*J. gen. Virol.* (1985), **66**, 1347–1351. *Printed in Great Britain*



Key words: *peanut clump virus/translation* in vitro/*polypeptides*

# Translation Products of RNA from Indian Peanut Clump Virus

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(*Accepted 18 February 1985*)

SUMMARY

When RNA from particles of Indian peanut clump virus (IPCV) was translated using messenger-dependent reticulocyte lysate the largest product had a mol. wt. of about 195000 and the other main products had mol. wt. of 143000 and 24500. The 24500 mol. wt. polypeptide co-migrated with IPCV coat protein and was the only product that reacted with antiserum to IPCV particles. Translation of separated RNA species showed that the 195000 mol. wt. and 143000 mol. wt., as well as some smaller less abundant products, arose from translation of RNA-1 (mol. wt. $1{\cdot}9 \times 10^6$) whereas RNA-2 (mol. wt. $1{\cdot}6 \times 10^6$) was translated to give only the 24500 mol. wt. product Translation of RNA of mol. wt. $0{\cdot}5 \times 10^6$ to $0{\cdot}9 \times 10^6$ gave a 50000 mol. wt. product that was present and sometimes prominent in translation products of unfractionated RNA. A product of about 20000 mol. wt. was made by translation of RNA of about $0{\cdot}2 \times 10^6$ mol. wt. IPCV resembles the viruses with bipartite genomes whose larger RNA species is translated into large polypeptides and whose coat protein gene is on the smaller RNA species. The other translation products may be those of subgenomic messenger, or of satellite, RNA species.

Indian peanut clump virus (IPCV) has rod-shaped particles mainly of two lengths (approx. 249 and 184 nm) (Reddy *et al.*, 1983) which contain RNA of mol. wt. $1{\cdot}9 \times 10^6$ (RNA-1) and $1{\cdot}6 \times 10^6$ (RNA-2) respectively (D. V. R. Reddy, D. J. Robinson & B. D. Harrison, unpublished). Its particles are similar in size, but serologically unrelated, to those of peanut clump virus (PCV) from West Africa (Reddy *et al.*, 1983). Moreover, there is evidence that both viruses are transmitted by *Polymyxa graminis* (Thouvenel & Fauquet, 1981*a*; D. V. R. Reddy, unpublished observations) and that their particle morphology resembles that of soil-borne wheat mosaic (SBWMV) and beet necrotic yellow vein (BNYVV) viruses, both of which are transmitted by *Polymyxa* spp. (Estes & Brakke, 1966; Thouvenel & Fauquet, 1981*b*). In this paper we describe the polypeptides made when IPCV RNA is translated *in vitro* and compare these results with those of similar studies with other *Polymyxa*-transmitted viruses.

The Ludhiana isolate of IPCV (Reddy *et al.*, 1983) was used throughout. Particles were precipitated from chloroform-clarified sap of systemically infected *Nicotiana clevelandii* plants by adding NaCl to 0·2 M and polyethylene glycol 6000 to 4%, resuspended and further purified by ultracentrifugation through a sucrose cushion. In most experiments particles were also centrifuged in 10 to 40% (w/v) sucrose density gradients prepared in 0·2 M-urea, 30 mM-$KH_2PO_4$, 20 mM-$Na_2B_4O_7$. RNA was extracted from particles suspended in 0·06 M-NaCl, 0·01 M-Tris–HCl, 3 mM-EDTA, pH 8·6, by adding SDS to 0·5%, incubating for 15 min at about 20 °C and extracting protein with phenol : *m*-cresol (9 : 1, v/v) containing 0·1% 8-hydroxyquinoline. RNA was then recovered by ethanol precipitation and re-precipitated from 0·3 M-potassium acetate.

Translation mixtures contained 66% (v/v) reticulocyte lysate (P & S Biochemicals, Liverpool, U.K.), 82 mM-KCl, 0·4 mM-$MgCl_2$, 8 mM-creatine phosphate, 0·4 mM-dithiothreitol, 40 μM each essential amino acid except leucine, 410 μCi/ml L-[4,5-$^3$H]leucine (124 Ci/mmol; Amersham)

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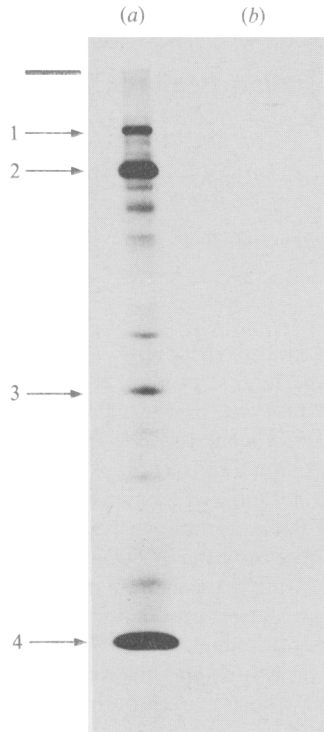


Fig. 1

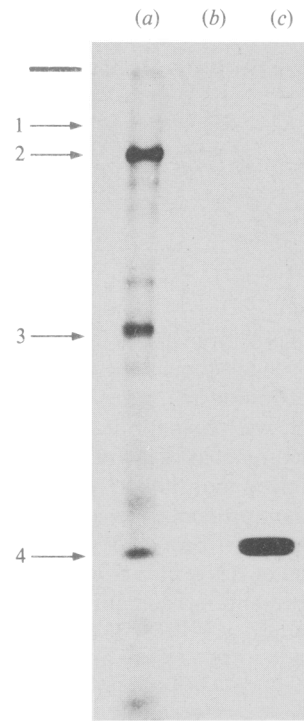


Fig. 2

Fig. 1. Fluorogram of IPCV RNA translation products made in reticulocyte lysate kept at 30 °C for 90 min containing (*a*) 78 μg/ml IPCV RNA or (*b*) no added RNA. Numbered arrows indicate translation products of mol. wt. 195000 (1), 143000 (2), 50000 (3) and 24500 (4). Arrow 4 also indicates the position of unlabelled virus coat protein in the corresponding stained part of the gel. The bar marks the top of the gel.

Fig. 2. Fluorogram showing the reaction between IPCV RNA translation products made at 37 °C and antiserum to IPCV. (*a*) Unfractionated translation products (the lane contains about 3% of the amount used in the serological reaction); (*b*) reaction with TBRV antiserum; (*c*) reaction with IPCV antiserum. Numbered arrows and bar are as in Fig. 1.

and 78 μg/ml RNA. Mixtures were kept at 30 °C or 37 °C, usually for about 90 min, and the reaction was stopped by adding 4 vol. 2% SDS, 0·15 M-dithiothreitol, 10% glycerol, in 0·125 M-Tris–HCl pH 6·8 (sample buffer). Samples were then heated in boiling water for 2 min and stored frozen. Translation products were analysed by electrophoresis in 10% polyacrylamide gels using a discontinuous buffer system (Laemmli, 1970) and detected by fluorography of gels impregnated with En$^3$Hance (New England Nuclear).

The products of translation are shown in Fig. 1(*a*) and Fig. 2(*a*). The largest polypeptide formed was of mol. wt. 195000 (arrowed 1) and the other abundant products were of mol. wt. 143000 and 24500 (arrowed 2 and 4). A 50000 mol. wt. polypeptide (arrowed 3) was prominent in some translation products (e.g. Fig. 2*a*) and was present but less abundant in others (Fig. 1*a*). Band 4 co-migrated with protein extracted from purified virus particles. Translation at 30 °C (Fig. 1*a*) resulted in proportionately more synthesis of 195000 mol. wt. polypeptide than did translation at 37 °C (Fig. 2*a*). No obvious bands were detected in protein from mixtures incubated without added RNA (Fig. 1*b*). The addition of 10 μg/ml calf liver tRNA (Boehringer) to translation mixtures did not alter the species of polypeptides made or change their relative abundance.

In further tests, the translation products were mixed with antiserum either to IPCV (homologous titre 1/600 in precipitin ring tests) or, as a control, with antiserum to tomato black

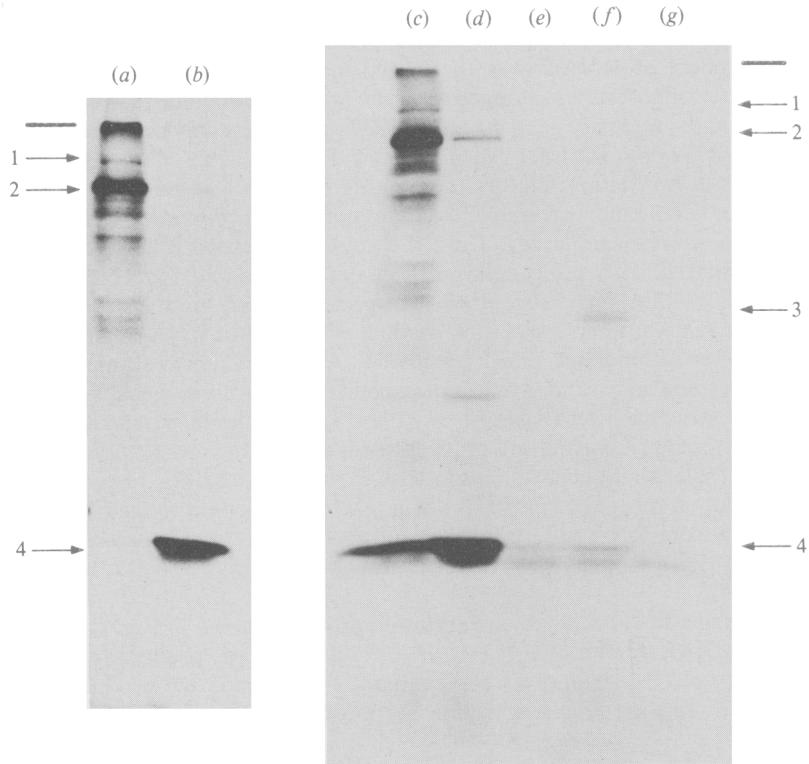


Fig. 3. Fluorogram showing translation of IPCV RNA separated in agarose gels. (*a*, *b*) Expt. 1 in which slices containing RNA-1 and RNA-2 were separated by a discarded region of gel; (*c* to *g*) Expt. 2 in which contiguous slices were used and in which RNA-1 and RNA-2 were poorly separated. (*a*) RNA-1; (*b*) RNA-2; (*c*) RNA-1; (*d*) RNA-2; (*e*) RNA of mol. wt. approx. $0{\cdot}9 \times 10^6$ to $1{\cdot}5 \times 10^6$; (*f*) RNA of mol. wt. approx. $0{\cdot}5 \times 10^6$ to $0{\cdot}9 \times 10^6$; (*g*) RNA of mol. wt. approx. $0{\cdot}3 \times 10^6$ to $0{\cdot}5 \times 10^6$. Numbered arrows and bars are as in Fig. 1.

ring virus (TBRV). After translation, samples of 40 μl were mixed with 13·4 μl 8% SDS, heated in boiling water for 2 min and mixed with 0·54 ml 1% Triton X-100 in 150 mM-NaCl, 50 mM-Tris–HCl pH 7·8, 2 mM-EDTA (TNTE). The sample was then divided into two parts which were mixed with 1 μl antiserum to IPCV or to TBRV. After 2·5 h at about 20 °C, 2·5 mg Protein A–Sepharose (Sigma) was added to each of the samples which were then shaken for 1 h at about 20 °C. The Sepharose was recovered by centrifugation, washed once in TNTE, twice in 0·95 M-NaCl in TNTE, and four times in TNTE. The final pellet of Sepharose was then suspended in 50 μl sample buffer and heated in boiling water for 3 min. Samples of the suspension were analysed by electrophoresis in 10% polyacrylamide gels.

Fig. 2 shows the result of one such experiment. The unfractionated reticulocyte lysate mixture produced the four products arrowed in Fig. 1 and several subsidiary bands (Fig. 2*a*). None reacted with antiserum to TBRV (Fig. 2*b*) but the 24500 mol. wt. polypeptide reacted with antiserum to IPCV (Fig. 2*c*). This polypeptide is therefore the virus particle protein.

To determine which of the two main RNA species carries the particle protein gene, RNA was separated by electrophoresis in 1·5% low-melting point agarose (Sigma) in 89 mM-Tris, 89 mM-boric acid, 2·5 mM-EDTA, pH 8·3. Bands were then located by staining parallel tracks with ethidium bromide and unstained regions of the gel were sliced to isolate each RNA species. Pieces of agarose were then melted and mixed with reticulocyte lysate and other components of the translation mixture as described by Brandt & Hackett (1983). Each mixture was as described above for conventional translation except that the RNA concentration was about 10 μg/ml.

The results (Fig. 3*a*, *b*) show that translation of RNA-1 yields the 195000 and 143000 mol. wt. polypeptides as well as several others with mol. wt. between 81000 and 140000, and that the only translation product of RNA-2 was the 24500 mol. wt. product (coat protein). In another experiment electrophoresis was stopped after the bromophenol blue in the sample had migrated only 20 mm and the region of gel between RNA-2 and the dye marker was cut into three pieces. These, and the poorly separated RNA-1 and RNA-2, were then translated. The RNA concentrations in translation mixtures containing the pieces of gel between the RNA-2 band and the bromophenol blue were much less than in mixtures containing the RNA-1 and RNA-2 because the gel pieces were larger. Thus, the abundance of the different translation products cannot be compared. The results confirm those described above for RNA-1 and RNA-2 (Fig. 3*c*, *d*) and show that the 50000 mol. wt. protein found in the translation products of unfractionated RNA is not a translation product of RNA-1 or RNA-2 but of an RNA of about $0{\cdot}5 \times 10^6$ to $0{\cdot}9 \times 10^6$ mol. wt. (Fig. 3*f*, arrowed). A band of about this mol. wt. was detected in some RNA preparations, and would correspond with a minor peak in the particle length distribution of virus particles (Reddy *et al.*, 1983; I. M. Roberts, personal communication). In this experiment some 24500 mol. wt. polypeptide is evident in lanes (*c*), (*e*) and (*f*) as well as (*d*), probably because of slight contamination with RNA-2. In addition, a polypeptide of mol. wt. 22000 is evident in lanes (*e*), (*f*) and (*g*) (Fig. 3). In another experiment this polypeptide was translated only from RNA with a mol. wt. of about $2 \times 10^5$. In the translation products of unfractionated IPCV RNA (Fig. 1) it would probably be obscured by the adjacent band of coat protein.

The results show that, of the main translation products shown in Fig. 1, the two largest, mol. wt. 195000 and 143000, are of RNA-1. The 195000 mol. wt. product represents most of the coding capacity of RNA-1 and the predominant 143000 mol. wt. product is therefore either from a different reading frame or comprises most of the sequence of the 195000 mol. wt. product.

In contrast to RNA-1, RNA-2 has a mol. wt. of about $1{\cdot}6 \times 10^6$ but the only translation product detected was coat protein. Thus, most of the coding capacity of RNA-2 (about 85%) is unaccounted for. It seems improbable that such large regions of RNA would survive evolution without a coding role and therefore other cistrons are likely to be present on IPCV RNA-2. One possibility is that these regions are transcribed in infected cells to give mRNA molecules but that, like the mRNA for tobacco mosaic virus coat protein (Hirth & Richards, 1981), these lack the sequence necessary for particle assembly and are thus absent from RNA extracted from virus particles.

The 50000 mol. wt. polypeptide is apparently the translation product of an RNA of mol. wt. about $0{\cdot}5 \times 10^6$ to $0{\cdot}9 \times 10^6$ and not of intact RNA-1 or RNA-2. This RNA may be either part of the untranslated region of RNA-2 or an overlap region on RNA-1. Alternatively, it may be a satellite RNA having little or no sequence homology with the genome RNA but becoming coated in IPCV coat protein and perhaps using its replication machinery. It would thus be similar to the satellite RNA found in some isolates of viruses belonging to several other virus groups (Murant & Mayo, 1982). Similar reasoning applies to the 20000 mol. wt. polypeptide.

The results of these translation experiments reinforce the similarity between IPCV and other viruses transmitted by *Polymyxa* (Shirako & Brakke, 1984). Thus, the translation product of RNA-1 of SBWMV is a 200000 mol. wt. polypeptide (Hsu & Brakke, 1983), and coat proteins of SBWMV (Hsu & Brakke, 1983) and BNYVV (Richards *et al.*, 1985) are translation products of a smaller RNA molecule. Also, like IPCV RNA, BNYVV RNA contains species smaller than RNA-2 (which codes for coat protein) that have messenger activity.

However, although such a correspondence of biological and molecular properties would seem to support the idea of classifying these viruses in a 'furovirus' group (Shirako & Brakke, 1984), this may be premature because whereas BNYVV RNA is polyadenylated (Putz *et al.*, 1983), IPCV RNA did not bind to oligo(dT)–cellulose in buffer containing 0·4 M-NaCl, suggesting that, like SBWMV RNA (Hsu & Brakke, 1985), it is not polyadenylated. Thus, further work on the genome strategy of IPCV and like viruses is needed both to understand their biology and to test the extent of their affinities with each other and with other more extensively studied viruses.

We thank Anne Jolly for skilled technical assistance. This paper was submitted as Journal Article no. 487 by the ICRISAT.

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(*Received 12 November 1984*)