Genome activation by Raspberry bushy dwarf virus coat protein

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Two sets of infectious cDNA clones of *Raspberry bushy dwarf virus* (RBDV) have been constructed, enabling either the synthesis of infectious RNA transcripts or the delivery of infectious binary plasmid DNA by infiltration of *Agrobacterium tumefaciens*. In plants, inoculation of RBDV RNA1 and RNA2 transcripts led to a low level of infection, which was greatly increased by the addition of RNA3, a subgenomic RNA coding for the RBDV coat protein (CP).

Agroinfiltration of RNA1 and RNA2 constructs did not produce a detectable infection but, again, inclusion of a construct encoding the CP led to high levels of infection. Thus, RBDV replication is greatly stimulated by the presence of the CP, a mechanism that also operates with ilarviruses and *Alfalfa mosaic virus*, where it is referred to as genome activation.

Mutation to remove amino acids from the N-terminus of the CP showed that the first fifteen RBDV CP residues are not required for genome activation. Other experiments, in which overlapping regions at the CP N-terminus were fused to the red fluorescent protein mRFP, showed that sequences downstream of the first 48 amino acids also are not absolutely required for genome activation.

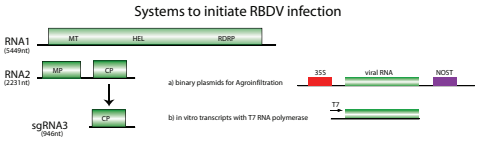
Characteristics

Raspberry bushy dwarf virus (RBDV) is one of the most important viral pathogens of red raspberry (*Rubus idaeus*) and is found world-wide. Plants infected with RBDV may exhibit "crumbly fruit" symptoms in which drupelet formation (raspberry fruits consist of a structured aggregation of fleshy drupelets) is severely affected, significantly reducing yield and fruit quality. In addition, when in combination with several other viruses, RBDV can cause stunting of plants and vivid "yellowing" of the leaves. RBDV is transmitted in the field by infected pollen and apparently without the intervention of any specific vector.

Symptoms

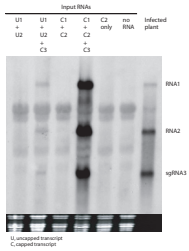
Structure

RBDV is the sole member of the virus genus *Idaeovirus* and has not been assigned to a family, although it has some similarities with ilarviruses from the family *Bromoviridae.* The virus genome consists of two positive-sense single strand RNAs; the larger RNA (RNA1) of about 5.4kb encoding a putative polymerase protein, and the smaller RNA (RNA2) of about 2.2kb encoding a putative cell-to-cell movement protein (MP) and the coat protein (CP). Virus particles, which are isometric in shape with a diameter of about 33nm, contain the two genomic RNAs as well as a third, subgenomic RNA (RNA3) of 946nt that is not replicated but is the template for CP expression. We have constructed two sets of infectious cDNA clones of RBDV, to produce either infectious *in vitro* RNA transcripts or binary plasmids that can be delivered by infiltration of plants with *Agrobacterium tumefaciens*.



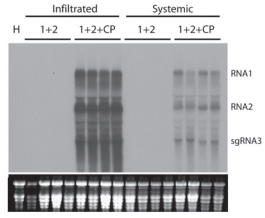
RNA3

In protoplast experiments no virus replication was observed unless subgenomic RNA3, encoding the CP, was included in the inoculum.



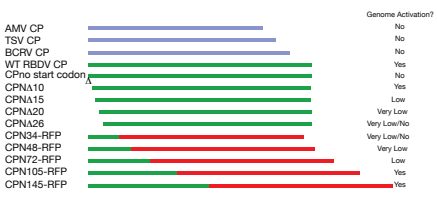
Agrobacterium

In whole plants infiltrated with Agrobacterium carrying RBDV full-length clones, co-expression with CP was required for detectable infection in either infiltrated or upper, non-infiltrated leaves. This is similar to the mechanism of replication initiation occurring with *Alfalfa mosaic virus* (AMV) and ilarviruses, and which is known as Genome Activation.



CP constructs

To identify specific regions of the CP that are required for genome activation constructs for agroinfiltration were made in which the RBDV CP gene was replaced by that of AMV, the ilarviruses *Tobacco streak virus* (TSV) or *Blackberry chlorotic ringspot virus* (BCRV), RBDV CP lacking a translation start codon, or other RBDV CP mutants missing various 5' sequences or having different 5' sequences fused to the red fluorescent protein (RFP). Successful infection was detected using an ELISA method.



Relative genome activation

Relative genome activation function of the various CP constructs. Data were taken from experiments using at least four plants per infiltration, and each plant sample was tested in duplicate. The bars (y axis) represent the average ELISA value for each construct expressed as a percentage of the average ELISA value of an infiltration containing wild type RBDV CP. The results show that the first fifteen amino acids of the RBDV CP are not required for genome activation, and that sequences downstream of the first 48 amino acids also are not absolutely required for genome activation. We are now in a position to carry out a more detailed investigation of the activation of replication by specific residues of the RBDV CP.