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Conserved regions of pestivirus E2 are essential for the formation of an E1-stabilizing E1-E2 homodimer and subsequently for virus assembly and egress

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Little is known about the interaction of the viral envelope proteins of Pestiviruses required for the assembly of infectious virus particles. Here, we analysed the role of two highly conserved regions within BVDV envelope protein E2 for assembly and egress: (1) CXXC, a disulfide isomerase motif and (2) a putative fusion peptide region. Single or multiple amino acids within the conserved regions were substituted or deleted in an infectious cDNA clone, and the putative fusion peptide encoding region was also exchanged with related sequences of tick born encephalitis virus (TBEV). The deletion or substitution of highly conserved amino acids (e.g. glycin_828 or tryptophan_899) completely abrogated the generation of virus progeny without influencing autonomous RNA replication. Mutants with substituted TBEV sequences did also not result in infectious virus progeny without impairing RNA replication. Western blot analysis demonstrated that all mutants failing to produce infectious progeny were unable to form detectable E1-E2 heterodimers as well as E2-homodimers. Furthermore, E1 was de-stabilized in those mutants. Real-time RT-PCR as well as electron microscopic analyses of cells transfected with the mutated constructs demonstrated that all defective mutants accumulated viral RNAs in transfected cells but did not allow the assembly and release of virions. In conclusion, we characterized conserved amino acids of BVDV-E2 which are essential for the formation of E2-E1 heterodimers and E2-homodimers, and subsequently for virus assembly and egress. Furthermore, an E1-stabilizing role of the E1-E2-heterodimer has to be assumed.

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