementation slightly affects the intensity of DNA synthesis under the PARylation catalysed by PARP1 and leads to a decrease of Polβ activity in case of PARylation by PARP2, in a NAD+-dependent manner. A remarkable result was obtained in the assay of DNA sealing by LigIIIα. This reaction is stimulated by the PARylation catalysed by PARP2. It is worth mentioning that outward-oriented lesions are more accessible to all the enzymes, and their processing requires a lower enzyme concentration and PAR level. More PAR is needed for the repair of more hidden damage sites.
Conclusion: Therefore, all obtained results and literature data indicate functional dependence of various repair enzymes' activities on PARylation and PAR synthesis and can be described by the following hypothetical model of the regulation of the basal BER process in the absence of massive DNA damaging exposure. DNA damage triggers the activation of a signalling pathway through PARP1. In this process, some regions of compacted chromatin could be converted to a partially de-compacted state. PARylation enables the dissociation of histone and non-histone chromatin proteins and involves specific repair proteins. The main role of PAR at this stage is to loosen chromatin structure and to attract BER proteins such as scaffold protein XRCC1 and possibly some key proteins such as APE1 and others. The cleavage of an AP site by APE1 leads to additional activation of PARP1 and PARylation resulting finally in the dissociation of PARP1. It makes way for subsequent stimulation of the BER process by the removal of APE1 from its product by Polβ. The weight of evidence suggests that the functions of PARP1 and PARP2 probably overlap partially after the AP site cleavage and supports a role of PARP2 in the regulation of BER activity. We believe that the contribution of PARP2 to BER becomes more specific starting from this point and begins to increase. The presence of a specific single-strand break DNA structure as well as PAR synthesised by PARP1 attracts PARP2, which in turn synthesises additional PAR molecules. PAR can regulate the turnover of BER proteins at various stages of the process. PARP1 and PARP2 regulate Polβ activity, whereas PARP2 stimulates LigIIIα activity to complete the repair process, indirectly through their interactions with XRCC1 and PAR. The synthesis of PAR that is catalysed by PARP1 and PARP2 during the interaction with damaged DNA helps the Polβ–XRCC1 complex to complete its action, and PARP2 providing PARylation stimulates the activity of LigIIIα–XRCC1 towards the NCP. The higher PAR level leads to a breakdown of the specific ligase complex, and repair is thus accomplished. We propose that the PAR synthesised at the initial stages by PARP1, and at the final steps also by PARP2, can act as a regulatory factor for the step-by-step progression of the BER process.
Acknowledgements: The study was supported by the Russian Foundation for Basic Research (No. 20-04-00674).