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REGULATION OF TRANSCRIPTION OF CORONAVIRUSES

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ABSTRACT

To study factors involved in regulation of transcription of coronaviruses, we constructed defective interfering (DI) RNAs containing sg RNA promoters at multiple positions. Analysis of the amounts of sg DI RNA produced by these DIs resulted in the following observations: (i) a downstream promoter downregulates an upstream promoter; (ii) an upstream promoter has little or no effect on the activity of a downstream promoter. Our data suggest that attenuation of upstream promoter activities by downstream promoter sequences plays an important role in regulating the amounts of sg RNAs produced by coronaviruses. Our observations are in accordance with the models proposed by Konings et al. (8) and Sawicki and Sawicki (16).

Coronaviruses produce a 3′-coterminal nested set of subgenomic (sg) mRNAs. All sg mRNAs contain a common leader sequence derived from the 5′ end of the genome. For mouse hepatitis virus MHV this leader sequence is 72 nucleotides (nt) in length (20). The joining of the 5′ leader RNA to the mRNA is believed to be a discontinuous transcription process (10,19), since the results of UV transcription mapping argue against RNA splicing (5,23). On the genome the transcription units for the mRNAs are preceded by the intergenic sequence (IS) (10,19). For MHV every IS contains a sequence element related to the consensus 5′ AAUCUAAAC 3′ (2,8,18). These IS elements function, on the negative stranded RNA template, as promoters for sg mRNA synthesis (14,22). On the negative strand the IS promoter elements are called intergenic promoter sequence (IPS).

The mechanism of coronavirus sg mRNA synthesis is a subject of considerable debate. In earlier experiments only genome length negative strands were found (11) and it was believed that genomic negative strands were the exclusive templates for the synthesis of sg mRNAs. To explain the synthesis of leader containing sg mRNAs, it has been proposed that short leader RNA species act as primers (9,20). According to this leader-primed

Corona- and Related Viruses, Edited by P. J. Talbot and G. A. Levy
Plenum Press, New York, 1995

transcription model, the leader RNAs are transcribed from the 3′ end of the genome, translocated to the several IPSs on the negative stranded template and then extended to form leader containing sg RNAs. The key observation that supports the priming of the leader during transcription initiation is the fact that the leader RNA includes an IS that allows base pairing between the 3′ end of the leader and the IPS (2).

The discovery in recent years of sg negative strands (4,6,16,17) has had consequences for the leader primed transcription model. The sg negative strands seem to be involved actively in the synthesis of sg mRNAs (3,16), although it has been argued that they are merely dead-end products synthesized from the sg mRNAs (6). Additional models have now been proposed for coronavirus transcription, in which the sg mRNAs are transcribed from sg negative stranded templates.

Sethna et al. (17) speculated that sg mRNAs produced in the classic leader-primed fashion, are amplified from negative stranded counterparts as replicons. However, to date all attempts to obtain direct evidence for mRNA replication have failed. Transfecting synthetic mRNAs into coronavirus infected cells did not result in replication of the sg RNA (1,12,14). However, it could well be that transfected sg RNAs are not suitable templates for replication.

Sawicki and Sawicki (16) proposed an alternative model. They suggested that sg negative strands are synthesized first to serve as templates for the synthesis of the corresponding mRNA and not vice versa. In this model transcription should be regulated on the level of negative strand synthesis.

Many of the basic features of coronavirus transcription are unclear because of the lack of an appropriate experimental system. Recently, it has been shown that full length cDNA clones of defective interfering (DI) RNAs can be used to study MHV mRNA transcription (7,13-16,22). Inserting an IS into the genome of a synthetic MHV DI-RNA and transfecting this DI-RNA into MHV infected cells gives rise to a DI derived sg RNA. To study transcription, we use a DI RNA vector based on a full length cDNA clone of a natural occuring 5.5 kb DI RNA of MHV-A59, pMIDIC (21,22).

Coronavirus mRNAs are, in general, synthesized in amounts that are inversely related to their size. Previously, we have proposed that the generation of this gradient of sg mRNA arizes because larger RNA molecules are more prone to premature transcription termination and therefore produced less abundantly than smaller RNAs (8). There are two stages in which transcription termination can occur. In one case transcription initiation events on downstream promoters on the negative strands are attenuating factors for positive strand synthesis (8). Alternatively, premature termination could occur during negative strand synthesis (16). This is based on the model in which a nested set of sg negative strands is synthesized first. In this case larger negative strands are produced in lower quantities because they encounter more transcription attenuating antipromoters on the positive strand during their synthesis then smaller ones.

To test the hypothesis of attenuation we inserted wildtype (wt) as well as mutant sg RNA3 promoters at different positions of our DI-RNA vector (Fig. 1). The mutant RNA3 promoter is inactive due to a single point mutation. DI-RNAs containing the wt and the mutant promoters replicated efficiently and produced sg DI-RNAs of the expected length. The DI-RNA constructs containing a wt RNA3 promoter at position A or C (Fig. 1), produced equal amounts of sg DI-RNA. However, analysis of the sg RNA synthesis of the DI-RNA containing wt promoters at positions A and C showed a difference in promoter activities. The activity of the promoter at position A was reduced by the presence of the wt promoter at position C, while the activity of the promoter at position C remained the same. The presence of an additional wt promoter at position B, downstream of A (Fig. 1), reduced the activity of the wt promoter at position A to an almost undetectable level. The activity of the promoter at position C was not affected by the presence of an additional upstream promoter.

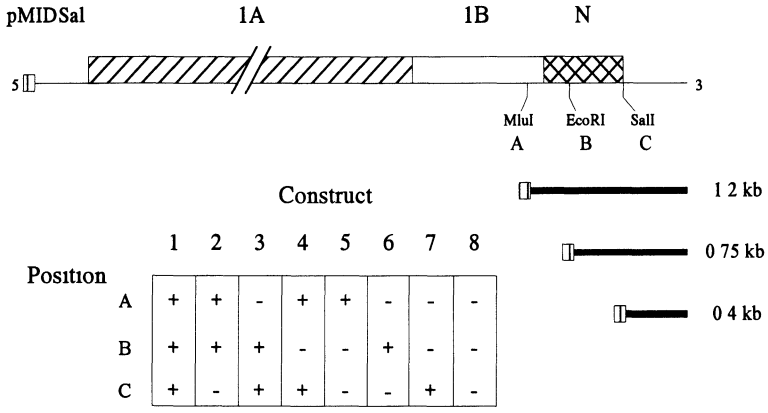

Figure 1. Schematic representation of the constructs containing RNA3 promoters the *Mlu*I, *Eco*RI and *Sal*I site of pMIDSal (positions A, B and C on the DI RNA) The black bars represent the subgenomic DI RNAs produced by the DIs The table shows which constructs contain which combinations of wildtype and mutant RNA3 promoters (+ = wildtype (UAAUCUAAAC), - = mutant (UAAUGUAAAC))

From these data we concluded that a downstream promoter attenuates the amounts of sg RNA generated by upstream promoter and not vice versa Our observations are in agreement with the models of Konings et al (8) and Sawicki and Sawicki (16) However, our data can not discriminate between attenuation during positive or negative strand synthesis We also observed that a wt RNA3 promoter at position B gave rise to more sg DI-RNA then at positions A and C Even in the presence of wt promoters at positions A and C, the sg DI-RNA produced by the promoter at position B is the most abundant This suggests that there are additional factors that regulate the accumulation of sg RNAs It could be possible that the polymerase initiates or terminates (depending on the model one prefers) transcription more efficiently at the promoter at position B Protein binding domains or the RNA secondary structure could play a role in this preference for the promoter at position B This could explain why for the coronaviruses TGEV and FIPV the smallest sg mRNA is not the most abundant as the gradient would predict Nevertheless, our data suggest that attenuation of promoter activities by promoter sequences is important in regulating the amounts of sg mRNAs of coronaviruses

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