RT-PCR and subcloning of MV and CDV envelope glycoproteins

In all cases, total RNA was prepared using the RNeasy Mini Kit (QIAGEN). Reverse transcription through Superscript II Reverse Transcriptase (Invitrogen) was initiated with random hexamer primers. Genome fragments containing the glycoprotein genes were then further amplified using TaqHiFi DNA polymerase (Invitrogen) and primers 5-gtccaccatgggtctcaaggtgaacgtctc and 5-CAAACAGAACAGCCAGCA (for MV F amplification), 5-ACAGCCGAAGCCCCA TGCAC and 5-CTTCAGAGTGATCTTAC ATAGG (for CDV F amplification), or 5-ATGCTCTCCTACCAAGACAAGG and 5-TCAAGGTTTTGAACGGTTACATGAGA ATC (for CDV H amplification)

For MV F subcloning, the 1736 bp *Hpa*I/*Pac*I F-containing fragment was replaced in pCG-F with the different equally digested cDNAs. For CDV F subcloning, recombination PCR was employed using the subcloned CDV F genes and a positive-strand cDNA copy of the MV-Edm genome (Radecke, F., Spielhofer, P., Schneider, H., Kaelin, K., Huber, M., Dotsch, C., Christiansen, G., and Billeter, M. A. (1995) *Embo J* **14**(23), 5773-5784) as templates to independently generate 5'- and 3'-fragments of CDV F flanked by MV non-coding sequences, which were subcloned into TOPO 2.1 vectors (primers for 5'-fragments: 5-GAATCAAGACTCATCCAATGCACA AGAGAATCCCC and 5-TATAGACGC ACAATTTGCGACG (for CDV), and 5-CACTTGGTGGGATAGG and 5-GTGCAT TGGATGAGTCTTGATTCTGGG (for MV); for 3'-fragments: 5-ATTGAATTCAG TCCTCGAACC and 5-TCAGAGTGATCT TACATAGG (for CDV), and 5-CCTATG TAAGATCACTCTGATCCTCTACAACT CTTGAAACAC and 5-CAAACAGAACA GCCAGCA (for MV)). Final constructs were then assembled through transfer of *Nar*I/*Pvu*II 5'- and *Pvu*II/*Pac*I 3'-fragments into the pCG-F backbone opened with *Nar*I/*Pac*I. For CDV H subcloning, MV-flanking sequences were introduced through PCR with primers 5-TTAATTAAA ACTTAGGGTGCAAGATCATCGATAAT GCTCTCCTACCAAGACAAGG and 5-ACTAGTGGGTATGCCTGATGTCTGGG TGACATCATGAGATTGGTTCACTAGC AGCCTCAAGGTTTTGAACGGTTACAT GAGAATC. Amplicons were digested with *Pac*I/*Spe*I and ligated with the pCG-H backbone opened with *Pac*I/*Spe*I.

Generation of chimeric constructs and mutagenesis

A *Bgl*II site was generated in CDV F with primers 5- GTGTCTCCAAAGGA GCGAGATCTCAGATCGAAAGGCGGC AACCC for F-Lederle and 5-GTGTCTCAAAAGGAGCGAGATCTCAG ATCGAAAGGCGGCAACCC for F-ODP. A *Kpn*I site was generated with primers 5-GGAATAATTTGTCAACTATTGGGATT ATTGGTACCGATAGTGTCCATTATAA GATCATGGC for F-Lederle and 5-GGAATAATTTGTCAACTATTGGGATT ATCGGTACCGATAGTGTCCATTACAA GATCATGAC for F-ODP.

To generate MV/CDV H chimeras, recombination PCR was employed. For MV H (1-138 CDV), primers were 5-TTAATTAAAACTTAGGGTGCAAGATC ATCGATAATGCTCTCCTACCAAGACA AGG and 5-GCGGGTTGATACAC CAGTGGAGATCGCGGAAATCG (for CDV), and 5-CTCCACTGGTGTA TCAACCCGCCAGAGAG and 5-CTGTAAGCGTGAGGGAC (for MV). For construct MV H (1-90 CDV), primers were 5-TTAATTAAAACTTAGGGTGCAAGAT CATCGATAATGCTCTCCTACCAAGAC AAGG and 5-GTGGTGTCAGCACGTC TATGACTTGATGATGTACGGCCTC (for CDV) and 5-GTCATAGACGTGCT GACACCACTCTTC and 5-CTGTAAG CGTGAGGGAC (for MV). For construct MV H (98-138 CDV), primers were 5-GCATCAGGTCAAGGATGTCTTGACAC CGCTCTTC and 5-CTGTAAGCGTG AGGGAC (template MV H (1-138 CDV)), and 5-CTTGCACAGAATTGACC and 5-CAAGACATCCTTGACCTGATGCTCGA TTGAG (for MV). All recombinant amplicons were sequence-confirmed and transferred into pCG-H with *Pac*I/*Nsi*I.