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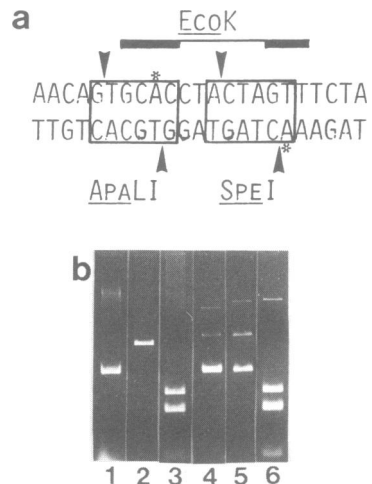
The sensitivity of DNA cleavage by *SpeI* and *ApaLI* to methylation by *M.EcoK*

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During work on site-directed mutagenesis of the human interleukin-2 gene an *EcoK* site (1) was created which overlapped recognition sequences for *SpeI* (1) and *ApaLI* (1) (Fig. 1a). Isolation of the DNA from m_K^+ strain DH1 (2) and m_K^- strain HB101 (3) and subsequent incubation with the two restriction endonucleases revealed that *EcoK* methylation completely or almost completely protected both DNA strands from cleavage by *SpeI*, but did not prevent cleavage of either strand by *ApaLI* (Fig. 1b). Thus, methylation of only one of the 5'-terminal A's of the *SpeI* site is sufficient to protect it against *SpeI*, whereas methylation of one of the two A's of the *ApaLI* sequence does not interfere with its cleavage by *ApaLI*.

Figure 1. a: Overlap of the *EcoK* site with the *ApaLI* and *SpeI* sites as created by site-directed mutagenesis. The *EcoK* heptanucleotide, separated by a 6 nt spacer, is overlined by bars. The A's modified by *M.EcoK* are marked with asterisks. The restriction sites are boxed; potential cleavage positions are indicated by arrows. b: Analysis of cleavage of the sequence shown in fig. 1a by *SpeI* and *ApaLI*. Lanes 1-3: unmethylated DNA, lanes 4-6: methylated DNA. Lanes 1 and 4: intact vector, lanes 2 and 5: vector incubated with *SpeI*, lanes 3 and 6: vector incubated with *ApaLI*.



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