Analysis of classical swine fever virus RNA replication determinants using replicons

Self-replicating RNAs (replicons), with or without reporter gene sequences, derived from the genome of the Paderborn strain of classical swine fever virus (CSFV) have been produced. The full-length viral cDNA, propagated within a bacterial artificial chromosome (BAC), was modified by targeted recombination within E. coli. RNA transcripts were produced in vitro and introduced into cells by electroporation. The translation and replication of the replicon RNAs could be followed by the accumulation of luciferase (from Renilla reniformis or Gaussia princeps) protein expression (where appropriate), as well as by detection of the CSFV NS3 protein production within the cells. Inclusion of the viral E2 coding region within the replicon was advantageous for the replication efficiency. Production of chimeric RNAs, substituting the NS2 and NS3 coding regions (as a unit) from the Paderborn strain with the equivalent sequences from the highly virulent Koslov strain or the vaccine strain Riems, blocked replication. However, replacing the Paderborn NS5B coding sequence with the RNA polymerase coding sequence from the Koslov strain greatly enhanced expression of the reporter protein from the replicon. In contrast, replacement with the Riems NS5B sequence significantly impaired replication efficiency. Thus these replicons provide a system for determining specific regions of the CSFV genome required for genome replication without the constraints of maintaining infectivity.

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