Cauliflower Mosaic Virus replication complexes: characterization of the associated enzymes and of the polarity of the DNA synthesized in vitro

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Abstract

The synthesis of both strands of CaMV-DNA has been studied in vitro using viral replication complexes obtained by hypotonic extraction of infected plant organelles. Hybridization of the DNA synthesized in vitro to single stranded CaMV DNA probes cloned in bacteriophage M 13 confirmed that the 35 S RNA served as a template for the synthesis of the (-) DNA strand. The response of CaMV DNA synthesis to various inhibitors suggests that a single enzyme directs both steps of the replication cycle. A comparative activity gel analysis of the DNA polymerases present in nuclear extracts from healthy and CaMV-infected turnips revealed an increase of a DNA polymerase species migrating in the 75 Kd range in infected tissue. When the enzyme activity associated with the isolated replicative complexes was similarly analyzed, the 75 Kd polymerase was markedly predominant, confirming that DNA polymerases of the α-type (MW in the 110 Kd range) are not involved in the aphidicolin-insensitive CaMV DNA replication. It seems therefore increasingly probable that CaMV codes for its own polymerase.

Introduction

The use of small animal DNA viruses as model systems to study genome replication and expression has led to spectacular advances in the knowledge of the molecular biology of eukaryotes. Our present understanding of the regulation of gene expression and control of replication owes much to the fact that some of these viruses replicate and transcribe their DNA as a minichromosome, using the enzymes provided by their host (35).

Although it is widely believed that the rules governing transcription and replication in animal cells should also apply to the plant kingdom, plant molecular biology has lagged behind for want of an adequate combination of suitable recipient systems and simple extrachromosomal elements that would use the cell machinery to express and amplify their genetic information.

This is why Cauliflower Mosaic Virus (CaMV) has attracted increasing attention in recent years, since it is one of the few plant viruses possessing DNA rather than RNA as genetic material. This surge of interest rapidly led to a better characterization of the viral polypeptides (2) and of the architecture of the virion (1, 5). The viral DNA has been entirely sequenced (12). The host RNA polymerase II transcribes supercoiled intranuclear CaMV DNA (21) in the form of a minichromosome (24, 22), producing a 19 S mRNA and 35 S long transcript (7, 15, 23). However, Pfeiffer & Hohn (25) have shown that these minichromosome structures are not involved in the replication of CaMV and that part of the DNA synthesis reaction directed by CaMV replicative complexes utilizes RNA rather than DNA as a template. This led these authors (25) and others (16, 17) to propose that CaMV replicates by reverse transcription of the 35 S RNA.

The host plant possesses a γ-like DNA polymerase A (37) which is capable of copying poly rA-oligo dT (33). This enzyme would therefore be in principle capable of directing the reverse tran-
scription step. However, recent evidence of extensive homologies in the aminoacid sequences of the virus-encoded reverse transcriptases of animal retroviruses and the polypeptide encoded by ORF V of CaMV DNA (34, 37) indicates that CaMV probably codes for its own polymerase which would function as a reverse transcriptase endowed with multiple enzymatic activities (13).

We report here further characterization of the DNA synthesis directed in vitro by CaMV replicative complexes, together with changes in DNA polymerase patterns occurring upon CaMV infection, and the specific association of one of the DNA polymerase species with the replication complexes.

Materials and methods

Virus

Cauliflower Mosaic Virus, Strain Cabb-S, was propagated in turnip (Brassica rapa, cv. Just Right) by mechanical inoculation of the plants at the 3-leaf stage. For the experiments described here, the systemically infected young leaves at the centre of the rosette were harvested 3-4 weeks after inoculation.

Isolation of subcellular particles (viroplasms and nuclei)

All steps were performed on ice and without interruption, and the procedure is given for 10 g of leaves. These were washed with 2 changes of cold deionized water, drained, and the midrib was removed. They were homogenized (3 × 30 sec pulses at full speed and 30 sec intervals) with 20 ml grinding buffer (50% glycerol in MMD containing 250 mM sucrose; MMD buffer is pH 6.0, 50 mM morpholinoethane sulfonic acid-NaOH, 1 mM DTT, 5 mM MgCl₂) with a Polytron homogenizer. The slurry was filtered through two layers of Miracloth and Triton X-100 was added as a 10% solution to 1% final. After 10 min stirring on ice, the detergent-treated filtrate was centrifuged through a 1.2 M sucrose cushion in MMD for 20 min at 5 000 g. The pellet of organelles was resuspended with a Pasteur pipette in 1 ml MMD containing 250 mM sucrose, centrifuged briefly in a Sigma refrigerated centrifuge, and resuspended again in 0.5 ml hypotonic 'H' buffer ('H' buffer is 50 mM Tris-HCl (pH 7.8), 1 mM MgCl₂, 1 mM DTT). These suspensions were observed in the electron microscope: they contained swollen nuclei and some starch grains, and also inclusion bodies or 'viroplasms' in the case of infected plants.

Extraction of CaMV replicative complexes

The organelle suspension was extracted for 1 h on ice in hypotonic buffer with occasional gentle homogenization. Particles were then pelleted by a 10 min centrifugation at 15 000 rpm and the supernatant containing replicative complexes was analyzed on a 5 to 20% sucrose gradient in 'H' buffer centrifuged for 3 h at 10 °C and 59 000 rpm in a Beckman SW 60 Ti rotor. When the effect of other divalent cations was to be studied after fractionation, the gradient buffer contained 0.1 mM EDTA instead of 1 mM MgCl₂.

DNA-polymerase assays

The DNA-polymerase activity present in the