Evidence for the polar nature of multipartite tubular rod-shaped virus particles and implications for systemic movement, genome integrity and stability

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Introduction

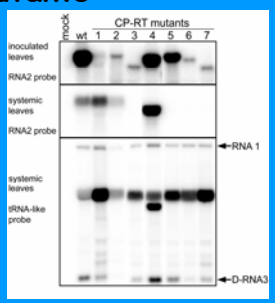
Potato mop-top virus (PMTV) has a tri-partite genome and tubular rod-shaped particles; previously we have shown that two of the RNAs, which encode replicase and movement proteins, when inoculated alone are capable of moving long-distance in *Nicotiana benthamiana* in the absence of coat protein encoding RNA [1].

Results

To examine the role of CP and CP-RT (minor CP) in PMTV systemic movement we engineered a set of mutants.

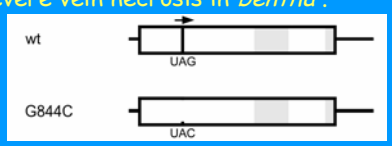
Systemic movement of PMTV CP/minorCP mutants

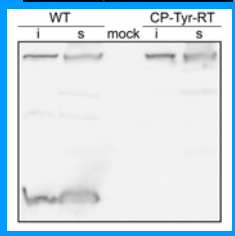
Northern blot analysis of the PMTV RT mutants in the inoculated and systemic leaves using two probes.
Minor CP is absolutely indispensable for the long-distance movement of RNA 2 and for symptom induction.



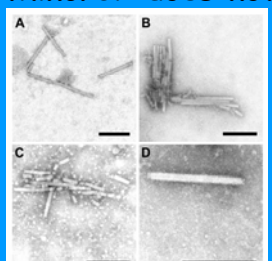
The mutant (G844C) in which the amber CP term. codon was replaced with a tyrosine codon was viable.

CP-Tyr-RT mutant moved systemically and induced severe vein necrosis in *bentha*.





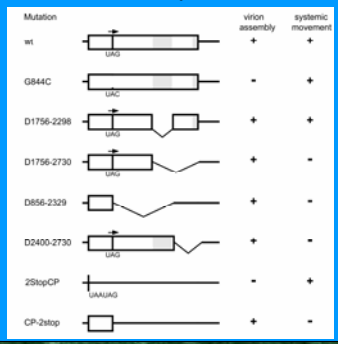
MinorCP does not affect virion assembly



Both CP-RT-null mutant and C-terminally truncated mutants of CP-RT were competent in virus assembly.

The G844C mutant produced only minor CP was deficient in viral assembly.

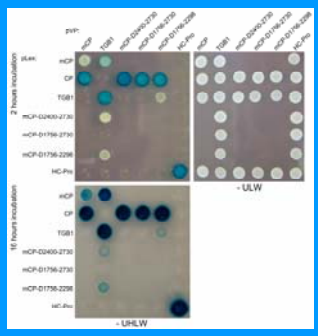
Virion assembly and sytemic movement of PMTV mutants



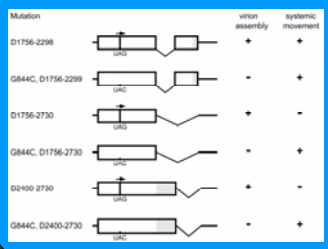
The minor CP is absolutely indispensable for the long-distance movement of RNA2 and for symptom induction.

minorCP interactions in YTHS

In yeast two-hybrid experiments minorCP (CP-RT), but not CP, interacted with the movement protein TGB1. C-terminally truncated mutants of CP-RT that did not interact with TGB1 were incapable of long-distance movement.



Mutants rescue



The mutants defective in systemic movement can be rescued by preventing CP expression; possibly because CP interacts more strongly with RNA than TGB1 forming movement defective particles.

Our model for PMTV movement

PMTV RNAs can move both as RNP complexes in association with TGB1 and as particles, and PMTV particles competent for systemic movement require the presence of CP-RT and TGBp1. CP-RT is associated with one extremity of the virus particles (probably the one that contains the RNA 5'end) [2]. Since TGB1 interacts with CP-RT, we suggest that TGBp1 may also be associated with this end. Thus our data indicate that terminal structures on virus particles are not only found in filamentous viruses (e.g. *poty*, *potex*- and *closterovoruses*), but in tubular rod-shaped vurises as well.

References: [1] Savenkov E.I. et al (2003) J. Gen Virol, 84: 1001-1005; [2] Cowan G.H. et al. (1997) J. Gen Virol, 78: 1779-1783.