and expression of viral genes. Specific viral proteins are involved in the initiation of rolling circle replication, *trans*-activation of the CP promoter, production of ssDNA, and viral movement functions (outlined in **Fig. 1**; *see* **refs. *1*** and ***2*** for comprehensive reviews). Upon entry into the nucleus, host DNA replication machinery converts the virion ssDNA into dsDNA. The double-stranded replicative form DNA (RF-DNA) then functions as both a template for transcription of viral genes and for genome amplification by rolling circle replication, initiated by the Rep protein, which binds specifically to sequences within the intergenic region and induces a nick in the conserved nonanucleotide motif (TAATATT-AC) in the loop of a conserved hairpin-loop structure essential for replication ***(5–9)***. The virion-sense strand released by rolling circle replication circularizes, and is made double-stranded or is encapsidated.

Isolation of geminivirus particles can be very difficult: The success of any isolation protocol is highly dependent on the virus and host plant. In this chapter, we describe a simple method developed at the University of Cape Town for isolating MSV virions from infected maize tissue ***(10)***: This employs a low pH extraction buffer and an acidification step, which serves to denature many contaminating plant proteins. We then simply subject the clarified sap to two cycles of differential centrifugation, which usually yields pure virus. For geminiviruses that may be more recalcitrant, some authors have found that stirring sap overnight in the presence of Triton X-100 helps to release virus particles from inclusion bodies, prior to two steps of differential centrifugation and purification in sucrose density gradients ***(11,12)***. Alternatively, it may be useful to include a chloroform emulsification step to denature plant proteins before further purification by PEG precipitation and differential centrifugation ***(12,13)***.

Initially, most researchers used virion-associated ssDNA to clone geminivirus genomes: Virions were isolated, ssDNA purified from the virions, and the complementary strand synthesized in vitro. This synthesis is simple for Mastreviruses, which have a short DNA primer molecule bound to the virion DNA ***(14–16)***; however, ssDNA from Curto- and Begomoviruses is not usually associated with DNA primers, so second-strand synthesis has to be randomly or specifically primed. The major limitation of this method is that geminivirus virions are frequently difficult, if not impossible, to isolate, and yields are usually low. The use of ssDNA as a starting point for molecular manipulations of geminivirus genomes has therefore generally been supplanted by the direct use of viral RF-DNA.

Geminivirus RF-DNA typically accumulates to high levels in the nuclei of infected cells: In our experience, the RF-DNA of MSV is often visible as discrete fast migrating bands in plant DNA extracts electrophoresed in agarose