***MscI*, a type II restriction endonuclease from *Micrococcus* species which recognizes 5' TGGCCA 3'**

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MscI, a type II restriction endonuclease, has been isolated from *Micrococcus species* (NEB #502). *MscI*, an isoschizomer of *BalI* (1), recognizes the palindromic hexanucleotide sequence 5' TGGCCA 3'. Like *BalI*, *MscI* cleaves the sequence, TGG/CCA, generating a blunt end.

The single cleavage site of *MscI* on M13mp18 DNA was mapped to approximately position 5095 by analysis against *BglI*, *BsmI* and *DraIII* cleaved M13mp18 DNA (figure I, lanes 2-5). The sequence 5' TGGCCA 3' occurs at position 5080. The number and sizes of the fragments generated by digestion with *MscI* on eight DNA molecules (39 sites) match the computer predicted (2) number and sizes of the fragments that would be generated by cleavage at the sequence 5'TGGCCA3'. *MscI* has the following number of recognition sites on these commonly used DNAs: pUC19 (0), pBR322 (1), phiX174 (0), SV40 (0), M13mp18 (1), T7 (2), Lambda (18) and Adeno2 (17), (figure I, lanes 6-8). From these data we conclude that *MscI* recognizes the sequence 5' TGGCCA 3'. The crude extract contains approximately 1000 units *MscI* per gram of cells.

The cleavage site of *MscI* was determined by cleavage of a primed synthesis reaction (2). Using a DNA fragment known to contain the sequence 5'TGGCCA3' as template with an appropriate primer, the four standard dideoxy sequencing reactions were performed and a fifth reaction was extended through the *MscI* site. The fifth reaction was terminated by heat treatment. *MscI* was added to the fifth reaction. The cleaved product resulted in a single band (figure II, lane -) comigrating with a band in the G channel of the sequencing reactions. The addition of Klenow fragment subsequent to *MscI* digestion resulted in a single band comigrating with the same G residue (fig. II, lane +). These results indicate that *MscI*, exactly like its isoschizomer *BalI*, cleaves after the second Guanine nucleotide within the recognition sequence in the 5' to 3' direction on both strands producing a blunt end.

REFERENCES

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Figure I. *MscI* digests of DNA. Lane 2, uncut M13mp18; lane 3, M13mp18 + *MscI* + *BglI*; lane 4, M13mp18 + *MscI* + *BsmI*; lane 5, M13mp18 + *MscI* + *DraIII*. Lanes 6-8: T7, Lambda and Adeno2, respectively. Markers: lane 1, *BstEII*-Lambda and *MspI*-pBR322; lane 9, *HindIII*-Lambda and *HaeIII*-phiX174.

Figure II.