On the variability of the 3' terminal sequence of the turnip mosaic virus genome

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Summary. The sequence of the 3'-terminal 1223 nucleotides (nts) of a Japanese isolate of turnip mosaic virus (TuMV-Jap) RNA has been determined. The sequence reveals a single open reading frame (ORF) which terminates at a position 212 nts upstream of the 3' poly(A)-tract. Determination of the N-terminal amino acids of TuMV-Jap coat protein (CP) mapped the CP cistron within this ORF and revealed a Glu-Ala dipeptide sequence as the putative cleavage site by which the CP is released from the viral polyprotein. The predicted amino acid sequence of the TuMV-Jap CP shows 97.2% identity with that of a Canadian isolate of TuMV (TuMV-Can) and 99% with a second, Chinese, isolate (TuMV-Chi). However, the 3'-terminal non-translated region (NTR) of TuMV-Jap RNA is significantly shorter (212 nts) than the 3'-NTR of TuMV-Can RNA (668 nts), but of equal length as the 3'-NTR of the TuMV-Chi isolate which also measures 212 nts. The 3'-NTRs of both the TuMV-Jap and TuMV-Chi RNAs show homology with the first 201 nucleotides of the TuMV-Can RNA 3'-NTR. A search in the EMBL nucleotide sequence database revealed that the 467 nt-long unique extension of the 3'-NTR of TuMV-Can RNA has 89.8% homology to a part of the chloroplast ribosomal protein 12 gene (rps12-gene). Irrespective of the origin of this extra sequence in the reported TuMV-Can sequence, which may have been introduced by a genuine RNA recombination event, it is concluded that the standard TuMV genome has a CP gene of 864 nts and an conserved 3'-NTR of approximately 212 nucleotides in length.
Introduction

Turnip mosaic virus (TuMV), member of the potyvirus group, is a world wide distributed pathogen infecting many plant species from 20 different dicotyledonous families [19]. The virus is sap-transmissible and spread by a considerable number of aphid species [19]. The genomic RNA of potyviruses encodes a single large polyprotein which is subsequently cleaved, by at least two virus-coded proteases, to yield mature viral proteins [3, 4]. Genome mapping and sequence analysis of several potyviral RNAs have revealed that the coat protein (CP) cistron is invariably located directly upstream of the polyadenylated 3' terminus of the genomic RNA.

Detailed sequence comparisons of a growing number of potyviral CP genes demonstrate that the internal, core region of the potyviral CP is conserved, whereas both the length and the amino acid sequence of the extended N-terminus may be highly heterogeneous among different potyvirus species [17, 18, 24]. In addition, the 3' non-translated regions (3'-NTRs) of the RNAs of different members of the potyvirus group show no significant homologies [8, 24]. For these reasons both the amino acid sequence of the CP, especially that of the variable N-terminal domain of this protein, as well as the nucleotide sequence of the 3'-NTR have been proposed as molecular criteria for potyvirus classification [8, 22, 24].

Recently the nucleotide sequence of the 3' terminal region, including the CP cistron, has been elucidated for two different TuMV isolates, one originating from Canada (TuMV-Can) and one from China (TuMV-Chi) [20, 10]. These two isolates share a high degree of homology, in both the length and amino acid sequence of their CPs, including the N-terminal region. In contrast to this, their 3'-NTRs show a great divergence in length. This observed discrepancy obscures the precise taxonomic relationship between both isolates, and moreover it breaks the generally accepted consensus by which potyviruses are nowadays classified [24].

In order to study the variability among TuMV isolates we decided to determine the 3'-terminal RNA sequence of an additional, third isolate. This paper reports the nucleotide sequence of the CP gene and 3'-NTR of a Japanese isolate of TuMV and its comparison to the isolates from Canada and China.

Materials and methods

A Japanese isolate of TuMV (TuMV-Jap), originally obtained from radish (Raphanus sativus L.) [16], was propagated in turnip (Brassica rapa) by successive sap-inoculation. Virus was purified from infected turnip leaves as described by Choi et al. [5], and genomic RNA was purified according to Brakke and van Pelt [2]. Complementary DNA (cDNA) clones corresponding to regions upstream of the poly (A) tract were constructed using the cDNA synthesis system plus kit (Amersham International PIC) with oligo(dT) as a primer, and subsequently cloned into Sma I-digested pUC 19 [25]. Additional cDNA clones were synthesized using a synthetic oligonucleotide 5'-TGAGCGGCTTGTGATCGGG-3', whose sequence was derived from obtained nucleotide sequence data. Several cDNA clones from the 3' end of the genomic RNA were obtained. These cDNA clones were subcloned into