M.*KpnI* is an adenine-methyltransferase

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The recognition sequence of the *KpnI* restriction-modification system is GGTACC (1). There are conflicting reports in the literature concerning the nucleotide methylated by the *KpnI* methyltransferase. In one paper (2) it was suggested that M.*KpnI* produces m⁴-cytosine. Other investigators obtained indirect evidence suggesting but not proving that it methylates the adenine in the recognition sequence (3). Here we present direct evidence showing that M.*KpnI* is an adenine-methyltransferase.

Ligated *KpnI* linker (approximately 130 pmoles monomer, dGGGTACCC) was methylated in a reaction containing 50 mM Tris-HCl pH 8.0, 7 mM 2-mercaptoethanol, 10 mM EDTA, 4 μM S-adenosyl-L-/methyl-³H/methionine (3 Ci/mmol) and cell-free extract of an *E. coli* clone that carries the cloned *kpnIM* gene (Finta *et al.*, unpublished). The unincorporated S-adenosyl-methionine and buffer components were removed on a Sephadex G-50 column in 0.1% NH₄HCO₃. The peak DNA fraction (18 000 cpm) was evaporated to dryness, then the DNA was dissolved in 10 μl 60% perchloric acid and heated to 100°C for 1 hour in a sealed capillary (4). Perchlorate ions were removed by chromatography on a DEAE-Sephadex A-25 (HCO₃⁻) column, then the hydrolyzed DNA was lyophilized and dissolved in water. DNA bases were analyzed in two thin layer chromatographic systems: Fixion 50×8 cation-exchange thin layer plates (Chinoin) in 0.4 M Na-acetate solvent (4, 5) and silica gel plates with a solvent containing n-butanol/ethanol/water (16:2:5). m⁴-cytosine, m⁵-cytosine and m⁶-adenine were used as standards. In both systems at least 95% of the radioactivity migrated in the m⁶A spot, thus we conclude that M.*KpnI* is an adenine-methyltransferase.

Consistent with this assignment is the observation that we made by testing the digestibility of *SmaI* and *MspI/HpaII* sites that

partially overlap with a methylated *KpnI* site. An 800 bp *NdeI*-*ClaI* fragment encompassing part of the pUC19 polylinker was isolated from pKpn4, a plasmid expressing *KpnI* methyltransferase. In the pUC19-derived region of this fragment there is a *KpnI* site which partially overlaps with a *SmaI* and a *MspI/HpaII* site: GGTACCCGGG. This sequence in the isolated fragment could not be digested by *KpnI* but it was cut by *SmaI*, *MspI* and *HpaII* (not shown). Since m⁵CCCGGG, m⁴CCCGGG and C^{m4}CCGGG methylations are known to block *SmaI* digestion and m⁴CCGG, m⁵CCGG methylations protect against digestion by *HpaII* and *MspI*, respectively (2 and references therein and 6), it must be the adenine of the sequence GGTACC that is modified by M.*KpnI*.

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REFERENCES

1. Tomassini, J., Roychoudhury, R., Wu, R. and Roberts, R.J. (1978) *Nucl. Acids Res.* **5**, 4055–4064.
2. Nelson, M. and McClelland, M. (1989) *Nucl. Acids Res.* **17**, r389–r415.
3. Hammond, A.W., Gerard, G.F. and Chatterjee, D.K. (1990) *Gene* **97**, 97–102.
4. Butkus, V.V., Klimasauskas, S.J. and Janulaitis, A.A. (1985) *Anal. Biochem.* **148**, 194–198.
5. Tomasz, J. (1979) *Chromatographia* **12**, 36–37.
6. Walter, J., Noyer-Weidner, M. and Trautner, T.A. (1990) *EMBO J.* **9**, 1007–1013.

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