The Polypeptides Induced in Drosophila Cells by a Virus of Heteronychus arator

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SUMMARY

The virus isolated from the black beetle, Heteronychus arator, is an icosahedral ribovirus with a diam. of 30 nm and contains one major structural polypeptide of mol. wt. 40,000. The virus can be grown in Drosophila melanogaster cells and also titred in this line using a plaque assay method. Infected Drosophila cells pulsed with $^{35}\text{S}$-methionine in the presence of actinomycin D induced three virus polypeptides of mol. wt. 110,000, 40,000 and 8000. Short pulses, chases with excess non-radioactive methionine and pre-pulse treatments with protease inhibitors failed to demonstrate processing of virus-induced polypeptides.

Black beetle virus (BBV) was first isolated from a black beetle, Heteronychus arator Fabricus (Coleoptera: Scarabaeidae), by Longworth & Archibald (1975) during routine surveys for pathogens of this beetle in New Zealand in the 1973–74 season. The virus is icosahedral in shape, 30 nm in diam. and lacks any obvious surface features. It has a sedimentation coefficient of 137S, a buoyant density in CsCl of 1.33 g/ml and RNA:protein ratio of 28:2:71:8 (Longworth & Carey, 1976). BBV contains two species of single-stranded RNA in equimolar amounts, which sediment at 22S and 15S corresponding to mol. wt. of $1.0 \times 10^6$ and $0.5 \times 10^6$ respectively. The virus was found to be infective for numerous species in the Lepidoptera and Coleoptera and also for Drosophila melanogaster cells in tissue culture (P. Scotti, personal communication). It was not found to be infective for suckling mice or BHK21 cells (Longworth & Carey, 1976). The presence of a split genome and similar buoyant densities and mol. wt. indicates that this virus is strikingly similar to Nodamura virus (Newman et al., 1978).

BBV was received from P. D. Scotti (Entomology Division, D.S.I.R., Auckland, New Zealand). D. melanogaster cells were grown in monolayers at 28 °C in 75 cm$^2$ plastic flasks (Nunc Plastics) for virus growth, and in 25 cm$^2$ flasks for radiolabelling experiments. Schneider’s Drosophila medium (revised; Gibco) containing 10% foetal calf serum, 100 units/ml penicillin–streptomycin, 40 μg/ml kanamycin (Gibco) and 50 μg/ml gentamicin (Nicholas Laboratories, Slough, U.K.) were used routinely. Confluent monolayers of cells were infected with 50 μl of the initial stock virus, which was allowed to adsorb for 1 h at 28 °C, after which time 5 ml maintenance medium (2% foetal calf serum and antibiotics) was added to each infected monolayer. The cells were maintained at 28 °C for 5 days, by which time c.p.e. was apparent in the form of floating and rounded cells. Purified virus was prepared as previously described (Moore et al., 1980).

The seed virus was initially titred using a 50% TCID$_{50}$ method as previously described (Moore et al., 1980). However, because this method was found to be inaccurate due to the growth inhibition around the end-point being difficult to assess, a plaque assay method was developed using Drosophila cells grown as monolayers in 5.8 cm plastic Petri dishes (Nunc Plastics). After the 1 h adsorption period the inocula were removed and the infected monolayers overlaid with 2 ml 0.75% agarose (Sea-Plaque, Marine Colloids, Rockland, Me., U.S.A.). After 4 days at 28 °C in a humidified atmosphere the plaques were visualized.
by the addition of a further 2 ml 0.75% agarose containing 2% neutral red (Flow Laboratories) for a further 6 h. Plaque numbers followed serial dilutions and the titres tended to be high, normally in the region of $5 \times 10^8$ p.f.u./ml.

For radiolabelling of the intracellular polypeptides 25 cm$^2$ monolayers of Drosophila cells were infected with 100 p.f.u./cell for 1 h at 28 °C. The inoculum was removed and 2 ml maintenance medium containing 5 µg/ml actinomycin D was added to each culture. At various stages post-infection, the overlay was removed and the monolayers washed with 2 × 2 ml methionine-deficient medium before incubating for 30 min in the same medium. After this starvation period the cells were pulsed with 100 µCi $^{35}$S-methionine (1350 Ci/mmol) (The Radiochemical Centre, Amersham, U.K.) for 30 min. Post-pulse cells were resuspended in ice-cold maintenance medium and pelleted at 2000 g for 5 min in an MSE Chilspin. Proteins were prepared for electrophoresis by resuspending the cells in 250 µl solubilization buffer containing 2% (w/v) SDS, 2% (v/v) 2-mercaptoethanol, 15% (v/v) glycerol and 0.001% bromophenol blue as a tracking dye. Samples were immediately heated for 2 min at 100 °C and for a further 30 s immediately before electrophoresis. Proteins were separated on 15 cm-long polyacrylamide slab resolving gels using the discontinuous tris–glycine buffer system described by Laemmli (1970). Gels were fixed in 45% methanol, 9% acetic acid and processed for fluorography (Laskey & Mills, 1975). Mol. wt. were determined using the direct comparative method of Shapiro et al. (1967).

The sequence of production of virus-induced polypeptides in infected Drosophila cells is seen in Fig. 1. Three virus-specific proteins with mol. wt. of 110,000, 40,000 and 8000 respectively (numbered 1, 2 and 3) were apparent in infected cells. These proteins appeared by 2 h post-infection and there was no evidence for a sequential appearance. Longer periods of infection before pulsing did not reveal any additional polypeptides. Virus proteins were still being actively synthesized at 24 h post-infection.
Fig. 2. Attempted pulse-chase labelling of virus polypeptides. Infected *Drosophila* cells were pulsed for 15 min at 4 h post-infection and chased for (a) 30 min, (b) 120 min and (c) 180 min, with excess non-radioactive methionine before separation of the $\text{^35S-methionine-labelled polypeptides on a 15\% polyacrylamide slab gel;}$ (d) is a 15 min pulse at 4 h post-infection without a pulse-chase.

The 40000 mol. wt. polypeptide co-migrated with the capsid protein, and there is no evidence of any immediate precursor of this protein. Newman *et al.* (1978) found with cell-free protein synthesis of the RNA from the related Nodamura virus that two major proteins of 105 000 and 43 000 mol. wt. were obtained. These authors presented evidence that the 43 000 mol. wt. protein was the precursor of the 40 000 mol. wt. capsid protein. They also suggested that the high mol. wt. protein of Nodamura virus synthesized in the cell-free system in response to the larger RNA molecule is a polymerase, but there was no evidence for a low mol. wt. protein in that system to correspond with the protein which we found in infected cells. This third protein presents difficulties in explaining how three proteins are being synthesized from two RNA species. If protein processing of one of the two protein species synthesized from the two RNAs is occurring, this should be visualized by pulse-chase. Fig. 2 shows there is no evidence of processing of any of the proteins after a 15 min pulse when the radioactive $\text{^35S}$ is chased with a 1000 times excess of cold methionine for up to 4 h. There was still some residual host-coded proteins being synthesized in these samples, as can be seen in Fig. 2, particularly a protein of a weight just below that of the highest mol. wt. virus-induced protein. With shorter pulses, i.e. 5 and 10 min, there is still no evidence that any of the proteins are being derived from higher mol. wt. proteins.

The possibility of any protein processing occurring was further tested using various protease inhibitors and amino acid analogues to attempt to inhibit any cleavages. However, addition of iodoacetamide (final concentration 2 mm), which has proven particularly successful in inhibiting the cleavages of high mol. wt. protein of insect entero-like viruses in *Drosophila* cells (N. F. Moore *et al.*, unpublished results), had no effect upon the polypeptide profile (data not shown). Short pulses, pulse-chases and cleavage inhibitors failed to demonstrate protein processing, for example, for the formation of the 110 000 and 8000 mol. wt. proteins from a polyprotein synthesized from the larger RNA. However, it is possible that there is an internal initiation site on either RNA. It is difficult at this stage to postulate a
function for a protein of mol. wt. 8000. There is no obvious parallel with VP4 of the mammalian picornaviruses and there is no evidence of this protein nor the high mol. wt. protein being incorporated into the virus structure.

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REFERENCES


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