Replication of maize streak virus lacking RepA or RepA-pRBR interaction

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Mastreviruses express two forms of replication-associated proteins, Rep and RepA, that arise from differential splicing of the C-sense transcripts. RepA binds the plant retinoblastoma-related protein (pRBR) and potentially transactivates V-sense gene expression. To examine the role of RepA for viral replication we investigated replicative forms by 2D-gel electrophoresis in maize streak virus (MSV) mutants that cannot bind pRBR or do not express RepA. Previous work on the pRBR-binding deficient mutant has shown that one of the three mutated nucleotides consistently reverts to the wt nucleotide. Interestingly, rather than restoring the wt amino acid sequence, the reversion apparently restores the mutant single-stranded DNA genome to a wild-type like secondary structure. Here we have found that pRBR binding is not essential for the formation of most replicative forms, but replication efficiency is reduced in the original mutant and gets restored by the single-nucleotide reversion. In addition, C1-intronless MSV clones, that cannot express RepA, led to lower replication levels in black mexican sweet (BMS) cells and to no detectable formation of single-stranded DNA (ssDNA) or rolling circle replication (RCR). We note that replication of RepA$^-$ MSV differs from replication of other RepA$^-$ mastreviruses, and discuss the possible roles of RepA for the viral replication in MSV.