Genome variations in *Carnation Italian ringspot virus isolates* derived from *Prunus avium, Dianthus, Gypsophila* and other plant species and from surface waters

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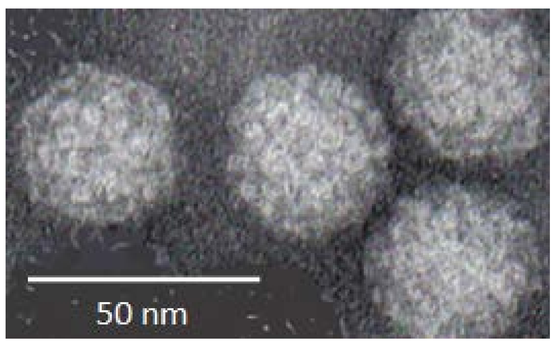


Fig. 1 Morphology of tombusvirus particles

Carnation Italian ringspot virus has been isolated for the first time in 1970 by Hollings et al. from carnations (isolate **CIRV-car**).

Its particles show the typical **tombusvirus morphology** (Fig. 1). **Serologically CIRV-car** is related to many other tombusviruses.

The **genome structure** of **CIRV-car** (Fig. 2b) resembles that of most other tombusviruses (Fig. 2a), except that the 5' terminal region of its ORF1/ORF1-RT (highlighted by a red color in Fig. 2b) shows very pronounced sequence differences to the corresponding regions in most other tombusviruses. A very similar deviating region is found in the genome of pelargonium necrotic spot virus (PelNSV) (Heinze et al., 2004; Fig. 3b)).

Tombusviruses induce in infected cells typical **multivesicular bodies (MVB)** (Figs. 2c and 2d) which are probably the site of viral RNA synthesis (DiFranco et al., 1984; Lesemann, 1991). With most tombusviruses the **MVB** originate from **peroxisomes.** The vesicles, the location of which is shown in Figs. 2c and 2d by white arrows, appear as invaginations of the single outer membrane of the **peroxisomes** (Fig. 2c). Only with **CIRV-car** and **PelNSV** the **MVB** develop from **mitochondria** (Fig. 2d). The vesicles, which are located between the outer and the inner **membrane** of the **mitochondria**, appear as invaginations of the outer **membrane** of the **mitochondria.** The black arrows in Fig. 2d point to remnants of the cristae of the **mitochondria.**

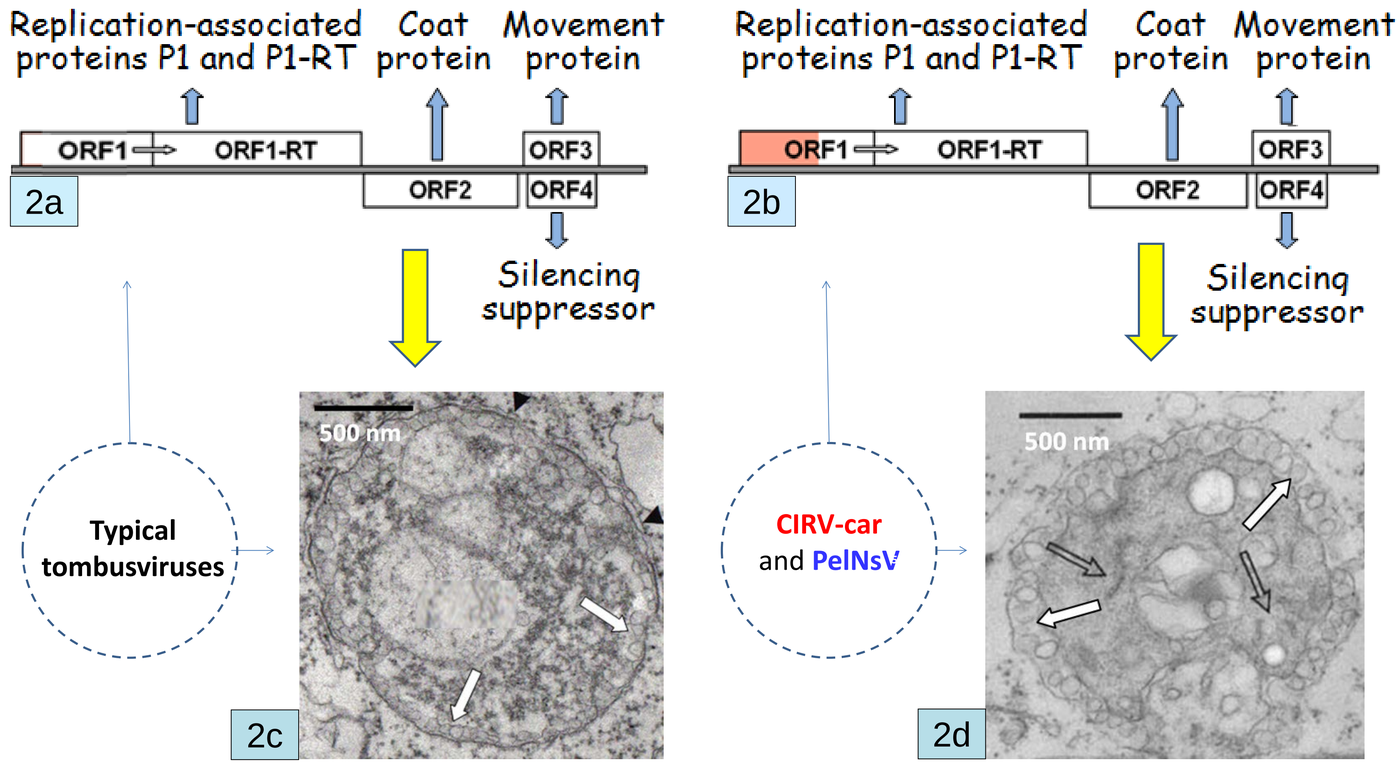


Fig. 2 **Genome structure** (2a and 2b) and **MVBs** (Fig. 2c and 2d) of the vast majority of tombusviruses (Fig. 2a and 2c) and of **CIRV-car** and **PelNSV** (Fig. 2b and 2d), respectively. For further details see text on the left side.

Burgyan et al. (1996) have used an infectious cDNA clone of **CIRV-car** to replace the deviating region in the 5' terminal area of its ORF1 by the corrspeonding region of a ‚normal' tombusvirus (Cymbidium ringspot virus). This hybrid virus induced the formation of **MVBs** which originated from **peroxisomes**. They concluded that the 5' terminal area of ORF1/ORF1-RT apparently determines whether the **MVB**s of a tombusvirus originate from **mitochondria** or from **peroxisomes**.

After the first isolation of **CIRV-car** in 1970, a number of other tombusvirus isolates have been described which were serologically indistinguishable from **CIRV-car.** Several of these isolates (i.e. OE11, OE14 and RSG, Fig. 3) were obtained from plantations of *Prunus avium* (Lesemann et al., 1989; Pfeilstetter, 1992). Infected plants showed typical symptoms of viral twig necrosis on shoot tips, leaves and fruit. Further isolates (Fig. 3) were obtained from Gypsophila (Koenig et al., 2004), spinach (Rabenstein et al., unpublished) and from a creek in a forested area (Büttner et al., 1987).

With the new isolates of CIRV, comparative molecular and cytopathological studies were done at the JKI in Braunschweig (Koenig et al., 2009). The amino acid sequences of the coat proteins of the new isolates show high percentages of sequence identity with the coat protein amino acid sequence of **CIRV-car** (Fig. 3a). They differ, however, greatly from the coat protein amino acid sequences of **PelNSV** and the other tombusviruses (shown in Fig. 3a only for tomato bushy stunt virus = TBSV). This confirms our earlier serological observations.

The sequences of the 82 N-terminal amino acids of P1 and P1-RT of the new CIRV isolates differ, however, considerably from the corresponding sequences of **CIRV-Car** and **PelNSV** (Fig. 3b). Smaller differences were found to the corresponding sequences of the other tombusviruses (shown in in Fig. 3b only for TBSV).

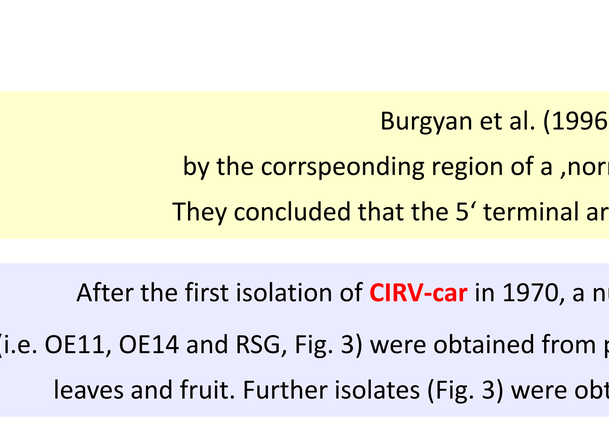
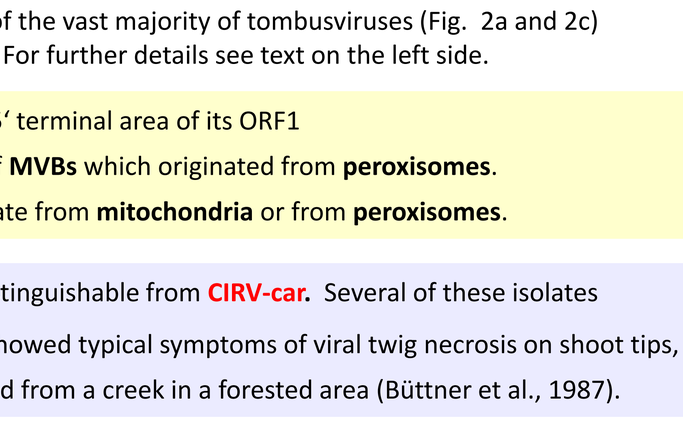


Fig. 3 Percentages of amino acid sequence identity

in the coat proteins → the amino acid sequence of **CIRV-car** shows a high degree of similarity with those of the new CIRV isolates and differs greatly from that of **PelNSV**

in the first 82 5' amino acids of the P1 and P1-RT proteins → the amino acid sequence of **CIRV-car** differs greatly from those of the new CIRV isolates, but resembles that of **PelNSV**



All new CIRV isolates induced **MVBs,** which originated from **peroxisomes** and not - as in the case of **CIRV-Car** - from **mitochondria** (Koenig et al., 2009)

Conclusion

Our observations confirm with natural isolates of CIRV a conclusion which has been reached previouly by Burgyan et al. (1996) on the basis of experiments with an artificially produced hybrid virus, i.e. that it is the 5' end of the P1 and P1-RT ORFs which determines whether the **MVBs** of tombusviruses are developping from **peroxisomes** or **mitochondria** (Koenig et al., 2009).

The genomes of **CIRV-Car** and of **PelNSV** are probably recombination products of typical tombusvirus genomes with genome portions of other, so far unknown viruses.

Literatur: Burgyan et al. (1996) J Gen Virol 77:1967; Büttner et al. (1987) J Phytopathol 118:131; Di Franco et al (1984) J Gen Virol 65: 1233; Heinze et al. (2004) Arch Virol 149:1527; Hollings et al. (1970) Ann Appl Biol 65:299; Koenig et al. (2004) Arch Virol 149:1733; Koenig et al. (2009) Arch Virol 154:1695; Lesemann et al. (1989) J Phytopathol 124:249; Lesemann (1991) Chapter 11 in: Electron Microscopy of Plant Pathogens. Springer Verlag, p 147; Pfeilstetter, E (1992) Dissertation, Technische Universität München.