論 文 の 内 容 の 要 旨

論文題目 **Function of base-excision restriction enzymes**

（塩基切り出し型制限酵素の機能）

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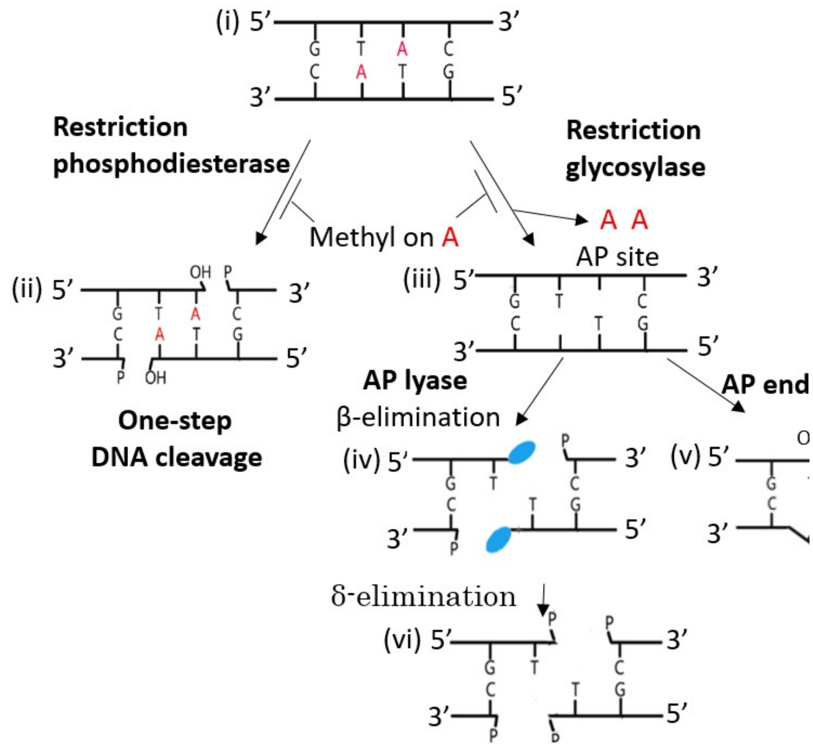
Restriction enzymes cleave DNA judged as non-self based on its epigenetic modification. All the restriction enzymes examined so far were phosphodiesterases that cleave a phosphodiester bond linking the monomer units and generate 3' hydroxyl (3' OH) and 5' phosphate (5' P) ends[1]. Our group identified a restriction enzyme that has a DNA glycosylase activity that excises a base (A) from its recognition sequence (5'GTAC) if it is not methylated. This enzyme, R.PabI[2][3], is from a hyperthermophilic archaeon, *Pyrococcus abyssi*. In the initial report, the enzyme was concluded not to be an endonuclease: the resulting AP site was assumed to be cleaved by heat but not by the enzyme. The following works, however, detected its weak and uncoupled AP lyase activity at a high temperature (> 60°C), which generates a 3'-phospho-α, β-unsaturated aldehyde (3'-PUA) and 5'P end pair and then a 3'P and 5'P end pair. In this work, we



examined role of the AP lyase in restriction *in vivo* using *E. coli*. We purified two R.PabI homologs from mesophilic eubacteria, *Campylobacter coli* (R.CcoLI) and *Helicobacter pylori* (R.HpyAXII). We demonstrated their DNA cleavage, glycosylase and AP lyase activities *in vitro* at 37°C. The AP lyase and glycosylase were more coupled in R.CcoLI than in R.PabI. R.CcoLI as well as R.PabI promoted restriction of incoming bacteriophage/plasmid DNA and chromosomal DNA in *E. coli* at 37°C, although the R.PabI-mediated restriction was promoted by AP endonuclease action *in vivo* or *in vitro*. These results reveal involvement of DNA breakage in restriction and diversity in the DNA breakage mechanism, their intrinsic AP lyase activity or an AP endonuclease. Plasmid transformation assay revealed that the ends generated by R.CcoLI and R.PabI *in vitro* are difficult to repair by ligation *in vivo*, which suggests biological significance of this family. The resulting high toxicity may explain strong avoidance of its recognition sequences in Helicobacter genomes and its only little contribution to the *H. pylori* transcriptome. The difficulty in repairing ends may be useful in targeted DNA inactivation.

1 **R.PabI homologs from mesophilic bacteria show glycosylase and AP lyase activities**

1) Purification of R.PabI homologs, R.CcoLI and R.HpyAXII

R.PabI is from a hyperthermophilic archaeon and shows no detectable AP lyase activity at 37°C, at which *in vivo* restriction can be studied with *E. coli*. We therefore turned to its homologs in mesophilic bacteria, R.CcoLI in *Campylobacter coli* and R.HpyAXII in *Helicobacter pylori*. We synthesized their genes, expressed them in *E.coli* T7 Express *lysY/I^{q}* (in the presence of a Chlorella virus DNA methyltransferase recognizing the same sequence), and purified the enzymes by Ni-NTA Agarose and Heparin HP column.

2) The homologs, R.CcoLI and R.HpyAXII, show cleavage activity at “GTAC” site at 37°C

The homologs, R.CcoLI and R.HpyAXII, show cleavage activity at “GTAC” site at 37°C while R.PabI works at 85°C to cleave at “GTAC”.

3) Glycosylase and AP lyase activities

The homologs R.CcoLI and R.HpyAXII also show sequence-specific glycosylase activity and AP lyase activity. R.CcoLI shows AP lyase activity un-coupled from the glycosylase as PabI but at 37°C. The R.CcoLI-DNA intermediate was captured.

2 R.PabI and its two homologs restrict bacteriophage propagation at 37°C

The restriction enzymes restrict propagation of DNA bacteriophages without proper DNA methylation. I demonstrated that R.PabI and its two mesophilic homologs are active in restriction of infecting bacteriophage lambda at 37°C when present in *Escherichia coli*. Because DNA cleavage activity of R.PabI and its AP lyase activity are weak, if any, at this temperature, this suggests that the base excision activity is responsible for the R.PabI-mediated restriction. This is consistent with previous experiments in which a plasmid DNA was treated by R.PabI and lost transformation ability in the absence of any strand

breakage.

3 R.PabI and its two homologs attack on bacterial chromosome.

Restriction enzymes attack on bacterial chromosomes under specific conditions. For Type II RM, such attack takes place in cells that have lost the rm genes and is called post-segregational killing. The chromosome breakage is repaired by homologous recombination involving RecA and RecBCD proteins. We tried to realize chromosome attack by suppressing expression of the Chlorella methyltransferase gene under arabinose inducible promoter by addition of glucose. The suppression led to decrease of viable cell counts in both R.PabI and R.CcoLI. But for R.PabI, The mutations in two AP endonuclease genes inhibited the killing. This indicates that AP endonucleases promote chromosome breakage and cell killing together with R.PabI.

4 R.PabI Restriction of transforming plasmid

When a plasmid was treated with R.PabI at 37°C, most plasmid molecules remain supercoiled, which means that it has no strand breakage at all, but they lost transforming ability to *E. coli*. This suggests base excision is responsible for restriction[1]. In this work, I found that the mutations in the two AP endonuclease genes of *E. coli* increased their transformation efficiency. This suggests that AP endonucleases promote restriction likely by causing strand breakage at the AP sites on the incoming plasmid.

5 Difficulty in repair by rejoining of the cleavage ends (AP lyase)

The AP lyase products are not rejoined by DNA ligase. Indeed DNA treated with R.PabI was not easily rejoined by DNA ligase different between other typeII restriction endonucleases[1]. In this work, we demonstrated it *in vivo*. The R.PabI and homolog generate the two types of cleavage ends. DNA ligase failed to rescue their biological activity.

Discussion: Biological significance of the toxic restriction enzyme

These results indicate that these restriction glycosylases are toxic to incoming DNA.
An earlier report revealed strong avoidance of their target sites[4]. Hirokazu Yano calculated ratio of the observed number of sites and the expected number of sites for related bacteria by a hiden Markow method. We found intermediate avoidance in Campylobacter and very severe avoidance in Helicobacter. This is compatible with long-term inheritance of pabI family in Helicobacter an in Campylobacter[5]. The site is frequent on rRNA genes, hotspot for homologous recombination in some bacteria. Presumably, the damage there may be easily repaired by recombination. Hirokazu Yano and collaborators compared transcriptome of pabI knockout mutant and parent by RNA-seq method. Only few genes were judged to be diffentially expressed. These include one specificity genes of a Type I RM system. This suggests

communication of PabI RM and this Type I RM systems. It also affected a gene for a membrane protein.

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