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Processing of envelope protein E^{rns} of bovine viral diarrhea virus: further characterization and identification of a stable E^{rns}-E1 precursor protein

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Bovine viral diarrhea virus (BVDV) belongs to the genus *Pestivirus* within the family *Flaviviridae*. The pestiviral RNA encodes a polyprotein which is co- and posttranslationally cleaved by cellular and viral proteases. The proposed processing scheme of the structural proteins of BVDV does not explain the processing between the glycoproteins E^{rns} and E1. In this study, bicistronic constructs with a deletion in the E^{rns}-encoding region expressing BVDV structural proteins from a synthetic open reading frame under control of a heterologous IRES element were developed on the basis of the infectious cDNA clone of BVDV strain CP7. Bovine cells transfected with the recombinant RNAs were characterized by immunofluorescence and western blot experiments. Furthermore, all structural protein sequences were cloned into the pCITE2a vector. With a new E1-specific antiserum an E^{rns}-E1 precursor protein could be identified and characterized. The biosynthesis of this E^{rns}-E1 precursor protein was studied by pulse-labeling of transfected cells with [^{35}S]methionine-[^{35}S]cysteine and radioimmunoprecipitation. After site-directed mutagenesis of putative cleavage sites and treatment with several protease inhibitors, the cleavage of the precursor protein was further analyzed in western blot experiments. With bicistronic and full-length mutants it could be shown that cleavage of the E^{rns}-E1 precursor protein is essential for the generation of infectious virus progeny, whereas the precursor protein itself is not essential but beneficial for the generation of infectious virus progeny.

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