Sequences Required for Replication and Packaging of IBV RNA

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1. INTRODUCTION

We have used a naturally occurring IBV defective RNA (D-RNA), CD-91 (9.1 kb; Penzes et al., 1994) to investigate IBV replication and packaging signals. This D-RNA comprised the 5'-most 1133 nucleotides (domain I, including the 528 nucleotide 5' untranslated region, UTR) and 3'-most 1626 nucleotides (domain III) of the genome and a middle section (domain II) comprising discontinuous parts (6322 nucleotides in total) of the 1b open reading frame (ORF) of gene 1, the polymerase gene. In a limited study (Penzes et al., 1996) 3 kb of the ORF 1b sequence was removed to produce CD-61 which was replicated and packaged as efficiently as CD-91. Removal of a further 1.4 kb from ORF 1b produced D-RNA CD-44 which was not rescued (replicated and packaged; detected by Northern blot analysis) by helper IBV. This led us to propose that the 1.4 kb sequence was essential either for replication or, more likely, for packaging of the RNA. We have extended that investigation to an additional 26 modified D-RNAs. Previously we relied upon several passages of the rescued D-RNAs to produce sufficient RNA to be detectable by Northern blot analysis. The incorporation of a chloramphenicol acetyltransferase (CAT) reporter gene into the D-RNAs (Stirrups et al., 2000b) has allowed us to detect replication

The Nidoviruses (Coronaviruses and Arteriviruses).
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of IBV D-RNA constructs in transfected cells, without reliance on packaging to indicate that replication had occurred. Thus we have been able to distinguish the processes of replication and packaging.

2. METHODS

A CAT reporter gene was inserted at one of two positions in CD-61, under the control of the transcription associated sequence of the IBV gene 5 (Stirrups et al., 2000b). D-RNAs were electroporated into IBV-Beaudette-infected cells (passage 0; Stirrups et al., 2000a). CAT (measured by an ELISA) in P0 was indicative of *replication* of a D-RNA. The progeny of P0 were passaged up to five times (P1-P5). CAT or Northern blot detection showed that a D-RNA had been *rescued* – indicative of *packaging*. Deletion mutants of CD-61 (± CAT) were mostly made using existing restriction sites.

3. RESULTS

Only a few of the constructs are illustrated here. A D-RNA with the first 544, but not as few as 338, nucleotides of the 5'-terminus was replicated (Figure 1).

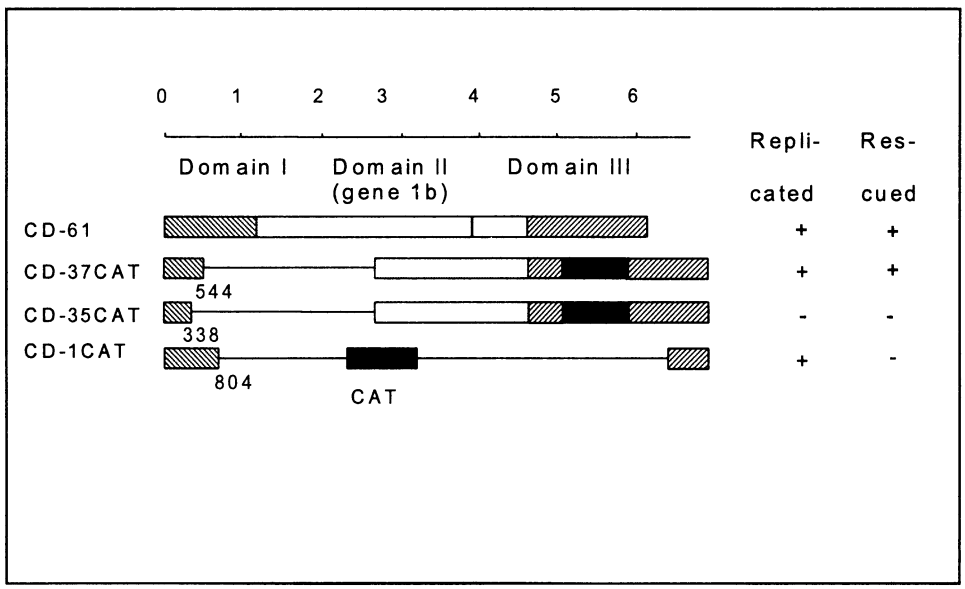

Figure 1. 5' sequences required for replication and packaging.

Region I of the 3' UTR, adjacent to the N gene, comprised 212 nucleotides and could be removed without impairment of replication or rescue of D-RNAs. A D-RNA with the final 338 nucleotides (CD-38CATstem+), including the 293 nucleotides in the highly conserved region II of the 3' UTR, was replicated (Figure 2). Thus the 5' terminal 544 and 3' terminal 338 nucleotides contained the necessary signals for RNA replication.

Phylogenetic analysis of 19 strains of IBV identified a conserved stem-loop at the 5' end of region II. Removal of the predicted stem loop (CD-38CATstem-) abolished replication (Figure 2).

D-RNAs in which the polymerase gene 1b sequence had been removed or replaced with all of the downstream genes were replicated well but rescued poorly, suggesting inefficient packaging. However, no *specific* part of the 1b gene was required for packaging.

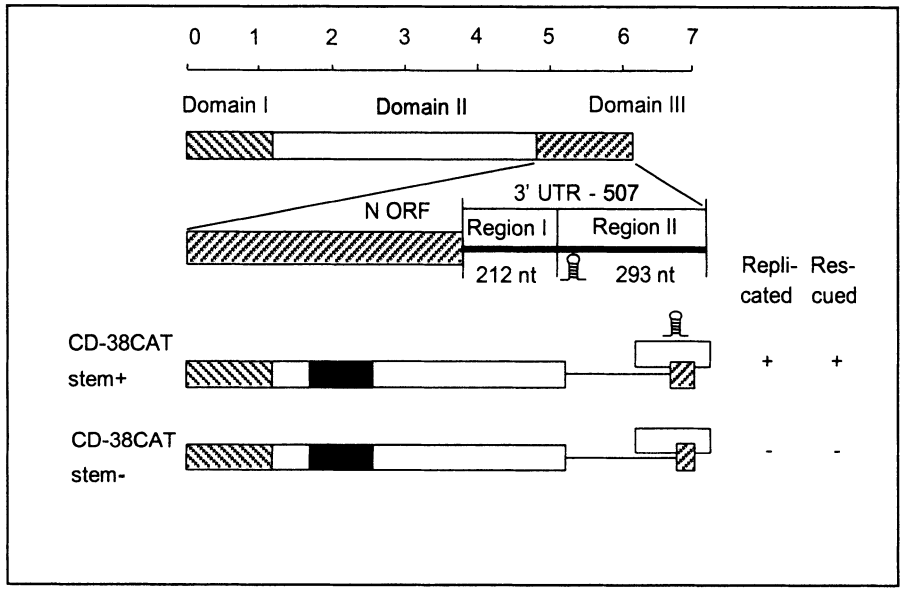

Figure 2. 3' sequences required for replication and packaging

4. DISCUSSION

The 5' UTR and region II of the 3' UTR contained the signals necessary for RNA replication; further deletions were lethal. Some gene 1b sequence, but not a specific part, was required for efficient packaging.

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