Structural Phosphoproteins Associated with Measles Virus Nucleocapsids from Persistently Infected Cells

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SUMMARY

Measles virus nucleocapsids were labelled with $^3$H-amino acids and $^{32}$P-orthophosphate, and purified from the cytoplasm of persistently infected human amnion cells (AV + ). When analysed by SDS-PAGE, the two major capsid-associated polypeptides (P, mol. wt. 69,000, and NP, mol. wt. 60,000) were shown to be phosphorylated. Subsequent characterization of the phosphorylated polypeptides by acid hydrolysis and high voltage paper electrophoresis showed that serine and threonine were the major phosphorylated amino acid species. The similarities between the peptide phosphorylation patterns obtained in these studies and those reported earlier for the virus phosphoproteins produced in acute infections (Robbins & Bussell, 1979) indicate that major phosphorylative modifications of the capsid proteins are not involved in measles virus persistence in AV 3 cells.

Previous studies conducted by Mountcastle & Choppin (1977) have demonstrated that measles virus nucleocapsids isolated from productively infected cells contain two major structural polypeptides: P, mol. wt. 69,000, and NP, mol. wt. 60,000. In addition to these two protein species, a variety of minor capsid-associated polypeptides has been reported (Stallcup et al. 1979). These minor capsid-associated species include a high mol. wt. protein (L, mol. wt. 200,000), the virus membrane protein (M, mol. wt. 36,000) and a number of even less prevalent peptides which appear to be degradation products of the major capsid protein, NP.

In a previous communication (Robbins & Bussell, 1979), we identified P and NP as phosphoproteins in measles virus capsids and virions from acutely infected cells. Characterization of these phosphorylated polypeptides by acid hydrolysis and high voltage paper electrophoresis showed that P contains only phosphorylated serine residues, while NP possesses both phosphoserine and phosphothreonine – the former predominating.

In this communication, we describe studies conducted on measles virus nucleocapsid phosphoproteins produced in a persistently infected human cell line (AV 3/MV). The results of our studies indicate that phosphorylative modification of the major capsid-associated proteins is not involved in persistence of measles virus in vitro.

The persistently infected measles virus-AV 3 cell line used in these studies was derived from AV 3 cells surviving a primary infection by the Edmonston strain of measles virus (Robbins, 1978). Anti-measles virus antibodies were not used in establishment or maintenance of this cell line. The passage history of the virus used to establish the persistent infection has been previously described (Bussell & Karzon, 1965). Confluent monolayers of the persistently infected cells were labelled for 24 h with $^3$H-amino acids (Schwarz/Mann, Orangeburg, N.Y.) and $^{32}$P-orthophosphate (carrier free, New England Nuclear, Boston, Mass.) as described earlier (Robbins & Bussell, 1979). Nucleocapsids were harvested from cytoplasmic lysates of the persistently infected cell cultures after 30 or more passages (approx. 10 months post establishment of the cell line). The capsids were purified by repeated centrifugation in alkaline pH CsCl density gradients according to modifications of
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Fig. 1. SDS–PAGE polypeptide profiles of measles virus nucleocapsids from persistently infected AV₃ cells. (a) ³H-amino acid (●—●) and ³²P-phosphate (○—○)labelled gel profile and (b) densitometer scan of unlabelled, stained gel. Migration is from left to right. The small black rectangles on the upper abscissa of each figure represents the relative migration of bovine serum albumin.

the procedures of Compans & Choppin (1967) and McSharry et al. (1975). Specific details of the capsid isolation procedure are available elsewhere (Robbins & Bussell, 1979). Identification of the capsid-associated phosphoproteins was by SDS–PAGE. Purified capsid materials from the persistently infected cell line were electrophoresed under reducing conditions on 5% polyacrylamide disc gels as described previously (Maizel, 1971).

Following electrophoresis gels were either stained with 0.5% Coomassie brilliant blue R-250 (Sigma Biochemicals, St Louis, Mo.) or were frozen, sliced into 1.0 mm discs, placed in scintillation cocktail-containing vials and counted in a β-scintillation counter. Stained gels were destained in a solution of 14% acetic acid and 50% methanol and the gels were scanned at 620 nm with a Standard Model Densitometer (E-C Apparatus Corporation, St. Petersburg, Fla.). The mol. wt. of the capsid-associated polypeptides were determined by electrophoresing marker proteins (β-galactosidase, mol. wt. 130000; phosphorylase A, mol. wt. 92500; bovine serum albumin, mol. wt. 68000; ovalbumin, mol. wt. 43000; all obtained from Sigma Biochemicals) on companion gels and comparison of their relative migrations. Identification of the phosphorylated amino acid species of the capsid phosphoproteins was accomplished by acid hydrolysis and high voltage paper electrophoresis as described previously (Robbins & Bussell, 1979).

When samples of capsid preparations from persistently infected AV₃ cells were electrophoresed on polyacrylamide gels, four polypeptide species were apparent: L, P, Np and VP₄ (see Fig. 1). As reported previously for the virus nucleocapsids isolated from acutely
Fig. 2. Electrophoretograms of acid-hydrolysed capsid phosphoproteins isolated from persistently infected AV8 cells. (a) P polypeptide and (b) NP polypeptide. Migration is from left to right. The black dots on the upper abscissa of the figure represent the relative migrations of unhydrolysed poly-peptide (P-peptide), phosphothreonine (P-threonine), phosphoserine (P-serine), and free phosphates (phosphate).

infected CV-1 and Vero cells (Mountcastle & Choppin, 1977), the major protein constituents of the nucleocapsids from persistently infected AV8 cells were P (mol. wt. 69,000) and NP (mol. wt. 60,000). In addition to these two protein species, we also detected two minor polypeptides associated with our capsid preparations (L, mol. wt. ~180,000, and VP4, mol. wt. 52,000). These two minor polypeptides were not initially observed by Mountcastle & Choppin (1977). However, a more recent study by Stallcup et al. (1979) has described their presence in capsid preparations from acutely infected CV-1 cells.

As is apparent in Fig. 1(a), the two major capsid polypeptides (P and NP) possessed co-migrating 32P-phosphate label. Treatment of the labelled capsid materials with RNase, DNase and phospholipase C before electrophoresis did not significantly modify the 32P-phosphate or 3H-amino acid profiles we obtained (data not shown). These results were the same whether the proteins were from purified capsids or from whole persistent cell extracts. Analysis of P and NP polypeptides by acid hydrolysis and high voltage paper electrophoresis (described below) showed that each peptide possessed phosphorylated amino acids and were, therefore, true phosphoproteins.

Paper electrophoretograms of P and NP acid hydrolysates are shown in Fig. 2. While serine was the primary phosphorylated amino acid species in both the P and NP polypeptides, significant amounts of phosphothreonine were also present in NP. Furthermore, the relative proportions of each phoshoamino acid in the P and NP polypeptides examined...
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in this study were very similar to those found previously for the capsid proteins produced in acutely infected cells (Robbins & Bussell, 1979).

Although Fujinami & Oldstone (1979) have reported that the membrane protein (M, mol. wt. 36000) of the virus is phosphorylated, we have not observed such phosphorylation in the virus membrane proteins produced in acutely infected AV3 and Vero cells or persistently infected AV3 cells (data not shown). While we have previously observed phosphate-labelled materials migrating ahead of the M polypeptide in preparations of virus and whole infected cell extracts, we have not detected phosphorylated amino acids in these materials (Robbins & Bussell, 1979 and unpublished observations). It is possible that the differences between our results and those of Fujinami & Oldstone (1979) are due to the use of different virus stocks, cells, electrophoresis or labelling procedures. However, previous studies of the phosphoproteins of the virus produced in acute infections by Vainionpää et al. (1978) and Wechsler & Fields (1978) are in agreement with our present findings.

The similarities between the peptide phosphorylation patterns obtained in these studies and those reported earlier for the virus phosphoproteins produced in acute infections (Robbins & Bussell, 1979) argue that major phosphorylative modifications of the two capsid proteins are not involved in the persistence of the virus in AV3 cells. It is important to note, however, that antibody to measles virus was not present during the establishment or maintenance of this persistent infection (AV3/MV). Since Fujinami & Oldstone (1979) have shown that phosphorylative modification of the P polypeptide occurs in antibody-modulated measles virus infections and as we have determined that such changes do not occur in an in vitro persistent infection, it would be of interest to determine whether phosphorylative changes occur in the capsid proteins produced in in vivo persistent measles virus infections.

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Department of Microbiology
University of Kansas
Lawrence, Kansas 66045, U.S.A.

Steven J. Robbins* 
Julie A. Fenimore
Robert H. Bussell†

REFERENCES


* Address for reprints: Department of Microbiology, The Milton S. Hershey Medical Center, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033, U.S.A.

† Deceased.

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