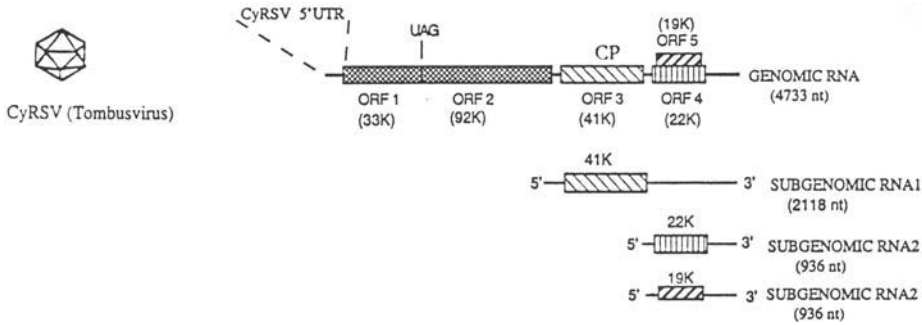

Fig. 1. Virus structure, genome organization, and expression strategy of a tombusvirus (CyRSV). Noncoding regions are shown as solid lines (UTR = untranslated leader sequence) and ORFs by boxes with different shading. The approximate mol wt of predicted translation products, and sizes and locations of subgenomic RNAs, are indicated. The virus CP (41 kDa) is encoded by ORF 3.

Tombusviruses may support the replication of two types of subviral RNAs: satellite and defective-interfering (DI) RNAs. Satellite RNA is a linear molecule of 619 nt, with little sequence in common with the genomic RNA; DI RNAs (400–800 nt) are deletion mutants of viral genomes that have generally lost all essential viral genes required for movement, replication, and encapsidation ***(9)***. Both satellite and DI RNAs require the presence of a helper virus for *trans*-acting factors necessary for replication ***(8)***.

2. Materials

2.1. Virus Purification

1. 0.5*M* sodium acetate buffer, pH 5.5 (stock solution).
2. Polyethylene glycol (PEG) 6000.
3. NaCl.
4. Homogenization buffer: 0.1*M* sodium acetate buffer, pH 5.5, containing 0.25% β-mercaptoethanol (freshly prepared).
5. High-speed (15K rpm) homogenizer.
6. Beckman J2-21 low-speed centrifuge, with rotor JA-20 or equivalent.
7. Beckman L7-55 ultracentrifuge, with rotor Type 40 or equivalent.
8. Eppendorf microcentrifuge, or equivalent.

2.2. RNA Extraction

1. RNA extraction buffer: 0.1*M* glycine-NaOH, pH 9.0, containing 100 m*M* NaCl, 10 m*M* EDTA, 2% sodium dodecyl sulfate (SDS), and 1% sodium lauroyl sarcosine.
2. 3*M* Sodium acetate, pH 5.5.