

Tobravirus Isolation and RNA Extraction

Sally C. Taylor

1. Introduction

The tobnaviruses have a genome consisting of two positive-sense, single-stranded RNA molecules. The two genomic RNAs, RNA-1 and RNA-2, are encapsidated separately in rod-shaped particles with lengths of 180–215 nm (L particles) and 46–115 nm (S particles), respectively (**Fig. 1**) (*1,2*). Both the RNAs are encapsidated by a single species of coat protein (CP) with a mol wt of approx 23 kDa. Tobnaviruses can be divided into three serologically distinct subgroups: tobacco rattle virus (TRV), which is the type member, pea early browning virus (PEBV), and pepper ringspot virus (PRV). In the field, these viruses are transmitted by soil-inhabiting nematodes of the family *Trichodoridae*, and certain isolates are also transmitted through the seed. Tobnaviruses can infect a wide range of plant species including economically important crops such as potatoes and ornamental bulbs.

The nucleotide sequence of several tobnaviruses has now been determined (*3–8*). This revealed that RNA-1 of each of the three virus subgroups is of a similar size (6.8–7.0 kb) and contains at least four open reading frames (ORFs) (**Fig. 1ai** and **bi**). RNA-2, however, varies considerably in length (1.8–4.5 kb), even between isolates of the same subgroup, and may contain 1–4 ORFs (**Fig. 1aii** and **bii**). Both RNA-1 and RNA-2 have a 7-methylguanosine cap structure at the 5' end and a tRNA-like structure at the 3' end (*2,9*).

The genomic organization of RNA-1 of tobnaviruses is very similar to the monopartite tobamovirus, tobacco mosaic virus (TMV) (*10*). The two 5' proximal ORFs on RNA-1 of the type member TRV encode proteins of 134 and 194 kDa. The 134-kDa protein terminates in an opal (UGA) stop codon and readthrough of this codon results in the production of a 194-kDa protein. The 134- and 194-kDa proteins have been found to share amino acid homology