ENCAPSIDATION OF NUCLEIC ACIDS BY CUCUMOVIRUS COAT PROTEINS

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Summary

An improved method with high efficiency for reassembly of both CMV and TAV was developed and physico-chemical, serological, and biological analyses showed that the reassembled particles were indistinguishable from the native viruses.

In vitro reassembly of CMV (T strain, TCMV) particles with separated CMV RNA species demonstrated that four types of particles encapsidating different RNA content were indistinguishable both in morphology and sedimentation rate in sucrose density gradients from native TCMV. These particles encapsidated: 1) one molecule of RNA 1; 2) one molecule of RNA 2; 3) one molecule each of RNAs 3 and 4; and 4) 3-4 molecules of RNA 4, respectively.

When heterologous virus particles were assembled with coat protein from the V strain of TAV (VTAV) or M strain of CMV (MCMV), all particles were cucumovirus-like, irrespective of RNAs from tobacco mosaic virus (TMV), velvet tobacco mottle virus (VTMoV), galinsoga mosaic virus (GMV), MCMV and VTAV. They were all infectious and able to induce symptoms characteristic of the encapsidated viral RNAs. The assembled particles with VTAV coat and TMV RNA sedimented faster than VTAV in sucrose density gradient.

Coat proteins of VTAV and TCMV encapsidated *in vitro* a range of single-stranded RNAs or DNAs, which were either linear or circular. The minimum length required for encapsidation was about 35 but not 20 nucleotides and the maximum length was possibly less than 7200 nucleotides. Double-stranded RNA and DNA were not encapsidated. The heterologously assembled particles retained the size of 28-30 nm in diameter. VTAV coat protein preferred homologous VTAV RNA to heterologous TMV RNA in competitive assembly.

Particles with mixed capsid were assembled when coat proteins of VTAV and TCMV were mixed and assembled with VTAV RNA. These particles reacted with antisera to VTAV and TCMV.

In experiments to investigate the encapsidation of satellite RNA in cucumovirus particles, native B1 satellite RNA (B1 satRNA) of CMV was encapsidated by either VTAV or TCMV coat proteins *in vitro* . The assembled particles were cucumovirus-like and had the same sedimentation rate as native TCMV. Assembly of ^{32}P-labelled satellite transcripts in the presence of viral genomic RNAs of VTAV with VTAV protein showed that satRNA was co-

encapsidated with the viral genomic RNA. The coat protein of TCMV was better than that of VTAV in encapsidating satRNA into cucumovirus-like particles.

The aphid transmissibility of VTAV and MCMV was shown to be determined solely by the properties of their coat proteins. The non aphid-transmissible viruses, TMV and MCMV were readily transmitted by the aphid *Myzus persicae* when their RNAs were encapsidated by coat protein from the aphid-transmissible VTAV. Conversely, aphids could not transmit infection with particles reassembled from coat protein of the non aphid-transmissible MCMV, and RNAs from VTAV and TMV.

In an experiment done to determine whether the non aphid-transmissible VTMoV could be transmitted by aphids from plants co-infected with VTMoV and VTAV, a low rate of transmission was observed. However, no transmission was found in three other subsequent experiments conducted. On the other hand, satellite RNA of VTMoV was transmitted by aphids in a relatively higher rate when the plants co-infected with VTAV and VTMoV plus its satellite RNA were used as virus sources.