Comparison of the Genome RNA Sequence Homology Between Cricket Paralysis Virus and Strains of Drosophila C Virus by Complementary DNA Hybridization Analysis

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SUMMARY

The sequence homology between the RNA genomes of Cricket paralysis virus (CrPV) and Drosophila C virus (DCV) strains have been compared using cDNA hybridization analysis. Results show 60 to 85% homology between DCV strains obtained from different geographical locations. There was no detectable sequence homology between DCV and CrPV.

Cricket paralysis virus (CrPV) and Drosophila C virus (DCV) are small RNA-containing viruses of insects which have been classified as members of the Picornaviridae (Matthews, 1982). Virions have a capsid diameter of 27 nm and a buoyant density of 1.34 g/ml in CsCl. CrPV and DCV each contain three structural proteins with mol. wt. of about 30 × 10³ (VPs 1, 2, 3) and DCV contains a fourth structural protein (VP4) of approximately 10 × 10³ (Moore et al., 1980; Moore et al., 1981 b). Virions of both CrPV and DCV contain a single-stranded, positive-sense, RNA genome with a mol. wt. of 2.8 × 10⁶, which is polyadenylated at the 3' end (Eaton & Steacie, 1980; Moore et al., 1981 a; Reavy et al., 1983). The primary product of protein synthesis is a precursor polyprotein which is subsequently processed to produce the virus structural proteins. CrPV and DCV can be distinguished by differences in the intracellular proteins expressed in infected cells as well as differences in host range and structural proteins (Plus et al., 1978; Moore et al., 1980, 1981a). DCV has been isolated in Morocco [Taroudant (DCV ธ) and Ouarzazate (DCV ธ)], France [Gif (DCV ธ), Vigier (DCV ธ) and Charolles (DCV ธ)] and the French Antilles (DCV ธ). Although these strains differ in host range and pathogenicity, they all have capsid proteins in common and the intracellular proteins that they induce are similar (Plus et al., 1978; Moore et al., 1982). All the DCV strains are serologically related to each other and to CrPV (Plus et al., 1978).

Clewley et al. (1983) compared the RNAs of the DCV strains by ribonuclease T1 fingerprinting and the six strains were found to have common large oligonucleotides. Two strains, DCV ธ and DCV ธ, were closely related, whilst DCV ธ was less related and the other three strains were only distantly related to the French strains. CrPV shared no common large oligonucleotides with DCV ธ (Pullin et al., 1982). Oligonucleotide fingerprinting, however, compares only about 15% of the RNA sequence. The present study compares the sequence relationship between the DCV strains, DCV ธ, DCV ธ, DCV ธ, DCV ธ and CrPV using the considerably more sensitive technique of complementary DNA (cDNA) hybridization.

CrPV and DCV were grown in Drosophila melanogaster cells and purified as described by Moore et al. (1980). The RNA was purified by digestion with proteinase K (200 μg/ml) in 10 mM-Tris–HCl pH 7.4 100 mM-NaCl, 1 mM-EDTA containing 1% (w/v) SDS for 1 h at 37 °C, followed by two extractions with phenol, two ether washes, and ethanol precipitation. RNA was pelleted, washed twice in 75% ethanol, dried and redissolved in sterile water. RNA prepared in this way gave a single discrete band in 1% agarose gels, in both non-denaturing and denaturing conditions. ³H-cDNA to CrPV, DCV ธ and DCV ธ RNA used in the Rثţ tests was prepared using random DNA fragments as a primer, and conditions for hybrid formation were as

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Fig. 1. Electrophoresis in a 1.4% alkaline agarose gel of $^{32}$P-labelled cDNA prepared against CrPV RNA, using oligo(dT)$_{12-18}$ as a primer (lane 1) or using random priming (lane 2). Lane 3 shows λ DNA, cut with HindIII and EcoRI, as molecular size (kbp) markers.

Fig. 2. The kinetics of hybridization of (a) DCV$_G$ cDNA with DCV$_G$ (●), DCV$_C$ (○), DCV$_T$ (■) and CrPV RNA (□); (b) DCV$_T$ cDNA with DCV$_T$ (●), DCV$_G$ (○), DCV$_C$ (■) and CrPV RNA (□). RNA and cDNA were prepared as described in the text. Hybridizations were at 60 °C for 2.5 h in 0.18 M-NaCl, 10 mM-Tris-HCl pH 7-0, 1 mM-EDTA, 0.05% (w/v) SDS. Hybrid formation was assayed using S$_1$ nuclease and adsorption to DEAE-cellulose filters (Maxwell et al., 1978).

described by Robinson et al. (1980). Hybrid formation was assayed using S$_1$ nuclease and adsorption to DEAE-cellulose filters (Maxwell et al., 1978). $^{32}$P-labelled cDNA was prepared using oligo(dT)$_{12-18}$ or random DNA fragments as a primer, under conditions described by Cann et al. (1983). $^{32}$P-cDNA was analysed in 1.4% alkaline agarose gels (McDonnell et al., 1977), using λ DNA cut with HindIII and EcoRI restriction endonucleases as markers.

Fig. 1 shows the autoradiograph of $^{32}$P-labelled cDNA synthesized from CrPV RNA, using either random priming or oligo(dT) as a primer. Random priming gave cDNA fragments less
than 300 base pairs in length and was therefore suitable for use in the $R_{0.0}$ tests. cDNA made against other virus RNAs under similar conditions has been shown to represent all RNA template sequences equally (Gould & Symons, 1977). By contrast, when oligo (dT)$_{12-18}$ was used as a primer, discrete bands of cDNA were seen, the largest of these corresponding to 8500 base pairs or a mol. wt. about $2.8 \times 10^6$. This suggested that full length cDNA was being synthesized, indicating that the complete RNA sequence was available to be copied.

Fig. 2(a) shows the kinetics of hybridization of DCVG cDNA with DCV$_G$, DCV$_O$, DCV$_T$ and CrPV RNA and Fig. 2(b) between DCV$_T$ cDNA and DCV$_T$, DCV$_G$, DCV$_O$ and CrPV RNA. At the highest concentration of RNA, in the homologous reactions, 65% (DCVG) and 60% (DCVT) of the cDNA became $S_1$ nuclease-resistant after hybridization. In the absence of RNA 6 to 8% of the cDNA was resistant. These values are taken to represent 100% and 0% hybridization respectively.

In Fig. 2(a), a single sharp transition was observed in the homologous reaction and gave a $R_{0.0}$ value (initial RNA concentration $\times$ time, for 50% hybridization) of $7.9 \times 10^{-3}$ mol.$\cdot$s/l. Hybridization with DCVG RNA also showed a single sharp transition but at one-quarter the rate of the homologous reactions. Since the maximum amount of hybrid formation was 85% that of the homologous reaction, DCV$_G$ probably has at least this amount of sequence homology with DCVG. Hybridization with DCVT RNA occurred only at very high values of $R_{0.0}$ and had a $R_{0.0}$ value of $1.41 \times 10^{-1}$ mol.$\cdot$s/l, indicating that DCVT and DCVG may share about 6% common nucleotide sequence (compare Gould & Symons, 1977). When DCVT cDNA was hybridized with the RNAs of DCVT, DCVO, DCVG and CrPV (Fig. 2b), similar $R_{0.0}$ values ($3.16 \times 10^{-2}$ to $3.9 \times 10^{-2}$ mol.$\cdot$s/l) were obtained, with the exception of CrPV RNA. The extent of hybrid formation was 71% for DCVG and 60% for DCVO. CrPV RNA did not hybridize with either DCVG or DCVT cDNA, even at high values of $R_{0.0}$, implying that any sequence homology between DCV and CrPV. Hybridization of CrPV cDNA with CrPV RNA gave a single sharp transition with a $R_{0.0}$ value of $3.54 \times 10^{-3}$ mol.$\cdot$s/l, whilst no hybridization was detected between CrPV cDNA and DCVG or DCVT RNA (data not shown). Since we did not observe any biphasic component in rates of hybridization we concluded that few, if any, of the RNAs had reiterated sequences.

Our experience with DCV and CrPV is consistent with the oligonucleotide fingerprinting data (Pullin et al., 1982); these viruses have little, if any, sequence homology. Hence, the serological interaction between DCV and antiserum raised against CrPV (and vice versa) cannot be explained by similarities in extensive regions of the primary sequence. By contrast, the DCV isolates from geographically distinct regions have many properties in common, with the French isolates (C and G) being most, and the Moroccan isolate (T) least similar in the sequence of their RNA. In infected D. melanogaster cells the different DCV isolates induce proteins with similar mol. wt. and partial proteolysis products. Consequently, the distinguishable RNA sequences revealed by cDNA hybridization analysis probably occur in regions of the genome that are not expressed. Recognizing the mechanism of RNA translation into a polyprotein (Moore et al., 1980), the distinguishable sequences probably occur at the 3' and 5' ends. Hence, to determine fully the differences between the strains it will be necessary to perform sequence analysis of the genome.

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**REFERENCES**


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