**RNA Polymerase-Binding and Transcription Initiation Sites Upstream of the Methyl Reductase Operon of Methanococcus vannielii**

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RNA polymerase, purified from Methanococcus vannielii, was shown by exonuclease III footprinting to bind to a 49-base-pair (bp) region of DNA in the intergenic region upstream of mcrB. S1 nuclease protection experiments demonstrated that transcription initiation in vivo occurs within this region at 32 or 33 bp 5' to the ATG translation initiation codon of mcrB and 19 or 20 bp 3' to a TATA box.

Although methanogens are archaeabacteria, the structure and organization of their polypeptide-encoding genes appear to be the same as those in eubacteria. Methanogen genes are often arranged in what seem to be multigene transcriptional units (operons), ribosome-binding sites precede genes, and to date, introns have not been detected (5). However, methanogen-derived DNA-dependent RNA polymerases (RNAP) are very different from eubacterial RNAPs (8, 9), and a major unresolved question is therefore the structure of methanogen RNAP in vitro. We also show that this RNAP-binding site is located 13 bp upstream of the mcrB translation initiation codon for mcrB. When the same procedure was used with the HindIII-PvuII probe (Fig. 1), derived from pMRP4, the 3' boundary of the RNAP-binding site was shown to be 13 bp upstream of the ATG codon. The site of transcription initiation in vivo for the mcrB operon was determined by the standard S1 nuclease protection procedure (4). RNAP was obtained by hot-phenol extraction of exponentially growing cells of M. vannielii (4). The DNA probe was derived from pET400 (Fig. 1). Transcription initiation was found to occur primarily at one of two adjacent bases (C and T) located 33 and 32 bp upstream of the ATG translation initiation codon (Fig. 2). There is therefore a nontranslated leader region containing the sequence previously predicted to be a ribosome-binding site (1, 5).

Our results defined the M. vannielii RNAP-binding site as being 49 bp in length, extending 29 bp 5' and 19 bp 3' from the in vivo site of transcription initiation. A DNA which we have shown to bind RNAP (Fig. 3) is the sequence 5' ATATA, located 19 or 20 bp upstream of the ATG translation initiation codon for mcrB.

There have been several reports cataloging conserved sequences upstream of archaeabacterial genes (2, 5, 6, 11, 12). The sequence 5' TATATA-(19 or 20 bases)-TGC has been proposed as a consensus sequence for promoters of stable RNA genes in both M. vannielii (11) and other archaeabacterial species (6, 12), with transcription initiating in vivo at the G residue of this sequence (11). Within the region of M. vannielii DNA which we have shown to bind M. vannielii RNAP (Fig. 3) is the sequence 5' ATATA, located 19 or 20 bp upstream of the in vivo sites of transcription initiation. A TATA box motif, ~19 bp upstream of the site of transcription initiation, could therefore be a common element of...
FIG. 1. Probes and experimental protocols used. Plasmid pET1400 contains part of the mcrB gene (---) and the intergenic region (---) upstream of the mcrB gene cloned in pUC19 (1, 10). Restriction fragments, 5' end labeled by means of polynucleotide kinase and [γ-32P]ATP, were subsequently cleaved by restriction enzymes as indicated to obtain the single-end-labeled molecules used as probes. Locations of 32P atoms are denoted by asterisks, and the in vivo initiation site and direction of transcription are indicated by the small rightward-pointing arrow. DNA which would be digested by ExoIII activity in the presence of RNAP is also indicated (---). Restriction sites are indicated by the standard abbreviations except for HindIII (HIII), EcoRI (RI), and BamHI (BHI). The sequence of the M. vanneillii DNA shown has been published previously (1). Standard techniques were used for the in vitro DNA manipulations, S1 analysis, and ExoIII footprinting (4, 7, 10, 13).
FIG. 2. ExolII determination of the 5' boundary of the \textit{M. vannielii} RNAP-binding site. RNAP-DNA complexes (+) prepared by the pMRP5-derived BamHI-EcoRI probe (Fig. 1) were digested with ExolII (28 and 56 U of ExolII-reaction mixture), and the products were visualized by autoradiography following electrophoresis through an 8\% polyacrylamide sequencing gel. Labeled probe DNA in control experiments without added RNAP (−) was completely digested by the added ExolII (28 and 56 U). DNA sequencing ladders produced from the probe DNA molecule, resolved in the adjacent tracks, were used to determine the exact size of the RNAP-protected fragment. The in vivo sites of transcription initiation indicated to the left of the figure.

promoters for archaebacterial genes encoding both stable RNA (6, 11, 12) and polypeptides (2, 5). The results presented here show that this conserved sequence is part of a sequence specifically bound by \textit{M. vannielii} RNAP.

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**LITERATURE CITED**


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