The restriction enzyme BanI is inhibited by dcm-methylation of the GGC\textsuperscript{m5}C site

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We constructed a synthetic tRNA\textsuperscript{bys,3} gene that was cloned into pUC9. A BanI site was introduced at the 3' end to allow run-off transcription by T7 RNA polymerase. Surprisingly, we were unable to digest this BanI site, while three BanI sites in the pUC9 plasmid were efficiently cleaved (Figure 1, lane 7). Similar results were obtained upon prolonged incubation at either 37°C or 50°C, which is the optimal temperature for BanI (results not shown).

Since BanI recognizes different sequences (G/GPyPuCC), this could be due to site-preference. However, the BanI site in the tRNA gene was identical to one of the pUC9 sites (GGCGCC).

Inspection of the downstream sequences indicated the presence of an overlapping dcm-methylation site (GGCGCCAGG). Because the plasmid was grown in a dcm+ host (DH5), this will result in C5-methylation of the final C of the BanI site (GGCGC\textsuperscript{m5}C). Next, we grew pUC-tRNA\textsuperscript{bys,3} in a dcm− host (GM48), which resulted in complete digestion of the plasmid (lane 3). These results indicate that the BanI enzyme is not active on GGC\textsuperscript{m5}C sites.

Endonucleases that recognize the same nucleotide sequence can differ in their sensitivity to methylation. Using the dcm-methylated and unmethylated control plasmid, we tested the activity of the restriction enzymes NarI (GG/CGCC) and KasI (GG/GCGCC).

It should be noted that the plasmid contains two sites for these enzymes. Both enzymes digested the methylated GGCGCC\textsuperscript{m5}C sequence (Figure 1, lanes 5 and 6, respectively), as well as the unmethylated control (lanes 1 and 2). A similar result was previously reported for the NarI enzyme (1). Interestingly, a striking different reactivity profile was reported for this same sequence when modified by the HhaI-methylase (2, 3). This sequence, GG\textsuperscript{m5}CGCC, is cleaved by BanI, but not by NarI and KasI. The ability of BanI (GPpPuCC) to recognize both C and T at the third base position may explain these results because T is C5-methylated. Our results indicate that of the three enzymes, BanI is exclusively inhibited by methylation of the sixth base position. Since BanI recognizes a degenerate internal dinucleotide, it is possible that this protein will recognize the flanking nucleotides with enhanced specificity. This may explain the inability of the BanI restriction enzyme to bind and/or cleave the GGC\textsuperscript{m5}C sequence.

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REFERENCES

Figure 1. Restriction enzyme digests were analyzed on a 5% polyacrylamide gel and stained with ethidium bromide. pUC-tRNA, grown in the dcm− host GM48 (lanes 1–3) or the dcm+ host DH5 (lanes 5–7) was digested overnight at 37°C with NarI (N; lanes 1 and 5), KasI (K; lanes 2 and 6) or BanI (B; lanes 3 and 7). A 100-bp ladder is shown in lane 4. Complete NarI or KasI digestion will produce a 175 bp fragment, complete BanI digestion will produce four fragments (of which the smallest two are 175 and 211 bp in length). The inability to recognize the methylated BanI site in the tRNA gene results in the production of a new 386 bp fragment (175 + 211).

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