Studies on a Novel DNA Repair Enzyme, Endonuclease Q from Archaea and Bacteria

by

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Abstract

DNA is constantly damaged by endogenous and environmental stress. Base deamination is one of the profound DNA damage in cells. The deamination of cytosine, adenine, and guanine gives rise to uracil, hypoxanthine, and xanthine, respectively. Deamination occurs under physiological conditions and is promoted in single-stranded DNA and at high temperatures. If not repaired, deaminated bases lead to point mutations during DNA synthesis because the base-pairing tendencies of their original bases are converted by deaminations. To maintain genome integrity, all organisms have developed counteracting systems against DNA damage, called DNA repair. However, whereas the repair pathway for uracil has been intensively studied over decades, little is known about the hypoxanthine or xanthine repair. Preliminary experiments conducted in our lab showed that a novel endonuclease activity on hypoxanthine had been detected from the hyperthermophilic archaeon *Pyrococcus furiosus*. In this study, I screened for the protein responsible for the activity and identified the corresponding protein. This newly identified protein bears no homology with any proteins of known function, hence this protein was designated as “endonuclease Q (EndoQ)”. Further biochemical analyses showed that EndoQ from *P. furiosus* cleaves the DNA backbone at the 5′-side of hypoxanthine, and most likely initiates a DNA repair pathway. The enzymatic activity is also exhibited towards uracil, xanthine, apurinic/apyrimidinic site, as well as hypoxanthine. The homolog from *Thermococcus kodakarensis*, belonging to the same order as *P. fuiorsus*, was also characterized as a repair protein possessing the same nuclease activity and substrate specificity. Furthermore, a better understanding of the EndoQ repair pathway was gained by the identification of proliferating cognate nuclear antigen (PCNA,

known as a ubiquitous and essential protein for DNA transactions) as an EndoQ-interacting protein. It was found that EndoQ from *T. kodakarensis* directly interacts with PCNA from *T. kodakarensis* through the consensus interacting motif (PIP box) and the nuclease activity was clearly enhanced by PCNA. Hereby, I propose the PCNA-dependent EndoQ-mediated repair pathway in which PCNA coordinates other interacting proteins and facilitates an efficient DNA repair. Interestingly, the conservation of EndoQ is only limited to the order *Thermococcales* and methanogens in Archaea, and some groups in Bacteria. To investigate the distribution of the functional EndoQ, a putative EndoQ homolog from *Bacillus pumilus* was characterized. This protein was found to exhibit the EndoQ activity, strongly suggesting EndoQ functions as a DNA repair protein in the bacterial domain of life as well. Further phylogenetic analysis showed that EndoQ might have been emerged in some lineage of Bacteria and Arcahea, yet the origin of this protein still remains unclear. Moreover, biochemical characterization of exonuclease III (ExoIII) and EndoQ from the mesophilic archaeon *Methanosarcina acetivorans* revealed that one of their activities is identical to each other. I speculate that whereas these proteins usually work in distinct pathways, they could function complementarily under some stress. Further analysis of DNA repair protein distributions in Archaea underlined the idea of a backup system. Thus, these series of findings provided us with clues to elucidate the mechanism underlying a unique DNA repair system to live in extreme environments and how cells have been evolved to counteract DNA damage.