Poster Presentation

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Sequence-specific DNA glycosylase found in a restriction-modification system

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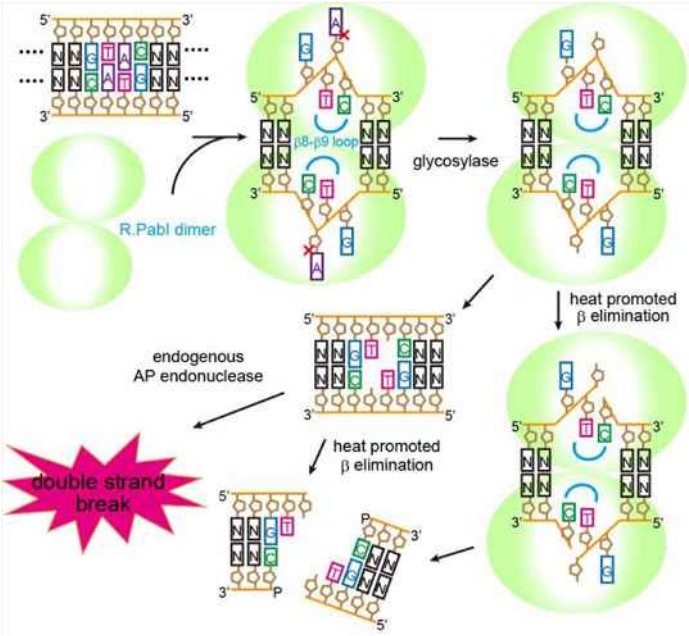
Restriction-modification systems consist of genes that encode a restriction enzyme and a cognate modification methyltransferase. It was believed that restriction enzymes are sequence-specific endonucleases that introduce double-strand breaks at specific sites by catalyzing the cleavages of phosphodiester bonds. R.PabI is a type II restriction enzyme from a hyperthermophilic archaea Pyrococcus abyssi that recognizes 5'-GTAC-3' sequence and cleaves DNA duplexes without the addition of a divalent cation. The structural and mutational analyses of R.PabI in our previous work showed that R.PabI forms a homodimer and has a novel DNA-binding fold called a "half-pipe," which consists of a highly curved anti-parallel β-sheet. Because the structure of R.PabI shares no structural similarity to any other protein, the structural basis of the sequence-recognition and DNA-cleavage mechanisms remained unclear. In this study, we report the crystal structure of R.PabI in complex with a DNA duplex containing the R.PabI recognition sequence. The structure of the R.PabI-DNA complex shows that R.PabI unwinds a DNA duplex at a 5'-GTAC-3' site and flips the guanine and adenine bases out of the DNA helix to recognize the sequence. The electron-density map of the R.PabI-DNA complex shows that R.PabI releases adenine bases from the R.PabI recognition sequence. Biochemical assays using HPLC and MALDI-TOF MS spectrometry also support the observation that R.PabI releases adenine bases by hydrolysis. These results show that R.PabI is not an endonuclease but a sequence-specific adenine DNA glycosylase. R.PabI is the first example of a restriction enzyme that shows DNA glycosylase activity. Mutational analysis reveals the active site of the adenine DNA glycosylase activity of R.PabI. The two opposing apurinic/apyrimidinic (AP) sites generated by R.PabI are cleaved by heat promoted β elimination and/or by endogenous AP endonucleases of host cells to introduce a double-strand break.

[1] *K. Miyazono, M. Watanabe, J. Kosinski, K. Ishikawa, M. Kamo, T. Sawasaki, K. Nagata, JM. Bujnicki, Y. Endo, M. Tanokura, I. Kobayashi, Nucleic Acids Research, 2007, 35, 1908-1918,* ***[2]*** *K. Miyazono, Y. Furuta, M. Watanabe-Matsui, T. Miyakawa, T. Ito, I. Kobayashi, M. Tanokura, Nature Communications, 2014, 5, 3178*



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