like protease ***(5,6)***, a NTPase/helicase ***(7)***, and a RNA replicase ***(8)***. After translation, the RP is hydrolyzed specifically by the protease to yield a 66-kDa C-terminal fragment containing the replicase, and a 140-kDa fragment ***(9)***.

Overlapping the 5'-terminal third of the RP, and always starting seven nucleotides to the 5' side of its start codon, is the least conserved ORF. It encodes the overlapping protein (OP) of approx 69 kDa that is very basic (pI 10.9–11.9).

The third and smallest ORF is between the RP gene and the 3' end of the genome. It encodes the virion protein (VP; 188–202 amino acid residues) of approx 20 kDa, and is expressed via a subgenomic RNA. A region of about 50 nucleotides to the 5' side of the start of the VP ORF, and including the 3' terminus of the VP ORF, has a closely similar sequence in all tymoviruses. One part of it, the tymobox, is probably the complement of the transcription promoter sequence of the VP gene, and is identical in eleven tymoviruses (5'-dGAGTCTGAATTGCTTC-3'), with a single difference in another three, and four differences in that of the wild cucumber mosaic virus genome (5'-dGAGTCTTCTTTGCATC-3') ***(10)***. An oligonucleotide with the tymobox sequence is thus useful as a probe for tymoviruses or as a PCR primer for isolating the virion protein gene of most tymoviruses.

2. Materials

1. Infected plant tissue: Tymoviruses are readily transmitted by manual inoculation, and their virions attain large concentrations in infected plants. TYMV infects a wide range of brassicas, but its virions attain very large concentrations (0.5–2.0 mg/g leaf tissue) in young plants of Chinese cabbage (*Brassica campestris* ssp. *chinensis* [Pak-choi] and ssp. *pekinensis* [Pe-tsai]) grown in rich, frequently watered compost, in bright light of 12–16 h/d, and a daytime maximum temperature of 25°C. The plants are best inoculated at the 3–6 leaf stage, and inoculated and systemically infected leaves harvested 2–4 wk later.
2. PA buffer: 100 m*M* Na_2HPO_4, 50 m*M* ascorbic acid, pH 7.0.
3. 50:50 chloroform:*n*-butanol: a 50:50 v/v mixture of chloroform and *n*-butanol.
4. TE buffer: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 7.0.
5. SSC: 150 m*M* NaCl, 15 m*M* sodium citrate.
6. RNA extraction buffer: 10 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.2% sodium dodecyl sulfate (SDS) in 10 m*M* Tris-HCl, pH 7.4.
7. TE-saturated phenol: phenol equilibrated with about 0.5 vol TE buffer by shaking them together several times, and then keeping the mixture overnight at 4°C for the phases to separate.

3. Methods

3.1. Virion Purification

Tymovirus virions are readily purified by a wide range of methods, because they are stable and attain large concentrations in the tissues of infected host