Replication Process of the Parvovirus H-1

IV. H-1-Specific Proteins Synthesized in Synchronized Human NB Kidney Cells

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The synthesis of two virus-induced proteins, VP1 (mol wt 92,000) and VP2' (mol wt 72,000), was detected in the cell and in purified virions from H-1 parvovirus propagated in synchronous cell cultures.

We previously reported that H-1 parvovirus, propagated in unsynchronized hamster embryo (HE) cells, showed a total molecular weight of virus capsid proteins which exceeded the coding capacity of its genome (7); this finding has been reported for other parvoviruses as well (4, 5, 14, 18). Since our initial attempts to detect virus-induced proteins in HE cells were hampered by a high background of cellular protein synthesis, we have reexamined the synthesis of H-1-induced proteins in synchronized NB cells (SV-40-transformed newborn human embryo kidney [16]). In this system, the yield of infectious virus is similar, but 10-fold larger amounts of hemagglutinating protein are synthesized. The kinetics of H-1 hemagglutinin, DNA, RNA and infectious virus synthesis are similar to those described for HE cells (12).

Identification of virus-induced proteins (VP) was achieved by co-electrophoresis of ^3H -labeled proteins from H-1-infected nuclei and those from ^{14}C -labeled uninfected nuclei with subsequent plotting of the $^3\text{H}/^{14}\text{C}$ ratios (Fig. 1A and B). The most striking finding was that two instead of the three virus capsid proteins previously described were detected in either the infected NB nuclear lysates or whole cell lysates (not shown). They corresponded in size to VP1 (mol wt 92,000) and VP2' (mol wt 72,000), formerly termed A and B proteins, respectively (7). VP1 averaged $25.3 \pm 1.8\%$ and VP2' averaged $74.7 \pm 1.8\%$ of the total viral-induced protein synthesis as determined by the radioactivity in the peaks. VP3 (mol wt 56,000), originally termed C protein when observed in H-1 preparations from unsynchronized NB cells or HE cells (7), and in purified rat virus (RV) preparations from randomly-grown rat nephroma cells (15), was not found in either the nuclear or whole cell lysates from infected NB cells.

The electrophoretic conditions used here will resolve the protein band VP2 into the previously described (8) VP2' (mol wt 72,000) and VP2

(mol wt 69,000) fractions (Fig. 2). When [^{14}C]amino acid-labeled proteins from purified H-1 propagated in synchronized NB cells were co-electrophoresed with unlabeled H-1 from randomly grown HE cells, the ^{14}C radioactivity was associated with the VP1 and VP2' bands stained by Coomassie blue, but not the VP2 band (Fig. 2).

The labeling pattern of viral proteins recovered from whole virions made in NB cells labeled and chased at different times after infection was also studied (Fig. 3). Again, only two distinct polypeptides, VP1 ($20.4 \pm 1.4\%$) and VP2' ($79.6 \pm 1.4\%$), were detected. These virion polypeptides migrated in the same relative positions in the gel as the virus-induced proteins in infected cells (Fig. 1) or as the ^{14}C -labeled proteins from purified H-1 (Fig. 2). VP3 was therefore conspicuously absent from our synchronized NB cell system. When we then purified labeled H-1 propagated in either HE synchronized as described (19) or human chondrosarcoma cells synchronized by the same method used for NB cells (Fig. 1) and examined the proteins produced, we found that these virions also contained only VP1 and VP2'. Two other parvoviruses, LU III and MVM, have been reported recently to consist of two polypeptides (ref. 2 and P. Tattersall, personal communication, respectively). We are currently investigating the origin and significance of VP3.

We have found that VP2' can be converted to a form which comigrates with VP2 by subjecting purified virus to a limited proteolytic treatment. If comigration of the two proteins indicates identity then this implies that the VP2 polypeptide is contained in the larger VP2' polypeptide (Kongsvik and Ellem, manuscript in preparation). This also implies that the proteolytic trimming to produce VP2 does not occur under the conditions used for synchronous infection. VP2 from purified H-1 produced in HE cells can also be resolved into VP2' and

FIG. 1. *H-1*-specific proteins synthesized in NB cells. NB cells were propagated in Eagle medium and synchronized by a double methotrexate block (3). At the end of the second 24-h block, the cells were infected with *H-1* at an MOI of 30 PFU per cell. At indicated times, the infected cultures were labeled with [³H]protein hydrolysate (25 μ Ci/ml) and uninfected, control cultures with [¹⁴C]protein hydrolysate (5 μ Ci/ml). After the labeling period the cultures were combined and the nuclei isolated by the method of Walter et al. (22). The nuclear pellets were lysed with 1% sodium dodecyl sulfate-1% mercaptoethanol in 0.01 M phosphate buffer, pH 7.2, for 1 min at 100 C and electrophoresed for 17 to 18 h on 10% gels using the system described by Summers et al. (17). Gel slices (1 mm) were allowed to swell overnight at 50 C in 1 ml of a 9:1 NCS-H₂O mixture (23) and counted. Sodium dodecyl sulfate-disrupted, purified *H-1*, the proteins of which have previously been characterized with respect to molecular weight (7), was run on parallel gels and the polypeptides stained with Coomassie Blue to determine their relative positions in the gel and to allow estimates of the molecular weight of the virus-induced proteins. (A) Proteins from nuclear lysates of cells labeled 13 to 14 h postinfection; (B) proteins from nuclear lysates of cells labeled 14 to 15 h postinfection, followed by a chase with unlabeled amino acids for 1 h. The ³H/¹⁴C ratios are illustrated above the respective fractions. Migration is from left to right.

FIG. 2. Coelectrophoresis of H-1 virion proteins from synchronized NB cells and randomly-growing HE cells. ^{14}C -labeled virus was purified by the method of Rhode (12) from infected NB cells harvested 30 and 96 h postinfection. The virions were then disrupted and analyzed on 10% sodium dodecyl sulfate-polyacrylamide gels in the presence or absence of disrupted virions propagated in unsynchronized HE cells. Gels were stained with Coomassie blue (7) and photographed. Following photography, the protein bands were cut out, digested with NCS and assayed for ^{14}C activity. (1) 30 h NB-H-1 plus unlabeled HE-H-1; (2) 30 h NB-H-1 only; (3) 96 h NB-H-1 plus HE-H-1; (4) 96 h NB-H-1 only; (5) unlabeled HE-H-1 used as carrier.

FIG. 3. H-1-specific proteins from disrupted, ^3H -labeled purified virions propagated in NB cells. NB cells were propagated, infected and labeled as described in Fig. 1. H-1 was purified from cells (12) that were pulse-labeled with ^3H amino acids at 0 to 8 (A), 8 to 16 (B), and 16 to 24 h postinfection (C), and then were chased with cold amino acids until the cells were harvested at 30 h postinfection. Virions were disrupted and the capsid proteins analyzed electrophoretically as described in Fig. 1.

VP2 on polyacrylamide gels prepared with ethylene diacrylate in place of bisacrylamide, as well as by using the discontinuous buffer system of Neville (11).

It may be noted that when infected NB cells were treated with the metabolic inhibitors, actinomycin D, cytosine arabinoside, or bromodeoxyuridine before HA-DNA synthesis (12), VP1 and VP2 synthesis was inhibited along with the inhibition of H-1 hemagglutinin and H-1 antigen synthesis as previously described (13).

Our findings have led us to a reconsideration of H-1 structure. Given that H-1 has icosahedral symmetry (6), and a reported capsid mol wt of 5×10^6 (10), then by the principles of Caspar and Klug (1) and the data described here, the most satisfactory capsid composition would be 60 VP2 and 12 VP1 polypeptides. The previously reported fivefold symmetry (6) makes 12 pentamers of VP2 with a molecule of VP1 situated at the vertex of each pentamer the most probable capsomere structure, instead of the 32 postulated for parvoviruses at a time when the virus proteins had not been thoroughly examined (6, 21). A model for a parvovirus constructed of 12 capsomeres was originally suggested by Mayor et al. (9) and has also been postulated for the phage ϕ X174 (20).

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