

Tobamovirus Isolation and RNA Extraction

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1. Introduction

The tobamoviruses produce rigid, rod-shaped particles, with dimensions of approx 300×18 nm, and form one of the most extensively studied groups of plant viruses (**1**). Members of the group infect a wide range of angiosperms, and individual members frequently have wide experimental host ranges. Tobamoviruses cause diseases in tobacco, tomato, pepper, orchid, cucumber, melon, bean, and crucifer plants. The characteristic symptoms of disease are stunting and chlorotic mosaics, mottles, or ringspots. These symptoms are often accompanied by distortion of leaves and fruits.

The tobamoviruses form particles that contain a single-stranded genomic RNA. Complete genomic RNAs of the majority of the definitive tobamoviruses have been cloned and their nucleotide sequences determined. The genomic organization of each of these viruses is very similar and each have four open reading frames (ORFs). The best characterized of the tobamoviruses is the type member tobacco mosaic virus (TMV) (**Fig. 1**). The TMV genomic RNA has a 7-methylguanosine cap structure at the 5' end (**2**), followed by an untranslated leader sequence of 68 nucleotides, which is an enhancer of translation (**3**). Downstream of the untranslated region are two ORFs that encode proteins of 126 and 183 kDa (**4**), which are involved in the viral replication process (**5**). Translation of the two proteins, which occurs from the genomic RNA, is initiated at the same methionine codon. The larger 183-kDa protein is produced by readthrough of a leaky amber stop codon at the end of the 126-kDa ORF, with a frequency of about 5% (**6**). The third ORF overlaps the 183-kDa ORF by 17 nucleotides, but is out of frame. The ORF encodes a protein of 30-kDa that is produced by translation of a subgenomic RNA, which is 3' coterminal with the genomic RNA. The 30-kDa protein is necessary for intercellular movement of