Replication modes of Maize streak virus mutants lacking RepA or the RepA-pRBR interaction motif

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Abstract

The plant-infecting mastreviruses (family Geminiviridae) express two distinct replication-initiator proteins, Rep and RepA. Although RepA is essential for systemic infectivity, little is known about its precise function. We therefore investigated its role in replication using 2D-gel electrophoresis to discriminate the replicative forms of Maize streak virus (MSV) mutants that either fail to express RepA or express RepA that is unable to bind the plant retinoblastoma related protein, pRBR. Whereas amounts of viral DNA were reduced in two pRBR-binding deficient RepA mutants, their repertoires of replicative forms changed only slightly. While a complete lack of RepA expression was also associated with reduced viral DNA titres, the only traces of replicative intermediates of RepA− viruses were those indicative of recombination-dependent replication. We conclude that in MSV, RepA, but not RepA-pRBR binding, is necessary for single-stranded DNA production and efficient rolling circle replication.

Introduction

Maize streak virus (MSV), the type member of the genus Mastrevirus in the family Geminiviridae, causes the most severe viral disease of maize in Africa (Shepherd et al., 2009). It has a 2.7 kb monopartite single-stranded (ss) DNA genome (Fig. S1, reviewed by Gutierrez et al., 2004) composed of a long intergenic region (LIR), a short intergenic region (SIR) and four open reading frames (ORFs). These express a movement protein (MP; from the V2 ORF) and a coat protein (CP; from the V1 ORF) from the virion-sense strand, and two replication-initiator proteins, Rep (spliced from the C1 and C2 ORFs) and RepA (C1 ORF alone), from the complementary strand. Rep and RepA share the same N-terminus and differ in their C-termini, which allows their different and multiple functions in the viral life cycle. Rep cleaves virion-strand DNA at the origin of replication in a hairpin structure situated in the LIR to initiate rolling circle replication (RCR; see Laufs et al., 1995), resulting in new circular ssDNA progeny which is converted to double-stranded DNA (dsDNA) by complementary-strand replication (CSR) and covalently closed circular DNA (cccDNA) upon packaging around host nucleosomes (Saunders et al., 1991; Stenger et al., 1991; reviewed by Hanley-Bowdoin et al. (1999), Jeske (2009)). The precise role of RepA is less well defined, although it is at least involved in the induction of a cellular environment that is amenable to viral replication (see Gutierrez, 2000, for a review).

In addition to RCR, recombination-dependent replication (RDR) has been found for plant begomoviruses (Jeske et al., 2001; Jovel et al., 2007), curtoviruses (Preiss and leske, 2003) and mastreviruses (Erdmann et al., 2010) as well as animal circoviruses (Cheung, 2012). RDR uses cccDNA as a template and does not rely on an origin of replication. Instead, replication is initiated by base-pairing interactions between the template cccDNA strand and the homologous nucleotides on the single-stranded 3' overhanging end of a linearised geminiviral genome or genome fragment. Replication then proceeds by extension of the free 3' end of this "invading" linearised DNA molecule (Jeske, 2007).

Since geminiviruses do not encode a polymerase, viral replication relies on host replication enzymes that are mainly inactive in differentiated cells, but which can be activated by the binding of...