Cleavage by *Apa*I is inhibited by overlapping *dcm* methylation

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While preparing restriction fragments of the *rbc* gene of *Rhodospirillum rubrum* cloned in *Escherichia coli*, attempts to cleave a unique *Apa*I site (GGGCCC) repeatedly failed. Upon examination of the DNA sequence [1]

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nt 645      Apa I      nt 665
      5'-GAGGCGGGCCCAGGACGAGACC-3'
              dcm

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I noted that the *Apa*I site overlapped a *dcm* methylation site (CmCAGG) [2]. After preparation of plasmid DNA (pFL23 [3]) from *Dcm*⁺ and *Dcm*⁻ hosts (ED8654 *dcm*⁺ *dam*⁺ *hsdR* [4] and GM31 *dcm*-6 *dam*⁺ *hsd*⁺ [5]), the *Dcm*⁻ DNA cleaved to completion with *Apa*I (Fig. 1; lanes f and g) while the *Dcm*⁺ DNA remained resistant to *Apa*I digestion (Fig. 1; lanes c and d).

Figure 1. The 4.1 kilobasepair *rbc* plasmid pFL23 has unique sites for *Apa*I and *Eco*RI. A double digest should produce 3.0 and 1.1 kbp *Apa*I-*Eco*RI fragments. Enzymes were obtained from New England Biolabs and were used according to the manufacturer's specifications. Legend: a, lambda *Hind*III standards; b,e, *Eco*RI digestion; c,f, *Apa*I digestion; d,g, sequential digestion with *Apa*I and *Eco*RI; b,c,d, *Dcm*⁺ pFL23; e,f,g, *Dcm*⁻ pFL23.

The known restriction modification methylase for *Apa*I methylates the internal cytosine (GGGmCCC) [6]. These data indicate that hemimethylated *Apa*I sites of the form GGGCCmC, arising from overlapping *dcm* methylation, are not cleaved.

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