Differences in Biological Activity and Structural Protein VP1 Phosphorylation of Polyomavirus Progeny Resulting from Infection of Primary Mouse Kidney and Primary Mouse Embryo Cell Cultures†

JOHN W. LUDLOW AND RICHARD A. CONSIGLI*

Section of Virology and Oncology, Division of Biology, Kansas State University, Manhattan, Kansas 66506

Received 28 July 1986/Accepted 7 October 1986

Both primary mouse kidney and primary mouse embryo cells in culture were used for polyomavirus progeny production. Examination of polyomavirus virion structural integrity revealed that mouse embryo cell progeny contained a threefold greater population of unstable particles when compared with mouse kidney cell progeny. Differences in biological activity between these two progeny virion types were also shown. Mouse kidney cell progeny compared with mouse embryo cell progeny exhibited a 10-fold greater ability to agglutinate guinea pig erythrocytes, a 3-fold lower ability to become internalized into monopinocytotic vesicles, and a 2-fold lower ability to initiate a productive infection based on positive nuclear immunofluorescence when mouse embryo host cell cultures were used. The mouse kidney progeny were also found to bind to host cells less specifically than the mouse embryo cell progeny. When these two progeny virion types were labeled *in vivo* with ^{32}P and subjected to isoelectric focusing followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the second dimension, differences in the phosphorylation pattern of the major virus-encoded structural protein VP1 species were observed. It was revealed that species D and E of mouse kidney cell progeny were phosphorylated to the same degree, while mouse embryo cell progeny species E and F were phosphorylated equally. These data suggest that the host cells play a role in modulating the biological activity of the virus by affecting the degree and site-specific phosphorylation of the major capsid protein VP1 which may influence the recognition of virus attachment proteins for specific cellular receptors.

The papovavirus polyomavirus provides a useful model for the study of oncogenic DNA tumor viruses and is a prime example of viral genetic economy. The virus genome codes for three structural proteins: VP1, VP2, and VP3 (7, 13, 16, 24, 27, 32). Approximately 80% of the total polyomavirus virion protein is composed of VP1, which forms the outer capsid structure (7, 12, 34). An expansion of this viral genetic economy is evident from the multiple functionality of this major structural protein, VP1. In addition to its structural role, VP1 appears to be involved in other important biological functions during the polyomavirus life cycle. A stable association between VP1 and nucleoprotein complexes released by *in vitro* dissociation of polyomavirus virions has been demonstrated (3, 7), and VP1 has also been shown to be involved in agglutination of guinea pig erythrocytes and specific cellular receptor recognition (3-5, 10-12, 24, 34).

Charge heterogeneity of VP1 can be demonstrated by isoelectric focusing (IEF), in which this protein can be resolved into six species designated A to F (2, 3, 38). Each of these species has the same amino acid sequence, as demonstrated by peptide mapping (2). This charge heterogeneity is due at least in part to phosphorylation (1-3, 15, 29, 30), since three of the identified species (D, E, and F) are phosphorylated (2, 3). Our laboratory is particularly interested in these three phosphorylated species since we have shown previously that these species correlate with the hemagglutination

ability of the virus as well as specific cellular attachment (1, 3-5).

The purpose of the studies presented in this report was to investigate the influence that host cells exert on the polyomavirus-coded structural protein VP1. We examined the polyomavirus progeny virions resulting from infection of primary mouse kidney cells (MKC-py) and primary mouse embryo cells (MEC-py). Evidence is presented that the phosphorylation of the polyomavirus major capsid protein VP1 is a host cell-dependent modification which varies according to the host cell type used for virus production. Differences in the degree to which the major structural protein VP1 is phosphorylated may, at least partly, be responsible for the biological activity of the virus as well as for recognition by virus attachment proteins of specific cellular receptors.

MATERIALS AND METHODS

Cells and virus propagation. The preparation of primary cultures of MKC and MEC has been described previously (21, 33). Stock polyomavirus from both MKC and MEC used for infection was prepared by two consecutive passages at low multiplicity in the respective primary cell type. Infected cell cultures were maintained in serum-free Eagle medium.

Virus purification. Polyomavirus virions were purified from infected cell lysates as previously described (22). CsCl gradients used to purify virus were prepared as described by Brunck and Leick (8) and were described in greater detail previously (6, 7).

Isolation of a population of stable and unstable assembled polyomavirus particles was achieved by harvesting infected cell lysates and by purifying immediately without interrup-

* Corresponding author.

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tion (lysates were never frozen or stored at 4°C). All reagents were prepared with serum-free Eagle medium to maintain physiological conditions. The virions were first purified through a 10 to 30% sucrose gradient. The 240S region, which contains both the stable and unstable assembled particles, was isolated for use in determining the percentage of unstable particles by further purification through a CsCl gradient (37).

Radioisotopic labeling of virus. ³H-amino acid labeling of polyomavirus was achieved by incubating infected cells in serum-free Eagle medium containing 10% amino acids supplemented with a ³H-amino acid mixture (ICN Radiochemicals, Irvine, Calif.) at a concentration of 10 µCi/ml of medium (23).

Virus was labeled with ³²P by the addition of serum-free, phosphate-free Eagle medium containing 60 µCi of ³²P_i (carrier free; ICN) per ml to the appropriate cell cultures 18 h postinfection as described previously (2, 3).

CsCl gradient-purified polyomavirus virions were labeled in vitro with ¹²⁵I (Amersham Corp., Arlington Heights, Ill.) by using the chloramine-T method as described by Frost and Bourgaux (14). Quenching of the labeling reaction was achieved by the addition of unlabeled tyrosine to a final concentration of 0.1 mg/ml. To remove unbound ¹²⁵I, the reaction mixture was centrifuged through a 2-ml Sephadex G-25 column at 2,000 × g for 10 min followed by overnight dialysis against 0.01 M Tris hydrochloride, pH 7.4. This method of radioiodination of purified virions does not cause a reduction in biological activity of the virus.

Gel electrophoresis. One-dimensional gel electrophoresis was performed in a 15% sodium dodecyl sulfate-polyacrylamide gel system as described by Laemmli (20). Two-dimensional gel electrophoresis was performed as described previously (2, 3, 28, 29), except that polybuffer 74 (Pharmacia, Inc., Piscataway, N.J.) (6% [vol/vol]) and polybuffer 96 (1% [vol/vol]) were used in place of ampholytes for the IEF. Proteins were visualized by Coomassie blue staining of the gels. Location of the ¹²⁵I- and ³²P-labeled polyomavirus structural proteins was achieved by autoradiography with XAR-5 film (Eastman Kodak Corp., Rochester, N.Y.) intensifying screens (Cronex Lightning-Plus; E. I. du Pont de Nemours & Co., Wilmington, Del.).

Immunofluorescence assay. Polyomavirus infection of MKC or MEC monolayers was determined by the indirect method of immunofluorescence. Quantitation was accomplished by counting multiple fields of cells and scoring for positive fluorescent nuclei. Antiserum to intact CsCl-purified polyomavirus was produced in New Zealand White rabbits as described by McMillen and Consigli (24).

Hemagglutination assay. Equal protein quantities of CsCl gradient-purified MKC-py and MEC-py virions were examined for their ability to agglutinate guinea pig erythrocytes (GPE). Virions were diluted 1:2, 1:3, and 1:5 in phosphate-buffered saline, pH 7.4, before serial dilution to improve titer accuracy. After the addition of GPE (0.75%), the hemagglutination pattern was allowed to develop at 4°C.

Binding of polyomavirus to host cell monolayers. A time course of ¹²⁵I-labeled MKC-py and MEC-py binding to MKC and MEC monolayers was performed as described previously (4). Cells were reacted with equal volumes of HEPES binding buffer (0.5 M HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid], 8 mM glucose, 1.6 mM bovine serum albumin, 0.15 M NaCl, 4.9 mM KCl, 0.6 mM MgSO₄, pH 7.4) containing equal radioactivity of ¹²⁵I-labeled CsCl gradient-purified virions with or without a 1,000-fold excess of unlabeled purified virions as determined by protein assay.

Cell-associated radioactivity at 1 h after the addition of virus was determined after washing the monolayers five times with cold phosphate-buffered saline, pH 7.4, and dissolving the cells in 0.5 N NaOH before gamma counting. Total binding was determined by reacting the cells with ¹²⁵I-labeled CsCl gradient-purified virions alone at 4°C and obtaining the cell-associated reactivity. Nonspecific binding was determined by mixing ¹²⁵I-labeled CsCl gradient-purified virions with a 1,000-fold excess of unlabeled CsCl gradient-purified virions before cell reaction at 4°C and obtaining the cell-associated radioactivity. Specific binding was taken as the difference between total and nonspecific binding.

Isolation of monopinocytotic vesicles. Monopinocytotic vesicles containing polyomavirus were isolated as described by Griffith and Consigli (17). Confluent monolayers of both MKC and MEC were infected with equal ¹²⁵I radioactivity of either MKC-py or MEC-py CsCl gradient-purified virions at 4°C for 1 h. Virus penetration was allowed to occur at 37°C for 1 h followed by washing of the monolayers and treatment with receptor-destroying enzyme (40 U/100-mm-diameter plate). The cells were then scraped into phosphate-buffered saline, pH 7.4, and pelleted by centrifugation at 8,000 rpm in a Sorvall HB-4 rotor. The cells were disrupted by homogenization in 0.25 M sucrose containing 0.01 M Tris chloride (pH 7.4), 1 mM MgCl₂, 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N'-tetraacetic acid, 0.2 U of neuraminidase per ml, 0.1 mM phenylmethylsulfonyl fluoride, using a Plexiglas mortar (Rohm & Haas Co., Philadelphia, Pa.) and a Teflon pestle (E. I. du Pont de Nemours) (20 strokes). The homogenate was centrifuged for 6 min at 2,000 rpm in a Sorvall HB-4 rotor, and the resulting cytoplasmic supernatant containing the vesicles was subjected to further fractionation on a 15 to 45% linear sucrose gradient containing 0.01 M Tris chloride (pH 7.4), 0.15 M NaCl, and 0.01% Triton X-100 centrifuged for 16 h at 32,000 rpm in an SW41 rotor (Beckman Instruments, Inc., Fullerton, Calif.) at 4°C. Gradient fractions were then collected for gamma counting to identify the peaks of vesicles containing the radioiodinated virus.

Quantitative assays. Protein concentrations were determined with a protein assay (Bio-Rad Laboratories, Richmond, Calif.) by using bovine serum albumin as a standard. ¹²⁵I-labeled samples were counted in a no. 1275 mini-gamma counter (LKB Instruments, Inc., Rockville, Md.). ³H samples were quantitated with an LS-233 liquid scintillation counter (Beckman) after suspension in toluene-Triton X-100 (3:1) scintillation fluid. ³²P was counted directly, without the addition of scintillation fluid.

RESULTS

Virion stability. Purification of polyomavirus from infected cell lysates by centrifugation through a CsCl gradient results in the separation of virions and capsids (9, 11, 35, 37). Our laboratory has recently shown that polyomavirus capsids are not found in vivo but instead are generated when infected cell lysates are subjected to the harsh conditions of CsCl gradient purification (37). An unstable population of virions eject their DNA genomes upon exposure to CsCl centrifugation, thus forming empty capsids. The stable population of virions is unaffected by the same CsCl treatment.

Equal amounts of both MKC-py and MEC-py 240S regions as quantitated by ³H-amino acid labeling of viral coat proteins isolated from a 10 to 30% sucrose gradient were used for the CsCl gradient purification. The gradients were then fractionated, and two peaks of radioactivity were

FIG. 1. Stability of polyomavirus progeny to CsCl gradient purification. ^3H -amino acid-labeled virus was first partially purified through a 10 to 30% sucrose gradient to isolate the peak of 240S virions. Equal amounts of radioactivity (2.6×10^4 cpm) of MKC-py and MEC-py from the 240S region were then subjected to CsCl gradient analysis. (A) MKC-py CsCl gradient profile showing intact virions (fractions 16 to 24) and empty capsids (fractions 27 to 33). (B) MEC-py CsCl gradient profile showing intact virions (fractions 16 to 24) and empty capsids (fractions 27 to 33). Density of virion peaks, 1.32 g/ml; capsid peaks, 1.28 g/ml.

resolved (Fig. 1). The virion peak (fractions 16 to 24) and the capsid peak (fractions 27 to 33) had buoyant densities of 1.32 and 1.28 g/ml, respectively. These densities are in agreement with those previously reported for polyomavirus virions and capsids (21). MEC-py virions (Fig. 1B) appeared to be more susceptible to CsCl treatment as evidenced by the greater capsid peak when compared with MKC-py (Fig. 1A) after the same treatment. The percentage of ^3H -amino acid label in the capsid peak with respect to the total radioactivity for the virion and capsid peaks was found to be 16% for MEC-py and 5% for MKC-py progeny. This threefold greater percentage of capsids for MEC-py over MKC-py after CsCl gradient purification indicated that, for a given population of fully assembled polyomavirus particles, MEC-py had a higher population of unstable particles than MKC-py did.

Phosphorylation of polyomavirus structural protein VP1. Autoradiography of purified ^{32}P -labeled MKC-py and MEC-py virions after sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that VP1 protein of both progeny types was phosphorylated. Since it was previously demonstrated that only three of six VP1 IEF species are phosphorylated (2, 3), we decided to further examine the phosphorylation of VP1 from both progeny types. The autoradiograms of purified ^{32}P -labeled MKC-py and MEC-py virion VP1 after IEF in the first dimension and sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the second dimension are shown in Fig. 2. While both progeny types exhibited ^{32}P incorporation only in the three acidic species D, E, and F, the amounts of ^{32}P contained within these VP1 species differed between MKC-py and MEC-py. Progeny resulting from both a homologous (host cells infected with virus type derived from the same cell type) and

a heterologous (host cells infected with virus type derived from the other host cell type) infection of MKC and MEC monolayers were examined. As illustrated by the autoradiogram, progeny resulting from homologous (Fig. 2, lane 2) and heterologous (Fig. 2, lane 3) infections of MKC monolayers incorporated the majority of the ^{32}P into species D and E, and much less into species F. Progeny resulting from homologous (Fig. 2, lane 4) and heterologous (Fig. 2, lane 5) infections of MEC monolayers incorporated the

FIG. 2. Two-dimensional gel electrophoresis of ^{32}P -labeled VP1 IEF species. Equal amounts of radioactivity (6.5×10^4 cpm) of each CsCl gradient-purified progeny virion type were separated by gel electrophoresis and visualized by autoradiography. Lanes: 1, Coomassie blue staining of VP1 species (A to F) used as markers; 2, autoradiogram of ^{32}P -labeled MKC-py species; 3, autoradiogram of ^{32}P -labeled species resulting from MEC-py infection of MKC monolayers; 4, autoradiogram of ^{32}P -labeled MEC-py species; 5, autoradiogram of ^{32}P -labeled species resulting from MKC-py infection of MEC monolayers.

TABLE 1. Biological activity of progeny virions purified from primary MKC or MEC cultures^a

Progeny type	Cell type	% Fluorescent nuclei ^b	Hemagglutinin titer/ μ g of virion protein ^c
MKC-py	MKC	20	13,940
	MEC	11	
MEC-py	MEC	17	1,330
	MKC	17	

^a Equal protein quantities (0.5 μ g) of CsCl gradient-purified MKC-py and MEC-py virions were used for homologous and heterologous infection of MKC and MEC monolayers and assayed 40 h post infection for positive immunofluorescent nuclei. Approximately 600 cells were counted from seven randomly selected fields.

^b Calculated by dividing the number of cells showing positive nuclear fluorescence by the total number of cells counted times 100.

^c Hemagglutinin titer of CsCl gradient-purified virion protein from the two host cell types was determined by calculating the final dilution of virions in the last well showing positive hemagglutination.

majority of the label into species D with much less being incorporated into species E and F, although these two species appeared to be phosphorylated equally. Anders and Consigli (2) have previously shown by phosphoamino acid analysis of VP1 that this modification resides on threonine and serine residues for MKC-py. We found the same to be true for MEC-py VP1 (data not shown). These results lend further support to the idea that it is the host cell type and not the infecting virus type which dictates these subtle differences in degree of phosphorylation. The difference in the amount of ³²P incorporated into each VP1 species is not due to different amounts of each species for the two progeny types. The amount of each VP1 species possessed by each progeny type appeared to be the same as that determined by densitometry of autoradiographs from ¹²⁵I-labeled samples and Coomassie blue staining (data not shown). Since there appear to be differences in the phosphorylation of these three VP1 species, one would expect differences in the pIs of these species between the two progeny types. However, pI determinations of ¹²⁵I-labeled MKC-py and MEC-py after IEF have not demonstrated any significant differences (data not shown).

Biological activity. Examination of the two progeny types with respect to their ability to productively infect host cell monolayers was achieved by measuring positive nuclear immunofluorescence. The number of immunofluorescent nuclei present indicates the number of cells actively producing polyomavirus structural proteins. Table 1 summarizes the

data obtained from a typical immunofluorescence experiment. When we used equal quantities of purified virion protein, homologous infection of MKC monolayers resulted in approximately twice as many positive immunofluorescent nuclei as those resulting from heterologous infection of MEC monolayers. Interestingly, MEC-py infection of both MKC and MEC hosts resulted in identical amounts of fluorescent nuclei, both of which were very close to the amount demonstrated by the homologous infection of MKC monolayers. A possible explanation for these observations may be the heterogeneous nature of the cells which compose MEC monolayers. The variety of mouse cell types available for polyomavirus infection in these monolayers may result in the production of a heterogeneous population of progeny virions capable of optimally infecting a wider variety of mouse cell types in culture. MKC monolayers, on the other hand, are more uniform in cell type. The resulting progeny from these monolayers may be more homogeneous in nature and thus may be more limited in the variety of mouse cell types which they can optimally infect.

Agglutination of GPE. One possible explanation for the difference observed in the ability of MKC-py and MEC-py to productively infect MKC and MEC monolayers is that there could be a difference in the ability of each virus type to bind to cells. In most binding studies, the hemagglutination model has been used to examine polyomavirus interaction with its receptor(s) on host cells (4, 10, 26). We therefore examined polyomavirus binding to GPE to determine if there were any differences in agglutinating ability. The hemagglutinin titer of purified virion protein per microgram of MKC-py and MEC-py was found to be 13,940 and 1,330, respectively (Table 1). This 10-fold greater ability of MKC-py to agglutinate GPE indicated greater reactivity between the MKC-py hemagglutinin and GPE than that between the MEC-py hemagglutinin and GPE.

Binding to host cells. MKC-py and MEC-py binding to both MKC and MEC monolayers was also examined. Equal amounts of ¹²⁵I-labeled purified virions, with or without a 1,000-fold excess of unlabeled virions, were allowed to bind to the cell monolayers at 4°C for 1 h before determining cell-associated radioactivity. The 1-h postinfection time for determining cell-associated radioactivity was chosen since it had been shown previously that maximum polyomavirus binding to host cell monolayers is achieved by this time (4). The data obtained from a typical adsorption experiment are shown (Table 2). Total binding of ¹²⁵I-labeled primary MKC-py was greater than ¹²⁵I-labeled MEC-py binding as indicated by higher cell-associated radioactivity. As expected, a

TABLE 2. Adsorption of purified progeny virions after homologous and heterologous reactions with primary MKC and MEC culture monolayers^a

Labeled progeny type	Host cell type	cpm ^b					% Total specific binding ^d	
		Total binding	Nonspecific binding		Specific binding ^c			
			MEC-py	MKC-py	MEC-py	MKC-py		
							MEC-py	MKC-py
MKC-py	MKC	10,710	9,140	6,860	1,570	3,850	15	36
MEC-py	MKC	9,440	6,960	5,250	2,480	4,190	26	44
MKC-py	MEC	7,120	5,330	2,750	1,790	4,370	25	61
MEC-py	MEC	4,670	2,800	2,220	1,870	2,450	40	52

^a ¹²⁵I-labeled MKC-py and MEC-py CsCl gradient-purified virions binding to MKC and MEC monolayers. Cells were reacted with 0.1 ml of HEPES buffer, pH 7.4, containing 2.1×10^4 cpm of virus with or without a 1,000-fold excess of unlabeled CsCl gradient-purified virions per 35-mm dish. Cell-associated radioactivity was determined at 1 h after the addition of virus.

^b Cell-associated radioactivity of ¹²⁵I-labeled virions alone (total binding), with a 1,000-fold excess of unlabeled MEC-py virions, and with a 1,000-fold excess of unlabeled MKC-py virions.

^c Determined by subtracting cell-associated radioactivity due to nonspecific binding from cell-associated radioactivity due to total binding.

^d Determined by dividing the cell-associated radioactivity due to specific binding by the cell-associated radioactivity due to total binding times 100.

FIG. 3. Sedimentation pattern of vesicles containing ^{125}I -labeled purified polyomavirus. Cells were infected with CsCl gradient-purified MKC-py and MEC-py virions having equal amounts of radioactivity (2.84×10^5 cpm). (A) MKC monolayer infected with ^{125}I -labeled MEC-py. (B) MEC monolayer infected with ^{125}I -labeled MEC-py. (C) MKC monolayer infected with ^{125}I -labeled MKC-py. (D) MEC monolayer infected with ^{125}I -labeled MKC-py. The percentage of ^{125}I -labeled virions contained within the vesicles isolated from each host cell type was calculated by totaling the radioactivity contained within peaks I and II (fractions 10 to 14 and 22 to 26 for MKC monolayers; fractions 14 to 18 and 30 to 34 for MEC monolayers) divided by the total radioactivity of the cytoplasmic fraction (8.4×10^4 cpm) placed on the 15 to 45% sucrose gradients for vesicle isolation times 100.

reduction in cell-associated radioactivity was observed when mixing the labeled virions with a 1,000-fold excess of unlabeled virions before reaction with the host cell monolayers. Mixing labeled virions with a 1,000-fold excess of unlabeled MKC-py, for reaction with either host cell monolayer type, resulted in higher specific binding as determined by the higher cell-associated radioactivity compared with mixing with a 1,000-fold excess of unlabeled MEC-py. Thus, when calculating the percentage of total specific binding, mixing of labeled virions with a 1,000-fold excess of unlabeled MKC-py before reaction with the host cell monolayers resulted in a higher percentage of total specific binding of labeled virions. These data were interpreted to mean that the binding of ^{125}I -labeled MKC-py to host cell monolayers is less specific in nature since we observed less competition with the respective unlabeled virus types than with ^{125}I -labeled MEC-py binding which demonstrated greater competition.

Isolation of monopinocytotic vesicles. Since we had evidence that these two progeny types differ in their ability to bind to both GPE and mouse cell monolayers, we next chose to investigate virion penetration into the cell. Polyomavirus enters the permissive cell by endocytosis after attachment and penetration into the cytoplasm and is contained in monopinocytotic vesicles (17, 21). By using the technique established by Griffith and Consigli (17), we isolated ^{125}I -labeled MKC-py and MEC-py virion-containing vesicles after infection of MKC and MEC monolayers (Fig. 3). Although equal quantities of ^{125}I -labeled purified infecting virions were used, differences were observed in the amount

of virus-containing vesicles isolated. More MEC-py-containing vesicles were isolated (Fig. 3A and B) than were MKC-py-containing vesicles (Fig. 3C and D), as indicated by the greater radioactivity in the two peaks. The percentage of ^{125}I -labeled MEC-py contained within the vesicles isolated from MKC (Fig. 3A) and MEC (Fig. 3B) monolayers was determined to be 19 and 18%, respectively. The amount of labeled MKC-py within vesicles isolated from cell monolayers was found to be 11% for MKC (Fig. 3C) and 6% for MEC (Fig. 3D). If the amount of polyomavirus-containing vesicles is indicative of the amount of virus which penetrates the cell in preparation to being transported to the nucleus for further steps in replication, the data presented in Fig. 3 suggest that MEC-py virus is superior to MKC-py virus in gaining entry into the host cell for transport to and replication in the cell nucleus.

DISCUSSION

In this report, evidence is presented that polyomavirus progeny resulting from propagation in two different primary host cell types exhibit differences not only in structural integrity and VP1 protein phosphorylation but also in their biological activity. The production of virus progeny is dependent on the host cell with respect to the biochemical pathways available for synthesis of virus protein. It is reasonable to speculate that different host cell types possess slight differences in certain biochemical pathways as essential characteristics of their metabolism. Slight differences in

biochemical pathways required for protein synthesis of the host cell might therefore be reflected in virus protein products. Several influenza A strains have recently been shown to exhibit differences in phosphopeptide fingerprints, depending upon the host cell type used for virus propagation (19). Our laboratory and others have found the polyomavirus major structural protein VP1, which forms the highly cross-linked protective outer capsid, to be modified by the presence of phosphate groups on serine and threonine residues (2, 15). These phosphate groups add negative charges to polypeptides, which may affect the tertiary structure and the biological activity of the protein (25). Anders and Consigli (1) have localized the major phosphorylation region to a central, cryptic portion of the VP1 sequence. Phosphorylation of VP1 may function to dictate protein conformation of capsomeres to fit into symmetrically distant environments as required by the all-pentamer capsid model of Rayment et al. (31). If phosphorylation does indeed function in dictating VP1 protein conformation, this modification may play a role in virion stability. It has been reported that both Ca^{2+} and disulfide bonds are at least in part responsible for maintaining polyomavirus virion integrity (6, 7). It seems reasonable that VP1 protein conformation may be important in determining the degree of bonding which involves Ca^{2+} and sulfur.

Our laboratory has had a long-standing interest in the mechanism by which polyomavirus interacts with the host cell, thereby resulting in a productive infection. This interest has directed us to investigate in detail the attachment species D, E, and F of the major structural protein VP1, since we have previously shown that these species are responsible for host cell receptor interaction as well as the hemagglutinating ability of the virus (3). Studies have been conducted which indicate that the polyomavirus hemagglutinin and receptor functions reside in, or at least have determinants contained in, the carboxy-terminal 153 amino acids of the VP1 sequence (1). Previous reports have suggested that polyomavirus binding to the host cell surface is a two-step process (3, 4, 18, 21): a nonspecific step involving VP1 species D and F and a specific step involving species E. Polyomavirus agglutination of GPE is believed to be nonspecific, involving species D and F, since both complete virions and empty capsids have this ability yet capsids lack VP1 species E. MKC-py, which exhibited a 10-fold greater ability to agglutinate GPE than that of MEC-py (Table 1), also demonstrated greater nonspecific binding ability to host cells than MEC-py did (Table 2). Although intact virions are internalized into monopinocytotic vesicles destined for transport to the nucleus for uncoating and further steps in the infection process (21, 36), the greater nonspecific binding by MKC-py to mouse cells may result in less of this progeny type being internalized into these monopinocytotic vesicles. As shown in Fig. 3, this was the case. Approximately twofold more MEC-py were internalized into vesicles from MKC monolayers (Fig. 3A) than were MKC-py (Fig. 3C), and about threefold more MEC-py vesicles were isolated from MEC monolayers (Fig. 3B) than were MKC-py (Fig. 3D). If more MEC-py can penetrate the cell and become internalized for further steps in the replicative process, one would expect a greater productive infection of host cells by this type of virus. MEC-py indeed productively infect both MKC and MEC monolayers to the same degree, as measured by positive immunofluorescent nuclei (Table 1). MKC-py, however, do not appear to infect MEC monolayers as well but do infect MKC monolayers to the same degree as MEC-py do. Phosphorylation may play a role in the binding ability of VP1

attachment species D, E, and F through conformation of these proteins. Although the host cell appears to dictate the phosphorylation pattern of these species (Fig. 2, compare lanes 2 and 3 with lanes 3 and 4), the differences between MKC-py and MEC-py phosphorylation may be related to the differences in biological activity. Subtle differences in phosphorylation may affect VP1 protein conformation sufficiently to alter binding of the virus to mouse cells.

It appears evident that host cells dictate subtleties in polyomavirus progeny structural protein VP1 that they produce. These subtleties in VP1 structure may in turn dictate the performance of the virus in subsequent infections at the level of viral attachment to the host cell receptor. It is tempting to speculate that host cell-contributed modifications of viral proteins play a role in determining the host range of the virus. Even though this study used polyomavirus, which has been shown to infect both MKC and MEC monolayers, differences in viral binding to mouse cells and biological activity were evident, depending upon the host cell type used for virus propagation. Whether the differences we reported here with the polyomavirus-primary mouse cell system are occurring in other virus systems remains to be determined.

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