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# CAULIFLOWER MOSAIC VIRUS ORF VII IS NOT REQUIRED FOR APHID TRANSMISSIBILITY

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Cauliflower mosaic virus (CaMV) is a plant virus containing a circular double-stranded DNA genome which replicates by reverse transcription (reviews **[2, 15]**). The virus is transmitted by aphids, but is maintained in the insect vector for only short periods of time. This non-persistent transmission is dependent on an «acquisition factor» **[12, 13, 16, 14]**.

The 8,000 base pair (bp) CaMV genome contains 8 open reading frames (ORF), ranging in size from 96 (ORF VII) to 680 codons (ORF V). Functions have been assigned to ORF II (aphid acquisition), IV (viral coat protein), V (reverse transcriptase) and VI (inclusion body matrix protein) (for ORF II, see **[3, 1, 8]**; for the others, see reviews **[2, 15]**). Gene products encoded by ORF III, IV and V have been detected in virus particles **[7 and 9]**; inclusion bodies contain virus particles and, in addition, products encoded by ORF I, II and VI. The 66 kilodalton (Kd) product derived from ORF VI is the main component of inclusion bodies and is essential for their formation. The 18-Kd product derived from ORF II is a minor component of inclusion bodies affecting the firmness of the structure **[8]**. Products derived from the two shortest ORF (VII and VIII) have not been detected *in vivo,* although ORF VII can be translated *in vitro* **[9]**. Indirect evidence indicates that it is also expressed *in vivo* **[2, 5, 6]**. Failure to detect the ORF VII product may be due to its instability. The high content in basic amino acids of ORF VII and VIII ($\simeq$ 25 % lys + arg) suggests a function of these proteins as nucleic acid binding regulators.

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ORF II and VII are not required for systemic spread of disease [**3, 1, 5, 6, 4**], but virus with mutations in ORF II cannot be transmitted by aphids without a wild type helper virus [**12, 13, 17**]. Several laboratory strains of CaMV have lost the ability to be transmitted by aphids without helper virus. The aphid non-transmissible strain CM4-184 has, as expected, a deletion in ORF II [**18**]. However, the aphid non-transmissible strains «Campbell» [**18**] and CM 1841 [**10**] contain ORF II of normal length and without dramatic sequence deviation compared to aphid transmissible strains. In their studies on chimaeric hybrid viral genomes of transmissible and non-transmissible strains, Woolston *et al.* [**18**] suggested that in «Campbell» and CM 1841 strains, sequences upstream of ORF II, encompassing ORF I and part of ORF VII, might affect transmissibility. The «Campbell» strain contains a stop codon in ORF VII that would cause premature termination of translation of the encoded protein (J. Davies, pers. commun.). It therefore seemed possible that the product of ORF VII is also needed for aphid transmissibility, either directly or as a factor controlling expression of ORF II.

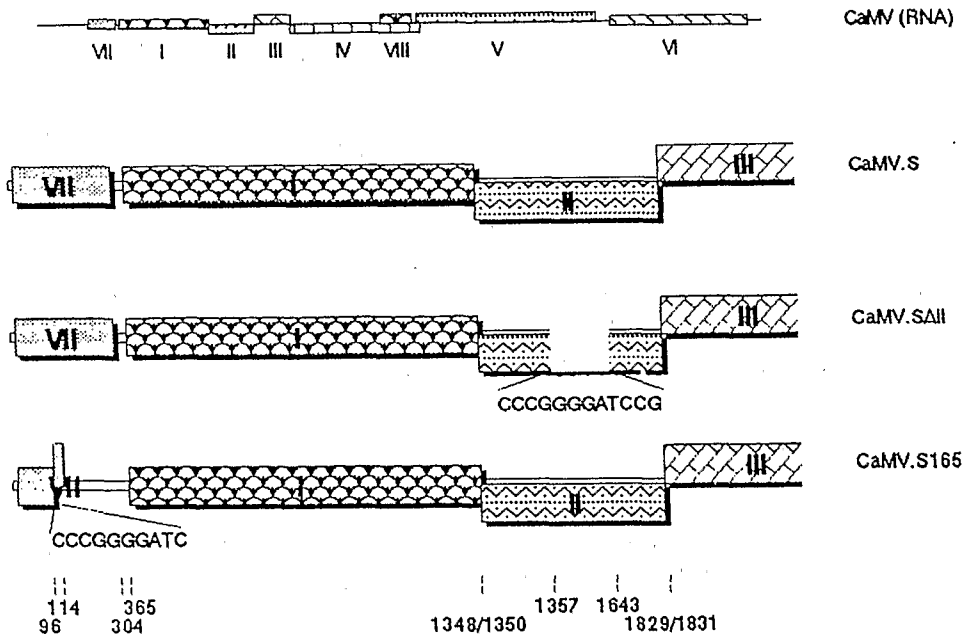


FIG. 1. — *Strains as described in the legend of table I.*

bp = base pair.
CaMV = cauliflower mosaic virus.
Kd = kilodalton.
ORF = open reading frame.

To examine this possibility, mutants were constructed *in vitro* in ORF II and VII of the aphid transmissible strain CaMV.S (fig. 1). These mutants were then tested and compared with wild type strain CaMV.S and with non-transmissible strain CM4-184 for their ability to be acquired and transmitted by aphids (table I). All strains tested were equally infective. Wild type CaMV.S and CaMV.S with a mutation in ORF VII could be transmitted equally well by aphids, whereas CaMV.S with a mutation in ORF II and CM4-184 were not transmissible by aphids.

These results indicate that ORF VII is not involved in aphid transmission of the virus. We would argue either that as yet undetermined specific interactions of the ORF II product with other virus (or host) proteins are affected in strains «Campbell» and CM 1841, or that the minor sequence variations in the ORF II of these strains affect functional domains of the gene product. If the expression product of ORF VII is indeed a regulator protein, its function has to be searched for at a level other than aphid transmissibility.

Recently, Woolston *et al.* **[19]** came to similar conclusions using recombinant DNA hybrids and *in vitro* mutagenesis of ORF VII.

TABLE I. — **Aphid transmissibility and infectivity test.**

| Strain | Infectivity by mechanical inoculation | | Aphid transmissibility | |
|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 |
| CaMV.S | 18/22 | 19/20 | 12/23 | 15/20 |
| CaMV.SΔII | 17/20 | 18/20 | 0/26 | 0/28 |
| CaMV.S165 | 17/20 | 17/20 | 16/32 | 21/24 |
| CaMV.CM4-184 | 19/20 | 16/20 | 0/23 | 0/20 |

Turnip plants were inoculated mechanically each with 5 μg virus DNA excised from hybrid plasmids harvested in *E. coli* as described **[11]** and symptoms were noted three weeks after inoculation. Aphid transmissibility test: aphids *(Myzus persicae* [Sulz] were maintained on radish *Raphanus sativus).* A subset of infected plants with fully developed symptoms was used for acquisition feeding three weeks after inoculation. Insects were starved for 2 h before the 2-3 min acquisition feeding; five insects were transferred to each test plant and allowed to feed on it for 16 h, after which time they were killed by spraying with an insecticide (Gesal). For each experiment and inoculum, a separate growth chamber was used. For positive counts noted, first symptoms appeared after 10 days and symptoms were clearly visible after 2 weeks. For negative counts, no symptoms were observed after 6 weeks. To check aphid transmission of CaMV.S/165, the DNA extracted from infected plants was tested with the restriction enzyme *Sma*I specific for CaMV.S/165 in this series.

*Strains.* — CaMV.S **[11]**; CAMV.SΔII is a deletion/insertion within ORF II (nucleotides 1538 to 1642 were replaced with CCCGGGGATCG **[8]**; CaMV.S/165 was constructed for this study from CaMV.S. It contains a 10-nucleotide insertion (CCCGGGGATC) within ORF VII (after nucleotide 96) causing a frameshift and shortening of the ORF. CaMV.CM4-184 is a non-transmissible aphid strain with a deletion within ORF II **[18]**. Constructions were confirmed by sequencing.

## RÉSUMÉ

LA PROTÉINE ORF VII DU VIRUS DE LA MOSAÏQUE DU CHOU-FLEUR N'INTERVIENT PAS DANS LA TRANSMISSION DU VIRUS PAR LES PUCERONS

Des mutants du virus de la mosaïque du chou-fleur ont été construits *in vitro*. La transmissibilité de ces mutants par les pucerons a été testée. Pour que la transmission par pucerons soit possible il faut un ORF II intact, alors que l'ORF VII n'est pas nécessaire.


MOTS-CLÉS : Virus phytopathogène, CaMV, ORF II, ORF VII ; Mutants, Transmission, Pucerons.


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