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Biochemistry

Host range transforming gene of polyoma virus plays a role in virus assembly

(virus encapsidation/transforming mutants/viral oncogene/post-translational protein modifications)

ROBERT L. GARCEA* AND THOMAS L. BENJAMIN

Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

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ABSTRACT **Polyoma virus host range transforming (hr-t) mutants are blocked in virion assembly. In normal 3T3 cells, a nonpermissive host, these mutants synthesize 30–40% as much viral DNA and 80–100% as much capsid proteins as does wild-type virus and yet produce only 1–2% as much infectious virus. Intermediates in virion assembly have been followed by [^{3}H]thymidine incorporation. hr-t mutants synthesize 95S replicating minichromosomes, which accumulate as 75S forms. However, the latter fail to undergo efficient transition to 240S virion structures. This block in encapsidation is overcome in permissive hosts such as primary baby mouse kidney (BMK) epithelial cells. The block in assembly of 240S particles is accompanied by a failure to induce a series of acidic isoelectric forms of the major capsid protein, VP_1. Multiple species of post-translationally modified VP_1 are seen by two-dimensional gel electrophoresis in wild-type virus-infected cells. These acidic VP_1 subspecies are decreased 6- to 10-fold in hr-t mutant-infected 3T3 cells but are produced in normal amounts when the same mutants infect BMK cells. When 3T3 cells are coinfected with hr-t mutant and wild-type viruses, normal amounts of the VP_1 subspecies are present, and hr-t mutant viral DNA is efficiently packaged into virions. These studies demonstrate an important role of the hr-t gene of polyoma virus in virus assembly. Specifically, we propose that VP_1 is a target for hr-t gene-controlled modification and that modified forms of VP_1 are essential for encapsidation of viral minichromosomes.**

The host range transformation-defective (hr-t) mutants of polyoma virus were selected originally for their ability to grow well on polyoma virus-transformed 3T3 (Py-3T3) cells and poorly on normal 3T3 cells. This selection was based on the assumption that genes essential for transformation should be identifiable on the basis of their serving an essential role in the virus growth cycle (1). Such dual functions for virus growth and transformation were thought to be expressed by the integrated viral genome in Py-3T3 cells and to be capable of complementing a superinfecting virus mutant defective in the homologous function. Mutants selected in this way form a single complementation group and have a uniform biological behavior (see ref. 2 for a review). They are completely deficient in transformation of cells *in vitro* (1, 3) and unable to induce tumors in newborn hamsters (4). The mutations map to a small segment of the early region (5–7) that encodes a common portion of the small and middle tumor-antigen proteins (8).

Analysis of the growth of hr-t mutants on different cell types has led to the hypothesis that cellular permissive factor(s) are required for efficient virus production and that the function of the hr-t gene is to regulate or induce the permissive factor(s) (3). Certain primary cells such as mouse embryo fibroblasts and baby mouse kidney (BMK) epithelial cells constitutively express permissive factors, allowing efficient growth of hr-t mutants (9). By using normal mouse embryo fibroblasts as a permissive host, it has been possible to isolate host range mutants that are nontransforming and otherwise identical to hr-t mutants isolated on polyoma virus-transformed 3T3 cells (10). Results of these biological experiments, together with those of mapping and sequence determination (5–7), establish that the growth and transformation defects of hr-t mutants are manifestations of single mutations, resulting in loss of a pleiotropic regulatory function (2). How the dual products of the hr-t gene elicit the multiple cellular changes that accompany malignant transformation and permissivity for virus growth is unknown.

A better understanding of how the small and middle tumor-antigen proteins exert their effects should come from biochemical studies comparing the lytic growth cycle of wild-type (wt) and hr-t mutant viruses. Studies in other laboratories on simian virus 40 (SV40) (11–15) have shown that virion assembly proceeds through several definable stages: (*i*) replicative intermediates that become labeled rapidly with [^{3}H]thymidine and sediment at 95S; (*ii*) minichromosomes that sediment at 75S and have a fraction active in transcription; and (*iii*) virion forms that sediment at 200–240S (which contain capsid proteins VP_1, VP_2, and VP_3) and include intact virions (240S) as well as salt-labile and other intermediates preceding the final virion structure. The transitions from 95S to 75S to 240S have been determined by pulse–chase labeling of viral DNA with [^{3}H]thymidine and by the use of a temperature-sensitive mutant in VP_1, which blocks the transition from 75S to the more rapidly sedimenting forms.

Our initial approach to studying the role of the hr-t gene in productive viral infection has been to determine the levels of viral DNA and capsid proteins present in hr-t mutant-infected cells and then to follow virion maturation through the defined intermediates. hr-t mutants show levels of viral DNA accumulation that are 30–40% of that of wt. However, the predominant defect in virus production lies in a failure to encapsidate the 75S minichromosome to form a 240S virion structure. The encapsidation defect is correlated with failure to generate post-translationally modified VP_1 species that are present in the mature virion and may be required for assembly of infectious virus particles.

MATERIALS AND METHODS

Cells and Viruses. Cell culture and plaque assays were performed as described (16). Primary culture of BMK cells were prepared as described by Winocur (17). NIH 3T3 cells were

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Abbreviations: BMK, baby mouse kidney; hr-t, host range transforming; 2-D, two-dimensional; wt, wild-type; pfu, plaque-forming unit; SV40, simian virus 40.

* Present address: Div. of Pediatric Oncology, Dana-Farber Cancer Inst., 44 Binney St., Boston, MA 02115.

mycoplasma-free and tested routinely for low permissivity for hr-t mutants (16); wt viruses were derived from hr-t mutant viruses by marker rescue (5). hr-t mutants used have been described (1, 3).

Chemicals and Isotopes. Electrophoresis reagents were obtained from Bio-Rad; [^{35}S]methionine (400–600 Ci/mmol; 1 Ci = 3.7×10^{10} Bq) and [^{3}H]thymidine (20 Ci/mmol), from New England Nuclear; type X neuraminidase, from Sigma; agarose, from Seakem; Ampholines, from LKB; and *Hpa* II, from Bethesda Research Laboratories.

Viral DNA Quantitation. Viral DNA was isolated from infected cell monolayers by the method of Hirt (18). Viral DNA was separated on 1% agarose gels, stained with ethidium bromide, and photographed with polaroid positive–negative film. Viral DNA standards of known concentration were run concurrently to insure linearity of staining. The negatives were densitometered, and DNA was quantitated by peak area. Specific activity of viral DNA was determined by cutting out the DNA bands, melting the agarose, and assaying radioactivity in Aquasol (New England Nuclear).

Preparation of Cell Extracts for Electrophoresis. Cells were plated into Linbro microwells (15-mm diameter) at 4×10^4 cells per well. For labeling periods longer than 2 hr, Dulbecco's modified Eagle's medium without methionine was used; for less than a 2-hr labeling period, Hanks' balanced salt solution was used. [^{35}S]Methionine was used at 0.1–1.0 mCi/ml. Cell lysates were made essentially by the method of O'Farrell and Goodman (19).

Two-Dimensional (2-D) Electrophoresis. Electrophoresis of proteins was carried out exactly as described by O'Farrell (20). In all cases, the second dimension separating gel contained a uniform concentration of 10% acrylamide. Ampholines for the first dimension were either pH 3.5–10/pH 5–7, 1:4 (vol/vol), for VP_1 quantitation or pH 3.5–10/pH 5–7/pH 7–9, 1:2:2 (vol/vol) for VP_1 subspecies separation.

VP_1 Quantitation. 2-D autoradiographs of [^{35}S]methionine-labeled infected cell extracts were measured by densitometry on a Quick-Scan scanner (Helena Laboratories, Beaumont, TX), and the VP_1 spot intensity was quantitated by peak area. Scans were performed in the isoelectric focusing direction, and minor cellular spots in the same region were subtracted by comparison to a mock-infected tracing. Exposures were made in the linear range of the film, and spot intensities were compared between autoradiographs by normalization to both the actin spot intensity and total cpm loaded onto the gel.

Preparation of Polyoma Virion Intermediates. Cells were infected at a multiplicity of infection of 1–10 plaque-forming units (pfu)/ml with infection begun at 50% confluency for NIH 3T3 cells. Labeling for the indicated intervals was done by directly adding 3 μCi of [^{3}H]thymidine per ml of media. Polyoma virion intermediates were prepared by a method similar to that described for SV40 (11, 12, 15) but modified to include a neuraminidase digestion step (unpublished data).

Virion Isolation and Restriction Digestion. Virions were isolated from infected cells by the method of Schaffhausen and Benjamin (21), and the virion band from the CsCl phase was dialyzed subsequently into 10 mM Tris (pH 9.0). [^{35}S]Methionine-labeled virions were prepared for 2-D electrophoresis in the same manner, except that the dialysate was precipitated with trichloroacetic acid and resuspended in lysis buffer. DNA was extracted from purified virions by addition of $NaDodSO_4$ to 1%, followed by two phenol extractions and precipitation by ethanol. The DNA was taken up in buffer for digestion with *Hpa* II according to the manufacturer's recommendations. Restriction fragments were resolved on a 4% acrylamide gel in Tris/borate/EDTA buffer.

RESULTS

hr-t Mutants Produce Sufficient Amounts of Viral DNA and Capsid Proteins for a Normal Virus Yield. We began our analysis of hr-t mutant virus infections by quantitating the amounts of viral DNA and capsid proteins made in permissive and nonpermissive hosts. To insure quantitative recovery of viral DNA, we compared extracts of virus-infected cells at various times after infection by the method of Hirt (18). Fig. 1 shows the accumulation of form I polyoma DNA during infection of permissive and nonpermissive cells. In permissive infection of BMK cells, wt and hr-t mutant viral DNAs accumulated to equal extents, whereas in nonpermissive 3T3 cells, the mutant DNA accumulated to levels 30–40% of those of wt. These infections were carried out at a multiplicity of infection of 2–10 pfu per cell; further increases in the virus input fail to increase the amount of DNA produced for either wt or hr-t mutant virus. Immunofluorescent staining for nuclear (large) tumor antigen confirmed that 70–80% of the cells in the monolayer were infected in both the hr-t and wt infections. The roughly 30–40% lower levels of DNA accumulation in hr-t mutant nonpermissive infections is insufficient to account for the 1–2% lower output of infectious virus particles.

To ensure quantitative recovery of capsid proteins, infected cell monolayers were completely solubilized with lysis buffer containing 9.5 M urea and 2% Nonidet P-40. [^{35}S]Methionine-labeled proteins in these extracts were analyzed by 2-D electrophoresis (19, 20) and quantitated by autoradiography and densitometry. Table 1 summarizes results on the major capsid protein VP_1. Both synthesis and accumulation of VP_1 are equivalent for wt and hr-t mutant viruses in nonpermissive and permissive cells. In repeated experiments comparing different matched pairs of hr-t mutants and their "marker-rescued" wt virus partners (5), the VP_1 levels achieved by the mutant were within 50% of those of the wt. VP_2 and VP_3, though more difficult to quantitate because they constitute a minor amount of the total ^{35}S-labeled proteins, also appeared by 2-D gel analysis to accumulate to within 50% of the wt amounts in nonpermissive infections. We conclude that the low yield of hr-t mutants in nonpermissive cells cannot be accounted for simply in terms of failure to produce adequate amounts of viral DNA or capsid proteins.

hr-t Mutants Are Blocked in the Encapsidation of the Viral Minichromosome. Because the growth defect of hr-t mutants does not result from a failure to synthesize viral DNA or capsid proteins, we next attempted to analyze the virus life cycle in terms of intermediates in virus assembly. At least three inter-

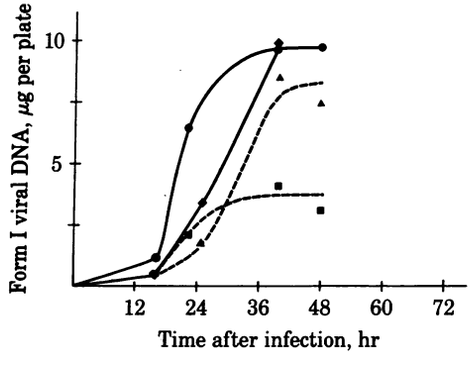

FIG. 1. Viral DNA accumulation in infections of permissive and nonpermissive hosts. At various times after infection, viral DNA was quantitated as described: ●, 59RA (wt) in BMK cells; ▲, NG59 (hr-t mutant) in BMK cells; ◆, 59RA in 3T3 cells; ■, NG59 in 3T3 cells. Only the NG59 (hr-t) viral infection of nonpermissive 3T3 cells shows a reduced accumulation of viral DNA (30–40%).

mediates in polyoma infection were identified: a 95S replicating complex, a 75S minichromosome, and a 240S virion structure. Fig. 2 compares profiles of subviral complexes from mutant- and wt-infected cells. In the infection of permissive BMK cells (Fig. 2 *Upper*), wt and mutant profiles appeared to be similar with regard to the relative amounts of the 75S and 240S species. However, in the infection of nonpermissive 3T3 cells (Fig. 2 *Lower*), the 240S virion peak was virtually absent from the hr-t mutant profile. Because differential recovery of viral intermediates could bias the interpretation of these results, we determined yields by comparing [^{3}H]thymidine recovered from the gradient to total [^{3}H]thymidine in viral DNA from Hirt-method supernatants. In the experiment of Fig. 2, the recovered viral complexes contained 90% of the total viral DNA. Therefore, we believe the profiles are representative of the total complexes present.

Quantitative comparison of the 240S species in Fig. 2 was made after subtracting the background of nonspecific [^{3}H]thymidine-labeled complexes and normalizing the peak intensities to the total viral DNA recovered. By this comparison, the 240S peak of the hr-t mutant was 15% of wt when grown on 3T3 cells (Fig. 2 *Lower*) and 90% of wt when grown on BMK cells (Fig. 2 *Upper*). Because DNA accumulation approximated 30–40% that in mutant-infected 3T3 cells (Fig. 1), the overall reduction in 240S production should be 5% of wt. Direct comparison of the 240S peaks in Fig. 2 *Lower* without normalization for DNA accumulation shows the mutant peak to be 6% of the wt.

The decrease in the 240S virion peak to 5% in the hr-t mutant-infected 3T3 cells is not surprising because outputs determined by plaque assay or by CsCl gradient isolation of virions are 1–2% that of wt. Because viral DNA accumulation accounts for only a 2- to 3-fold difference in virion production, we conclude that encapsidation of the 75S minichromosome is the major block (5- to 10-fold) in hr-t mutant infection of nonpermissive cells. Because the transition of 75S to 240S complexes in SV40 virion maturation is accompanied by the addition of the major capsid protein VP_1 to the minichromosome (11, 15), we next investigated VP_1 in more detail.

hr-t Mutants Show Host Cell Dependence of VP_1 Modification. In both polyoma and SV40, studies of the major capsid protein VP_1 have revealed multiple isoelectric subspecies attributed to post-translational modifications (19, 22–24). We compared the stoichiometry of the various VP_1 subspecies in hr-t mutant and wt infections. To ensure quantitative and nonselective extraction, entire infected cell monolayers were solubilized, and VP_1 subspecies were separated by 2-D gel electrophoresis by using an expanded isoelectric focusing range for resolving VP_1 forms. The following criteria were used to identify VP_1 subspecies: (*i*) absence in mock-infected cells, (*ii*) comparisons of mobilities with purified virion proteins, and (*iii*) comparison to previously published molecular weight and pIs for VP_1.

Table 1. Relative VP_1 synthesis

Infection	Pulse label	Continuous label
59RA BMK	1.10	1.26
NG59 BMK	1.06	0.89
59RA 3T3	0.89	1.52
NG59 3T3	1.06	1.58

Synthesis and accumulation of VP_1 during infection of permissive (BMK) and nonpermissive (3T3) cells with wt (59RA) and hr-t mutant (NG59) viruses. Total VP_1 was quantitated and expressed as relative units of autoradiographic density normalized to total cpm of [^{35}S]methionine loaded onto the 2-D gel. Normalization to the density of the actin spot gave similar results. Pulse labeling was carried out for 1 hr, and continuous labeling for 15 hr.

Fig. 3*B* shows a typical wt VP_1 pattern in 3T3 cells showing at least four of the six identified subspecies. Subspecies 1 (pI 6.30) was the primary translation product as identified by rapid pulse-labeling with [^{35}S]methionine. The label moved rapidly into the more acidic forms (pI 6.05–5.85), suggesting that the latter are post-translational derivatives (data not shown). Although only five subspecies are indicated in Fig. 3*E*, an additional subspecies migrating as a shoulder of form 1 was often detected (for example, see Fig. 3*D*). There was a notable absence of all modified VP_1 subspecies in the hr-t mutant infection of nonpermissive cells (Fig. 3*C*). Only a small amount of form 3, the major variant, and no detectable form 2 variant were present in mutant-infected 3T3 cells, as compared to infection

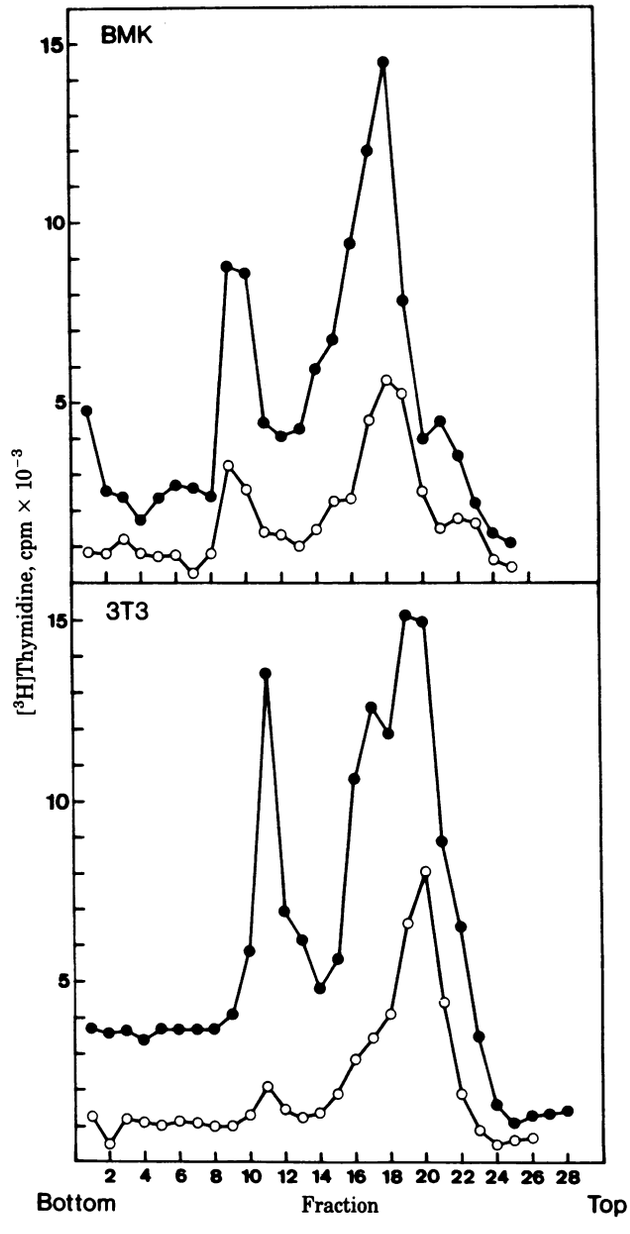

FIG. 2. Comparison of the profiles of viral intermediates from wt and hr-t mutant viral infections. (*Upper*) Sucrose gradient profile from infection of BMK cells with either 59RA (—●—) or NG59 (—○—). (*Lower*) Profile from infection of 3T3 cells. The major nucleoprotein species are identified as 75S to 95S (fractions 16–21) and 240S (fractions 9–12). As shown, the profiles are similar in the BMK infection but are different in the infection of 3T3 cells in that there is a greatly decreased amplitude of the 240S peak and a decreased 240S:75S ratio in the NG59 profile when compared to the wt.

of the same cells by the wt virus with an identical late region (Fig. 3*B*). We also tested two additional hr-t mutant/wt virus marker-rescued pairs and obtained identical results. Because the growth cycle of hr-t mutants in permissive cells is prolonged compared to that of wt, we looked at VP_1 by [^{35}S]methionine-labeling up to 60 hr after infection and found no difference from the pattern seen in Fig. 3*C*. Densitometry revealed that modified subspecies comprised 35–50% of the total VP_1 for wt, compared to 5–10% for mutant upon infection of 3T3 cells.

When hr-t mutants infected a permissive host, modified VP_1 subspecies were present in amounts comparable to a wt infection (Fig. 3*D*). Therefore, the VP_1 molecules produced by hr-t mutants are capable of being modified, and the deficiency of modified forms in nonpermissive cells is not a result of undetected late gene mutations. Production of increased amounts of the modified VP_1 subspecies in hr-t mutant-infected BMK cells correlates with increased amounts of the 240S virion peak in the same infections (Fig. 2 *Upper*). Conversely, the decrease in VP_1 modification to 5–10% seen in nonpermissive 3T3 cells correlates well with the failure of the 75S minichromosome to undergo transition to 240S virions (Fig. 2 *Lower*).

Fig. 3*E* shows the VP_1 subspecies present in purified virions. All virion preparations, including those obtained in low yields from hr-t mutant infections of nonpermissive cells showed this pattern. Thus, a precise stoichiometry of VP_1 subspecies may be required for virion assembly. This stoichiometry suggests that the yield of hr-t mutant virus in various host cells is limited by the level of modified VP_1 subspecies in the total VP_1 pool. The relative intensities of subspecies 2 and 3 were different in infected cell extracts (Fig. 3*B*) and purified virions (Fig. 3*E*). This difference suggests a selection of some subspecies over others in the final virion structure.

Coinfection With wt Virus Results in Packaging of hr-t Mutant Minichromosomes. To test the hypothesis that lack of VP_1 modifications results in the failure of hr-t mutant virions to assemble, a double infection experiment was performed. Nonpermissive 3T3 cells were coinfected with wt and hr-t mutant viruses, the resulting intact virions were purified by CsCl centrifugation, and the amounts of encapsidated wt and mutant DNA were determined by restriction endonuclease digestion of extracted viral DNA. A deletion mutant (NG18) and its wt rescued partner (R4) were used because of the easily recognized difference in the *Hpa* II cleavage patterns (3). Fig. 4 shows the cleavage pattern of Hirt-method supernatant viral DNAs of R4 (lane A), NG18 (lane B), and the mixed NG18/R4 infection (lane C); lane D shows the digestion pattern of virion DNA from CsCl-purified virions isolated from the mixed infection. A comparison of lanes C and D in Fig. 4 shows that there was no preferential gain or loss of either R4 or NG18 DNA during virus packaging. Virus outputs (pfu) were determined from parallel infections: R4, 4×10^8; NG18, 1×10^7; and R4/NG18, 4×10^8. Because there was an ≈1:3 ratio of NG18 to R4 virions produced, the mixed infection resulted in a titer rise of NG18 from 1×10^7 to 1–2 $\times 10^8$ pfu. When VP_1 subspecies were assayed by 2-D gel electrophoresis in the mixed infection, a wt pattern was observed. Thus, hr-t mutant DNA is packaged efficiently

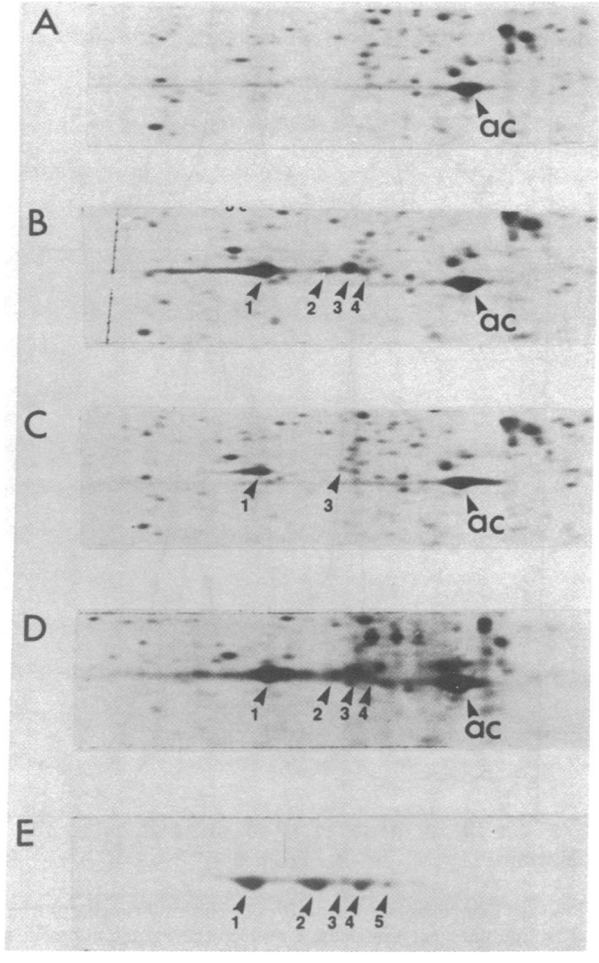

FIG. 3. VP_1 subspecies seen during polyoma virus infections. Shown are regions of 2-D gels of total cell extracts from [^{35}S]methionine-labeled cells (decreasing pH to the right). VP_1 subspecies are shown from mock-infected 3T3 cells (*A*), 59RA-infected 3T3 cells (*B*), NG59-infected 3T3 cells (*C*), NG59-infected BMK cells (*D*), and virions (*E*). ac, actin spot.

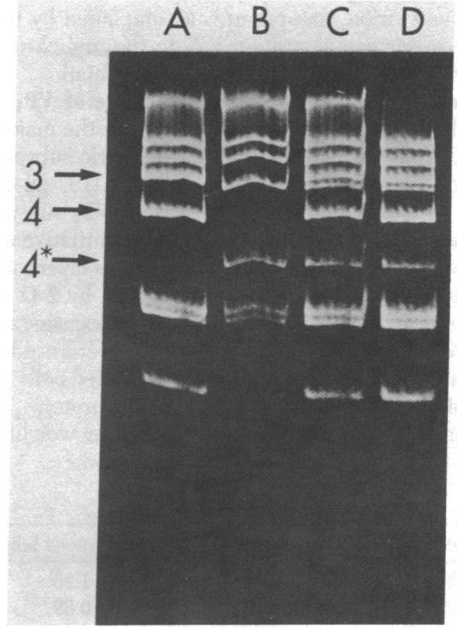

FIG. 4. *Hpa* II restriction digests from a mixed infection of 3T3 cells with R4 (wt) and NG18 (hr-t mutant) viruses. Shown are *Hpa* II digests of Hirt-method supernatant viral DNAs: R4 (lane A), NG18 (lane B), and NG18/R4 (lane C); lane D shows the digestion of viral DNA isolated from virions produced in the NG18/R4 double infection. *Hpa* II fragments 4 and 4* indicate the major restriction fragment difference between R4 and NG18 DNAs, although a slight difference involving fragment 3 can be seen also. As shown in lanes C and D, there is no preferential packaging of viral DNA.

in nonpermissive cells when complemented by the wt function, which supplies the appropriately modified forms of VP_1. This result is supported by complementation for virus growth between hr-t mutants and late mutants affected in VP_1 (25).

DISCUSSION

Transforming genes of tumor viruses can be classified into two groups: (*i*) those genes that serve an essential role in the virus life cycle and (*ii*) those that are nonessential. Cellular oncogenes ("proto-oncogenes") transduced by retroviruses, such as the *src* gene of Rous sarcoma virus, are examples of the nonessential class. These genes may be expressed at low levels in normal cells, but no function has been ascribed as yet to any gene of this class. The hr-t gene of polyoma virus is an example of the essential class; hr-t mutants define a single complementation group for virus growth and transformation (25, 3), and these two aspects of their phenotype are inseparably linked at the genetic level (5). Two genes in adenovirus, identified in a manner analogous to hr-t mutants, are additional examples of essential or "dual-function" transforming genes (26). Although the transforming genes of these DNA tumor viruses have no close homologue in the normal host cell, there are compelling reasons to believe in the case of polyoma virus that the hr-t gene acts to regulate or redirect normal cellular functions for the purpose of virus growth (3). In the present study, we have sought to define the nature of these cellular permissivity factors and the role of the hr-t gene in the polyoma virus growth cycle.

The hr-t mutant class presents a novel phenotype in that the genetic alterations are in the early region of the viral DNA but cause a "late" block affecting virus assembly. The result is a decrease to 1–2% in virus output when hr-t mutants are grown on nonpermissive cells. We showed that the defect in virus production may be accounted for by a combination of two effects: a decrease to 30–40% in the amount of viral DNA accumulated and a decrease to 5% in the ability to encapsidate the viral minichromosome (75S) to a final virion structure (240S).

The defect in encapsidation of hr-t mutant minichromosomes is associated with a failure to produce modified subspecies of the major capsid protein VP_1 that are normally present in the final virion structure. Four lines of evidence support a cause-and-effect relationship between VP_1 post-translational modification and encapsidation: (*i*) when nonpermissive cells are doubly infected with hr-t mutant and wt viruses, modified VP_1 subspecies are present and mutant minichromosomes are packaged with the same efficiency as those of wild type; (*ii*) when hr-t mutants infect permissive cells, a normal complement of modified VP_1 subspecies is present and encapsidation proceeds normally; (*iii*) polyoma virions show a fixed proportion of modified subspecies regardless of virus genotype or host cell on which they are produced; and (*iv*) a decrease to 5–10% in modified VP_1 subspecies in hr-t mutant infection of 3T3 cells correlates with a decrease to 5% in the efficiency of encapsidation of 75S minichromosomes to 240S virions.

The capsid protein modifications have not been fully characterized, although acetylation and phosphorylation have been reported for VP_1 from SV40 and polyoma (19, 23, 24). Our results suggest that the hr-t gene product(s) is in some manner responsible for these post-translational modifications of VP_1 and that the modified subspecies are necessary for final virion assembly. hr-t mutant virus particles purified from low-yield nonpermissive infections show the same stoichiometry of modified VP_1 subspecies as do wt virions. This fixed VP_1 stoichiometry in virions is distinct from the distribution of VP_1 forms in the intracellular pool, implying either nonrandom selection of modified subspecies for encapsidation or further modification/demodification reactions after assembly.

Our data do not rule out the possibility that some of the VP_1 modifications occur after virion assembly. However, other work supports a model where modification of VP_1 precedes assembly. Hunter and Gibson (22) in their analysis of VP_1 mRNA showed that VP_1 post-translational modifications occur in a wheat germ translation system, independent of viral DNA and virion assembly. Additionally, VP_1 modifications in cells infected with SV40 or polyoma occur extremely rapidly, with pulse-labeling periods as short as 10–15 min (ref. 11; unpublished data), making it unlikely that all such modifications would follow assembly.

It is remarkable that permissive cells infected by hr-t mutants produce a series of modified forms that is indistinguishable from that induced in nonpermissive cells by a functional hr-t viral gene. Earlier observations on the growth of hr-t mutants led to a model in which the hr-t gene acts to induce the expression of cellular permissive factor(s) required for virus production (3). In terms of the present findings, the cellular permissive factors previously hypothesized would be responsible for post-translational modifications of VP_1 and possibly for a stimulatory effect on viral DNA replication. The constitutive level of these modification enzymes would determine the yield of hr-t mutants in any given cell type. The hr-t gene products may act at the genetic level by inducing higher levels of these cellular enzymes or at the protein level by modifying the enzymes to have a higher activity toward VP_1.

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