GENES 3 AND 5 OF INFECTIOUS BRONCHITIS VIRUS ARE ACCESSORY PROTEIN GENES

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1. INTRODUCTION

Avian infectious bronchitis virus (IBV), a group 3 member of the genus *Coronavirus*, is a highly infectious pathogen of domestic fowl that replicates primarily in the respiratory tract but also in epithelial cells of the gut, kidney, and oviduct.[1-3] Interspersed amongst the IBV structural protein genes are two genes, 3 and 5 (Fig. 1),[4] whose role is unknown.[5-7] Gene 3 is functionally tricistronic,[8] expressing three proteins, 3a, 3b, and 3c, the latter being the structural E protein of IBV.[9] Expression studies have indicated that translation of the E protein is initiated as a result of ribosomes binding to a structure formed by the preceding 3a and 3b sequences.[10, 11] Gene 5 is functionally bicistronic and expresses two proteins, 5a and 5b, which are expressed in IBV-infected cells.[12] To investigate the requirement for the 3a, 3b, 5a, and 5b proteins for replication, we have used our reverse genetic system[13-17] to produce isogenic recombinant IBVs (rIBVs), after site-specific mutagenesis of the appropriate sequences, with specific modifications in genes 3 and 5.

2. MATERIALS AND METHODS

2.1. Modification of IBV cDNAs by PCR Mutagenesis

Overlapping PCR mutagenesis was used to scramble the initiation codons of 3a, 3b, 5b, delete the 3ab coding sequences and introduce a *Kpn*I restriction endonuclease upstream of the gene 5 TAS. The scrambled gene 5 TASs and scrambled 5a initiation codon were introduced using adapters to replace the 45 bp *Kpn*I-*Spe*I fragment. The modified sequences comprising two scrambled initiation codons ScAUG3ab and ScAUG5ab were generated from sequences containing a singly scrambled ATG. The modified cDNAs are shown in Fig. 2.

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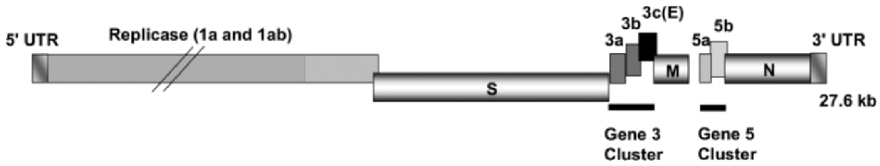

Figure 1. Schematic diagram of the IBV genome indicating the positions of genes 3 and 5.

2.2. Generation of rVVs with Modified IBV Full-Length cDNAs

Our IBV reverse genetics system is based on the use of vaccinia virus (VV) as a vector for the IBV full-length cDNA.[14] Recombinant VVs (rVV) containing the gene 3 and 5 modified cDNA sequences were generated by transient dominant selection (TDS)[18] using the *Eco gpt* (GPT) gene as the transient selectable marker.[13, 16] The modified IBV cDNAs were inserted into the Beaudette sequences in vNotI/IBV_{FL} as a result of homologous recombination and selection of rVVs expressing GPT in the presence of mycophenolic acid (MPA). MPA-sensitive vaccinia viruses, potentially containing the modified IBV cDNAs, were then generated from the MPA-resistant vaccinia viruses after the spontaneous loss of the GPT gene by three rounds of plaque purification in the absence of MPA.[13, 16] Two rVVs, representing each modification, identified by PCR amplification and sequence analysis, were isolated after two independent TDSs.

2.3. Recovery of Recombinant IBVs

Recombinant IBVs, containing each of the modified gene 3 and 5 sequences, were recovered from DNA isolated from the rVVs as shown in Fig. 3 and described in Refs.[13–16.] Recombinant IBVs were characterized and used for subsequent experiments after three passages in CK cells. Two independent clones of each rIBV were rescued from each of the two rVV DNAs, except for rIBVs ScAUG3b and ScAUG3ab, for which only one rIBV was recovered.

2.4. Growth Kinetics of rIBV

The growth kinetics of the rIBVs were analyzed on chick kidney (CK) cells, and the amounts of progeny virus produced, at specific time points, were determined by plaque titration in CK cells and compared with those produced from Beau-R.

3. RESULTS AND DISCUSSION

IBV and the coronaviruses isolated from other avian species, turkey,[19] pheasant,[20] peafowl (accession no. AY641576) and partridge (accession no. AY646283), all contain a tricistronic gene 3 and a bicistronic gene 5, the latter located between the M and N genes. The conservation of the gene 3 and 5 sequences in IBV and IBV-like viruses, isolated from other avian species, indicate they may play a role in the virus replication cycle. In order to determine whether the 3a, 3b, 5a, and 5b proteins are required for the replication of IBV, we have used a number of alternative ways to oblate the expression

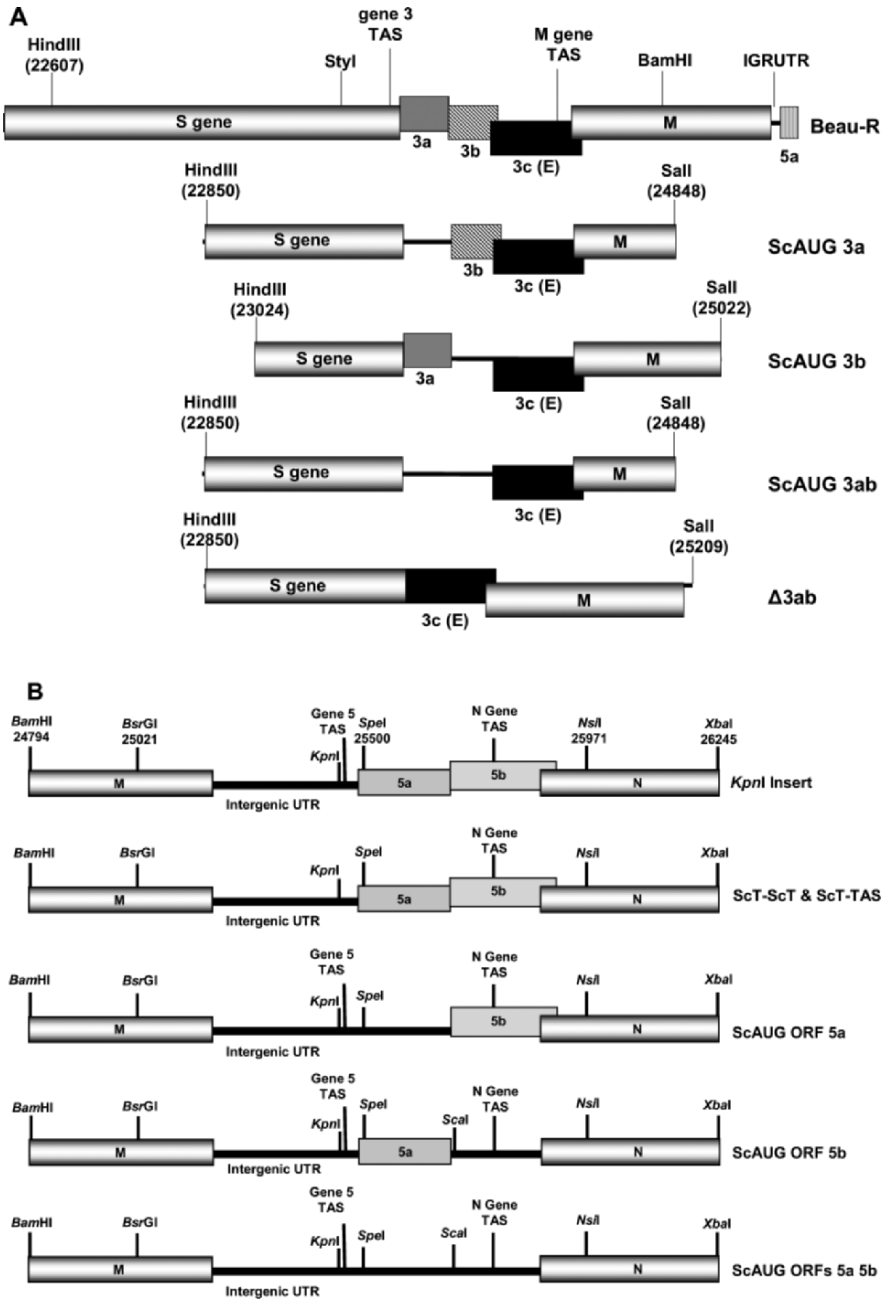

Figure 2. Summary of the modified (A) gene 3 and (B) gene 5 sequences. The positions of the IBV genes are shown with the horizontal black lines indicating that the coding sequences are retained but that translation of the gene product is lost. ScAUG-scrambled initiation codon. ScT-scrambled transcription associated sequence.

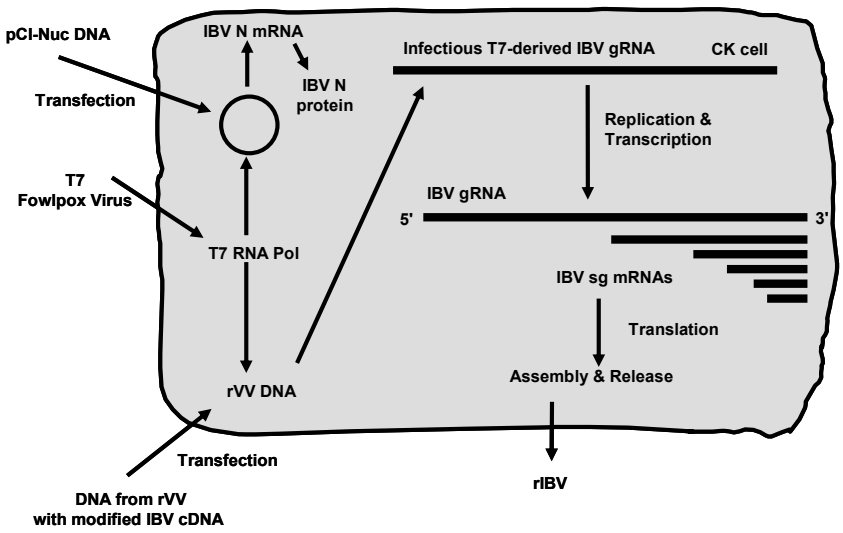

Figure 3. Schematic diagram representing the recovery of rIBV from DNA isolated from a rVV containing a full-length IBV cDNA under the control of a T7 promoter. A plasmid expressing the IBV nucleoprotein is required for successful rescue of IBV. The infectious IBV RNA is generated using T7 RNA polymerase expressed from fowlpox virus.

of these gene products. We modified the IBV genome corresponding to gene 3 by scrambling the 3a and 3b initiation codons, either singly or together, and by deleting the sequence corresponding to 3a3b. We have shown that 3a is no longer produced after scrambling of the AUG or deletion of the sequence (Unpublished data, Hodgson *et al.*). We modified gene 5 by scrambling the 5a and 5b initiation codons, either singly or together, and by scrambling the sg mRNA 5 TAS preventing expression of the sg mRNA. We have shown that sg mRNA 5 is no longer produced after scrambling of the TAS and that 5b is no longer produced after scrambling of the AUG.[16] Comparison of the growth kinetics of the rIBV with Beau-R, on CK cells, showed that there were no differences (Fig. 4), demonstrating that neither the IBV 3a, 3b, 5a, nor 5b proteins are essential for replication *per se*; they can be considered to be accessory proteins. We have rescued a rIBV that lacks expression of 3a and 3b, after deletion of their sequences, and lacks expression of 5a and 5b after scrambling of the gene 5 TAS indicating that both sets of gene products are dispensable *in vitro*.

4. ACKNOWLEDGMENTS

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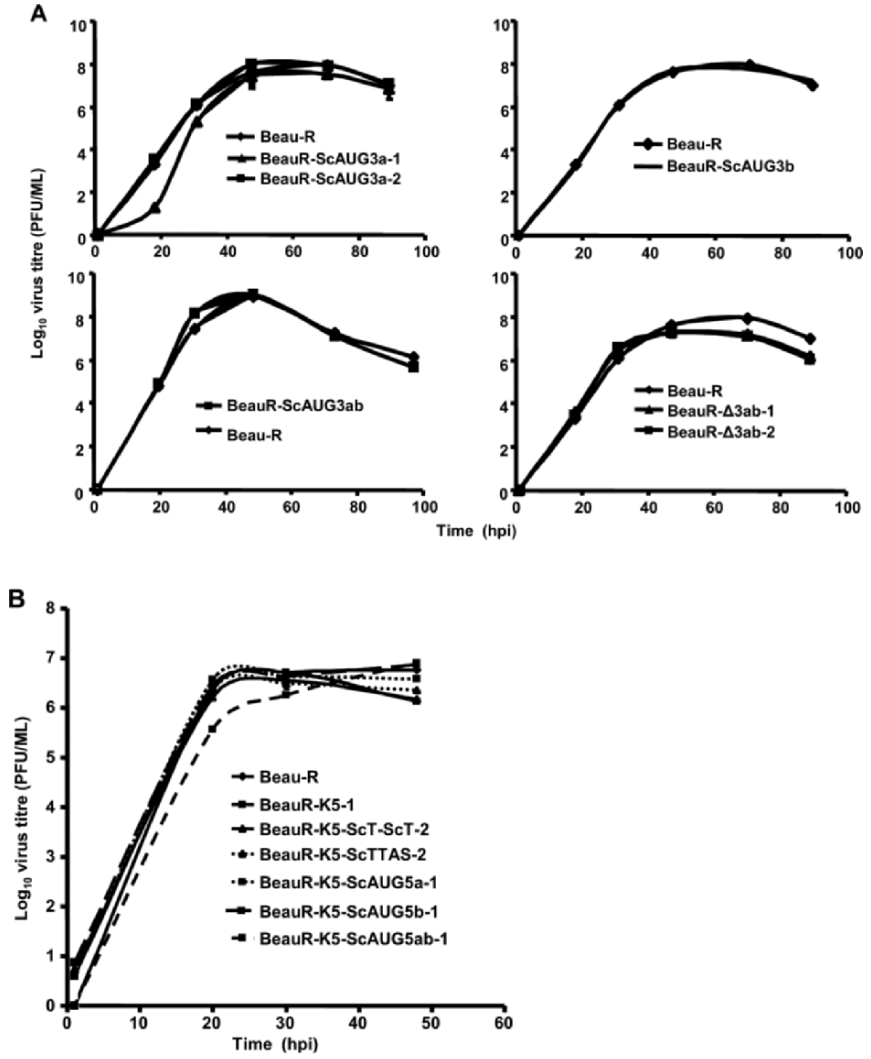

Figure 4. Comparison of the multistep growth kinetics of the (A) gene 3 and (B) gene 5 modified rIBVs on CK cells.

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