

$\times 10^6$  Dalton (about 7.5 kb) that accounts for 5% of the particle weight. The particles contain a single coat protein (CP) species with a mol wt of 22–27 kDa.

### 1.3. Molecular Properties

The genomic RNA of ACLSV is polyadenylated (**16**) and is probably capped at its 5' end (**8**). The determination of the complete or partial nucleotide sequence of the RNA of some members of the group (**8,12–14**) has provided knowledge on the genome organization and the gene expression strategy of trichoviruses (**9**). The ACLSV RNA contains at least three open reading frames (ORFs), encoding proteins with approximate mol wt of 216, 50, and 22 kDa (**Fig. 1**).

The 5' large ORF1 codes for a protein that contains three signature sequences, typical of replicase-associated proteins of the  $\alpha$ -like supergroup of plant viruses: The methyl-transferase, nucleotide-binding site helicase, and polymerase signatures (**8**).

The ORF2 of ACLSV shares distant similarities with the cauliflower mosaic virus gene I and TMV 30-kDa movement proteins (MP) (**8**). The ORF2 encoded protein has been included in the proposed family I of MP (**17**), which also includes the MP of tobamoviruses, tobnaviruses, comoviruses, caulimoviruses, and geminiviruses.

The capsid protein ORF is located at the 3' terminus of the genomic RNA. The CP contains motifs that are present in a highly conserved region of filamentous virus CPs, hypothesized to be involved in the formation of a salt bridge, and possibly vital for tertiary structure formation (**18**).

As shown in **Fig. 1**, GVA and GVB have an additional small ORF at the 3' end of the genome, downstream of the CP gene, which is missing in ACLSV and PVT (**14**). In the case of GVB, this small ORF has homologies with the small, cysteine rich, nucleic acid-binding proteins found in the genome of other plant viruses, such as hordeiviruses and carlaviruses. Distinct sequence homologies exist between the movement proteins and CPs of all members of the trichovirus group.

### 1.4. Virus Purification

The trichovirus group includes viruses that are routinely propagated in different herbaceous hosts, so that different protocols for virus purification are used for the various members. This chapter describes in detail the method of purification currently used in our laboratory for ACLSV, the type member of the trichoviruses. The reader is directed to the references for sources on other trichoviruses.

ACLSV purification is done by the bentonite–polyethylene glycol procedure adapted from Lister and Hadidi (**19**), with modifications by Dunez et al. (**20**) and other unpublished modifications.