TEMPORAL REGULATION OF RNA SYNTHESIS OF BOVINE CORONAVIRUS

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It has been shown with mouse hepatitis virus (MHV) and avian infectious bronchitis virus that all of the subgenomic mRNAs and genomic RNA are synthesized at the same rate throughout the viral replication cycle (Leibowitz et al., 1981; Stern and Kennedy, 1980). The viral genomic RNA gradually accumulates late in infection. However, no clear switching from transcription of subgenomic mRNAs to replication of genomic-sized RNA is seen. Thus, it is not known whether there is a temporal regulation of viral RNA synthesis in coronavirus-infected cells.

Since the replication cycle of MHV is approximately 12 hours, examination of temporal regulation of RNA synthesis is difficult. We therefore utilized bovine coronavirus (BCV) which has a growth cycle of 48 to 72 hours, to study the kinetics of viral macromolecular synthesis. Our studies indicate that there is a differential regulation of transcription and replication of coronavirus RNA.

BCV-specific intracellular RNA was studied in infected, actinomycin D-treated Bovine Embryonic Kidney (BEK) cells. A genome-sized RNA and seven subgenomic RNAs with molecular weights of 3.3×10^6 , 3.1×10^6 , 2.65×10^6 , 1.08×10^6 , 0.99×10^6 , 0.84×10^6 and 0.56×10^6 were detected on 1% agarose gels after labeling intracellular RNA from 2-4 hours post-infection with ³²P-orthophosphate (Fig. 1). Comparisons of BCV with the JHM strain of MHV revealed the presence of a new RNA, 2a, of 3.1×10^6 in BCV-infected cells. In addition, BCV RNAs 4 and 5 are smaller than the corresponding RNAs of MHV. The RNA 2a has a capacity to encode a polypeptide of approximately 430 amino acids based on the nested set structure of mRNAs, which was demonstrated by Northern blot using a BCV-specific 3'-end clone (data not shown).

Kinetic studies by pulse-labeling RNA at two-hour intervals demonstrated that the synthesis of subgenomic mRNAs and genomic RNA are differentially regulated (Fig. 2). At four to eight hours post-infection (p.i.), genomic size RNA accounts for approximately 7% of the total intracellular viral RNA. Later in infection, 70 to 72 hours p.i., this RNA accounts for 88% of the total RNA with a corresponding decrease in the synthesis of subgenomic RNAs. Immunoprecipitations of ³⁵S-methionine pulse-labeled viral proteins during the same time periods demonstrated that viral protein synthesis occurs early in the infection at the same time as the peak of viral subgenomic RNA synthesis. Western blot analysis suggests that these proteins are stable since the proteins

Figure 1. Comparison of ^{32}P -labeled BCV and JHM intracellular viral RNA. A) BCV; B) JHM.

Figure 2. Kinetics of ^{32}P -labeled intracellular BCV RNA synthesis. The numbers on top of each lane indicate the hours p.i. when the cells were labeled.

are present in high level as late as 70 to 72 hours p.i. The kinetics of production of viral particles coincides with the synthesis of genomic RNA. These studies thus indicate that there is a differential temporal regulation of the synthesis of genomic RNA and subgenomic mRNAs, and that the synthesis of genomic RNA is the rate-limiting step regulating the production of viral particles.

ACKNOWLEDGMENTS

We thank Carol Flores for typing the manuscript. This work was supported by the U.S. Public Health Research Grants A1 19244 and NS 18146 and National Science Foundation Grant DMB-9332.

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