A Novel Repetitive Sequence from Mycoplasma hyopneumoniae

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ABSTRACT. We have isolated a novel repetitive DNA element from Mycoplasma hyopneumoniae strain VPP11, the entire structure of which is distinct from those of prokaryotic transposons, insertion sequences or eukaryotic retroelements reported. Southern blot hybridization experiments indicate that at least eight copies of this element locate on the M. hyopneumoniae genome. The size of this repetitive sequence is 4,193 bp, which includes 270- and 272-bp direct long terminal repeats at each terminus. The internal domain of this element defines three open reading frames. —KEY WORDS: Mycoplasma hyopneumoniae, repetitive sequence, transposon-like sequence.


Mycoplasma hyopneumoniae (synonym M. suis pneumoniae) is a causative agent of mycoplasmal pneumonia in swine, which is one of the most important pathogens in swine diseases because it causes the growth retardation and reduction of feeding efficiency. Molecular genetics of M. hyopneumoniae is not well understood. The genome size for M. hyopneumoniae has been estimated to be 1,070–1,140 kbp by pulsed-field gel electrophoresis [4, 10–12]. We have previously isolated a recombinant DNA clone from a genomic DNA library of M. hyopneumoniae strain VPP11, which contains an approximately 5.4-kbp DNA fragment unique to the M. hyopneumoniae species [5]. In the present study we have sequenced the entire 5.4-kbp DNA fragment, and found that this DNA fragment contains a transposon-like element of 4,193 bp which possesses 270-bp and 272-bp direct long terminal repeats (LTR) at the 5' and 3' ends, respectively (Fig. 1). Southern blot hybridization experiments suggest that this element is transposable because at least eight copies of this transposon-like sequence locate on the chromosome (data not shown). The eight copies of this element amount to 33 kbp, or about 3% of the genome for M. hyopneumoniae. The internal domain of this transposon-like element defines three open reading frames (ORFs). The translational capability of ORF was assessed, taking into account that the Mycoplasma species utilize the UGA codon to incorporate tryptophan into the nascent polypeptide [13]. ORF1 codes for 87 amino acids. ORF2 and ORF3 are overlapped, and code for 150 and 590 amino acids, respectively. The nucleotide sequence and deduced amino acid sequence have no homology to those of the authentic transposon [6] or copia elements [1], and are distinct from other repeated sequences reported for swine mycoplasmas so far [2, 7, 8]. Ferrell et al. [2] reported the presence of two copies of a repeated sequence, 1,550 bp in size, flanked by 28-bp inverted repeats, in the M. hyopneumoniae strain J genome, which has no homology to the repetitive sequence shown in Fig. 1.

The repetitive sequence that we found is flanked by direct LTR. These LTR are smaller than bacterial insertion sequences and lack inverted repeated sequences at each terminus. These features of this repetitive sequence are distinct from those of bacterial transposons or insertion sequences. This repetitive sequence resembles the retroelements such as the copia element in eukaryotes in that it possesses direct LTR at each terminus, but it differs from the copia element in following features: (1) The authentic LTR in the copia element is bracketed by 5'-TG and CA-3', but this rule is not observed by the LTR of this repetitive sequence; (2) the primer binding site downstream from the 5'-LTR and the polyurine tract upstream from the 3'-LTR are not evident in this repetitive sequence of M. hyopneumoniae. A hypothetical secondary structure was predicted for the LTR of this repetitive sequence by minimizing the free energy of the overall structure (Fig. 2). The LTR is predicted to form stable stem-loop structures, with free energy of -277.44 kJ/mol calculated by the method of Freier et al. [5]. Although the biological function of these LTR has not been examined, a prokaryotic promoter sequence 5'-TATAAAT-3' is found within the LTR.

The entire structure of this repetitive element is different from those of transposons reported and is totally unique among prokaryotes. Biological significance of this repetitive sequence is currently unknown, but presence of eight copies of this element is remarkable because the mycoplasmas seem to have evolved to minimize their genome size [9].

The nucleotide sequence presented in this paper will appear in the DDBJ, EMBL, GSD, and NCBI nucleotide sequence databases under the accession number D26053.

REFERENCES

Fig. 1. Complete nucleotide sequence of the transposon-like repetitive element in M. hypogea. LTR: long terminal repeat. Small boxes: ATGs of the three ORFs. Underlined: stop codons of the three ORFs.

Fig. 2. Hypothetical structure of the 5′-LTR flanking the repetitive sequence of M. hypogea. Minimum free energy of the overall structure is calculated to be -277.44 KJ (1 cal = 4.184 J)/mol.