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Structural Roles of Polyoma Virus Proteins

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The superhelical, closed circular form of polyoma deoxyribonucleic acid (DNA) (Co 1) is bound in a 25*S* DNA-protein complex to the viral histone-like proteins after alkaline disruption of the virion. Nicked viral DNA or linear DNA are largely free of protein. Most of the viral protein disruption is in the form of capsomeres, sedimenting principally at 10*S* and 7*S*. Despite the relatively constant ratio of 10*S* to 7*S* material in many preparations, (1:5.5 to 1:6.0, respectively), the two classes of capsomeres are indistinguishable by electron microscopy and contain only P_2, P_3, and P_4 in molar ratios of approximately 5:1:1 or 6:1:1, respectively. Material with sedimentation rates of approximately 1 to 3*S* is enriched for P_5 and contains small amounts of P_2, P_3, and P_4. During the in vitro reassembly of DNA-free, shell-like particles from disrupted virus, proteins P_1, P_2, P_3, P_4, and P_7 are reincorporated efficiently, whereas P_5 and P_6 are not. The presence in empty reassembled particles of histone-like protein, expecially P_7, implies that at least this one of the minor protein components of the virion may participate in protein-protein interactions with other components of the capsid.

It is known that even some of the simplest icosahedral mammalian viruses, such as polyoma, simian virus (SV40), and the adenoviruses contain several proteins in addition to a major capsid protein (3, 7, 16, 21). Some proteins are in the capsid, whereas others are thought to bind to the viral nucleic acid and are designated "core" proteins (1, 2, 7, 17, 20). In the case of the adenoviruses, one kind of polypeptide is used in the assembly of hexons, whereas different proteins are used for penton and penton base assembly, respectively (17). The location and functions of the core proteins are unknown, although there is reason to suspect that the maturation of the virion and the encapsidation of nucleic acid is associated with the synthesis and binding of "core" proteins. For instance, arginine deprivation during infection with adenovirus results in the synthesis but not the assembly of most of the viral proteins. These proteins are assembled into mature virions, containing deoxyribonucleic acid (DNA), after arginine is restored (8). These events may coincide with the synthesis and incorporation into the virion of an arginine-rich core protein.

Highly purified polyoma virus contains at least six or seven proteins, as judged by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Little is known about the location or the structural roles of any of the proteins. The three smallest proteins contain no tryptophan and have lysine and arginine contents characteristic for histones (21). Frearson and Crawford have recently shown that the peptide maps of these basic proteins, after labeling with ^{35}S-methionine, are similar to those of uninfected host cell histones. Since they also can be labeled prior to infection, these workers have suggested that these proteins are host cell proteins (9).

In the related virus SV40, four basic proteins are found associated with the viral DNA after disruption of the virion (7), and this has been taken as evidence for an "internal" position for these proteins. There is some evidence that antisera directed against purified SV40 core proteins interact with native virions, suggesting that DNA-binding virion proteins may be at least partly exposed to the surface of the virion (14).

One of us has recently described an in vitro method for the assembly of empty, noninfectious particles from disrupted polyoma virus (10). Using alkaline disruption and reassembly, we report here evidence for the binding of histone-like proteins to polyoma viral DNA and describe interactions of these proteins which suggest that at least one of the small basic proteins may constitute a structural component of the capsid.

MATERIALS AND METHODS

Radioactively labeled large-plaque virus was purified from infected primary baby mouse kidney cells as previously described (10).

Alkaline disruption. Purified virus was dialyzed at 4 C for 2 hr with vigorous stirring against 100 volumes of 0.2 M Na_2CO_3-$NaHCO_3$, *p*H 10.6, with 3×10^{-3} M

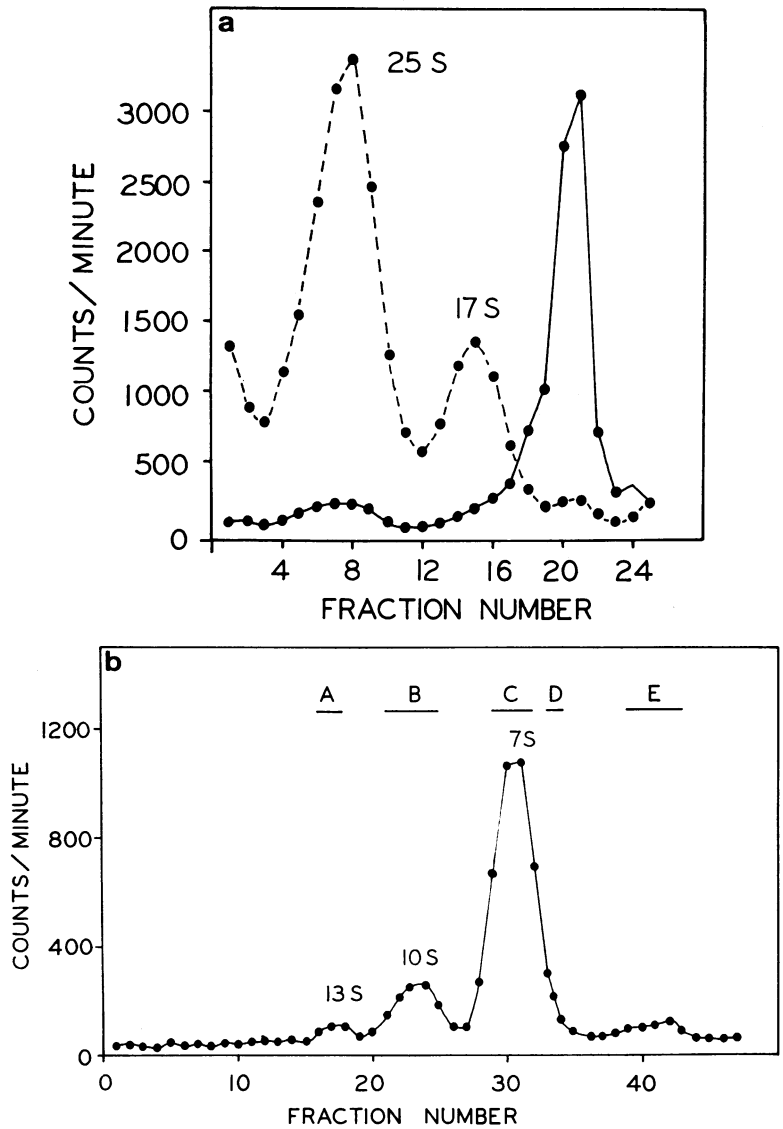

FIG. 1. *a, Alkaline disruption of polyoma. Purified virus labeled with ^{14}C-leucine, lysine, and valine and ^{3}H-thymidine was dialyzed against disruption buffer (0.2 M $NaCO_3$, pH 10.6, with 3 × 10^{-3} M dithiothreitol) at 0 C for 2 hr. Centrifugation was then carried out using a linear 10 to 30% glycerol gradient in the disruption buffer for 12 hr at 39,000 rev/min. Samples were collected and the positions of the products were located by scintillation counting, ^{14}C (●——●), ^{3}H (●- - -●). Sedimentation coefficients were calculated from the position of closed circular, 20S component 1 viral DNA in a parallel tube. b, Disrupted virus prepared as in 1a, but centrifuged for 20 hr. Only the ^{14}C-protein radioactivity is plotted.*

dithiothreitol (disruption buffer). The disrupted virus was then layered onto a gradient of 10 to 30% v/v glycerol in disruption buffer and centrifuged at 4 C in an SW41 Beckman rotor at 40,000 rev/min either for 12 hr to isolate the DNA-protein complex, or for 20 hr to resolve the major slower protein-containing peaks. Samples of 0.2 ml were collected through puncture holes in the bottom of the tube, and peaks were located by measuring radioactivity in 10-μliter samples. Component I viral DNA, with a sedimentation coefficient of 20S, was used as a sedimentation marker in parallel tubes.

Reassembly. Disrupted virus was dialyzed overnight without stirring against 200 volumes of 0.15 M NaCl, 0.05 M tris(hydroxymethyl)aminomethane (Tris), 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM β-mercaptoethanol, pH 8.0, at 4 C. The resulting hemagglutinin-positive material was treated with 20 μg of

pancreatic deoxyribonuclease per ml in the presence of 10 mM Mg^{2+} for 30 min at 37 C and then spun through a 10 to 30% glycerol gradient in 0.05 M Tris, *p*H 8.0, 1 mM EDTA, 0.05% bovine serum albumin (fraction V, Miles Laboratories, Inc.) in a SW50L rotor at 45,000 rev/min for 40 min. Each fraction was assayed for hemagglutination activity, and hemagglutination-positive portions of the gradient, corresponding to 140*S* and 180*S*, were collected and pooled separately.

Analytic polyacrylamide gel electrophoresis. The protein composition of samples was examined by electrophoresis in SDS-12.5% polyacrylamide gels by the method of Laemmli (15). Samples for electrophoresis were precipitated in cold 5% trichloroacetic acid in the presence of 100 μg of carrier bovine serum albumin, centrifuged in a swinging bucket Sorvall rotor at 7,000 rev/min for ten min, washed with acetone, and air dried. After electrophoresis, the gels were crushed in a Savant autogel divider, and samples were collected into scintillation vials. Autoradiographs were performed by exposing dried longitudinal gel slices to Kodak Royal Blue X-ray film.

Scintillation counting. The radioactivity of samples was measured in a Beckman scintillation spectrometer, using a toluene Triton-X scintillation mixture (1 volume of Triton X-100, 2 volumes of toluene, 4 g of 2,5-diphenyloxazole per liter). Double label settings were as described previously (10).

Electron microscopy. Samples were examined in a Siemens Elmiskop 1 at a magnification of 40,000, operating at 80 kv. Samples were stained with 1% phosphotungstate.

RESULTS

Disruption of purified virus under the above alkaline and reducing conditions results in the disruption of the capsid largely to capsomeres. Sedimentation of disrupted virus through a glycerol gradient in disruption conditions (Fig. 1a) showed the released virion DNA to be found as a 25*S* peak associated with approximately 4 to 6% of the recovered virion protein, as well as a second, DNA-containing peak at approximately 17*S*, largely free of protein. The small amount of ^{14}C-labeled material under the 17*S* peak in Fig. 1a was shown to represent the leading edge of the major protein peak, and somewhat longer centrifugation showed the 17*S* material to have no protein associated. After centrifugation of a similarly prepared sample for 20 hr under identical conditions, proteins derived from disrupted virus were resolved into four peaks as shown in Fig. 1b. Material at 7*S* and 10*S* contained many capsomeres which are indistinguishable by electron microscopy (Fig. 2), whereas material near the top of the gradient did not contain capsomeres. The small amounts of material recovered from the 13*S* region also contained capsomeres.

The DNA peaks at 25*S* and 17*S* were collected separately and examined by alkaline glycerol gradients (24) to determine the DNA configuration (Fig. 3). The 25*S* material was found to consist almost entirely of viral DNA (superhelical closed circular double-stranded), whereas 17*S* material contained no component I, but only nicked viral (viral component II) or linear forms of DNA, or both. Some of this DNA probably represents cell DNA derived from pseudovirions (18).

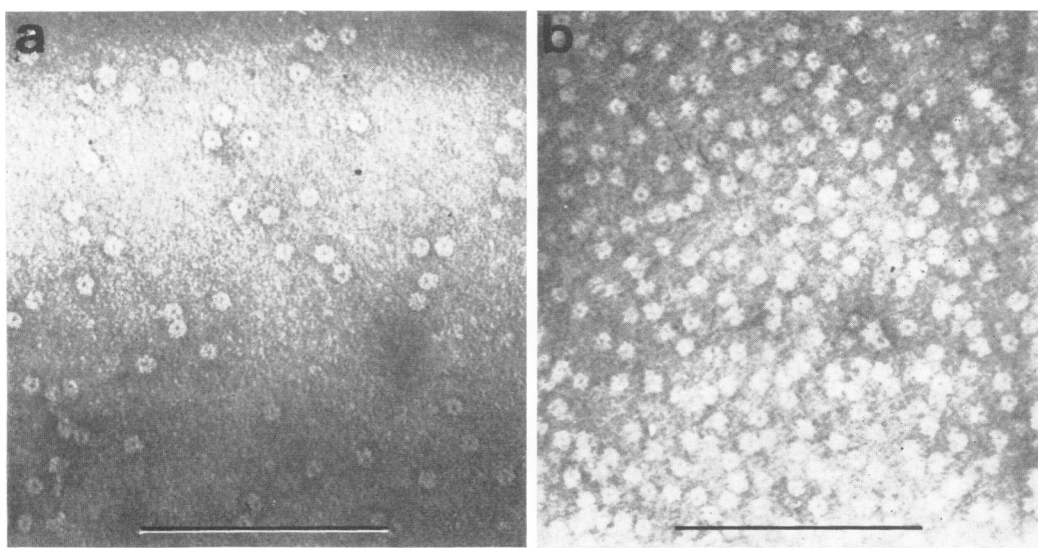

FIG. 2. *Electron microscopy of purified 10S (a) and 7S (b) capsomeres. Samples from corresponding parts of the gradient were applied directly to carbon grids, washed with 1% Na phosphotungstate, and air dried. The bar indicates 100 nm.*

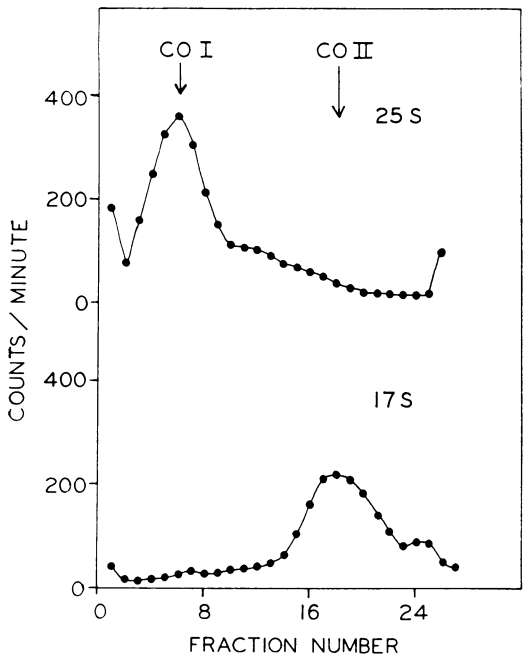

FIG. 3. *Centrifugation of 25S DNA-protein complex and 17S DNA peak through 10 to 30% glycerol in 0.1 N NaOH, at 38,000 rev/min for 90 min at 20 C. Samples were collected into scintillation vials, neutralized with 1 M Tris, pH 8.0, and counted in Triton-toluene scintillation fluid. The positions of component I viral DNA and of component II plus linear cell DNA are given by the arrows. In the alkaline conditions, component I viral DNA has a sedimentation of approximately 50S.*

Protein composition of disrupted particles. Autoradiographs of the proteins from radioactive purified virus, labeled with ^{14}C-leucine, lysine, and valine and separated by SDS-polyacrylamide gel electrophoresis showed seven major bands, designated P_1 through P_7, according to Roblin et al. (21) after short exposure to X-ray film (Fig. 4a). Estimates for the number of each of these proteins are given in Table 1 and are calculated assuming a virion protein content of 22×10^6 daltons. An additional 12 or 13 bands were identifiable after prolonged exposure to X-ray film (Fig. 4b). As with SV40 (23), some of these proteins are probably present in quantities sufficient to allow more than one copy to be present per virion, and so it is not clear whether these proteins are true virion proteins or merely contaminants. Crushing gels in the auto gel divider and counting fractions reveal at least seven distinct peaks, as seen in Fig. 5a.

The protein compositions of the products of disruption are shown in Fig. 5. The 25S DNA-protein complex contained only a small amount of the major capsid protein in P_2, a variable amount of material in the region of P_5, and virtually all of the recovery P_6 and P_7 (Fig. 5b). Both the 10S and 7S protein peaks contain proteins P_2, P_3, and P_4 in molar ratios 5:1:1 or 6:1:1 (Fig. 5c, d). The amount of P_1 in these preparations has not been estimated due to the variable amount of aggregated material in the heavy region of the gel. The material found between 1 to 3S contained some P_2, P_3, and P_4, but was enriched for P_5 (Fig. 5e).

Reassembly. Reassembled shell-like particles formed after exposure of 100S material to deoxyribonuclease were found in a neutral glycerol gradient at approximately 140S, 180S, and on cushions of half-saturated CsCl (10). Samples were taken from each of these areas, precipitated with cold 5% trichloroacetic acid in the presence of 100 μg of albumin, washed with acetone, and air dried. Gels of the 140S region and of the start-

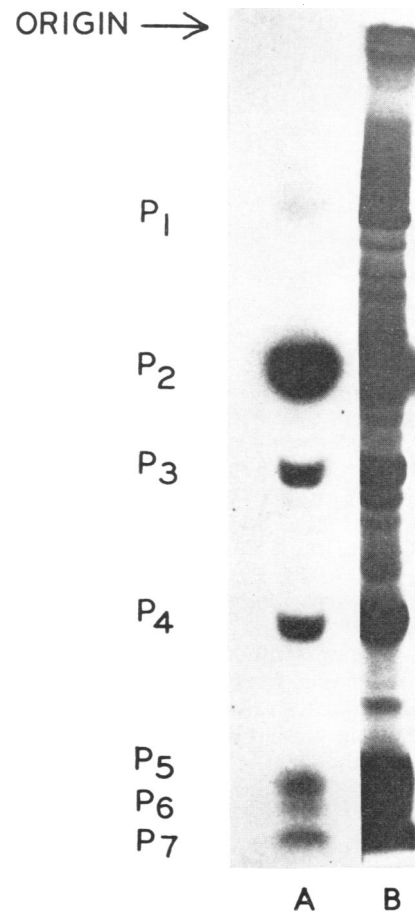

FIG. 4. *Autoradiograph of ^{14}C-labeled purified polyoma for A, 10 days and B, 43 days. Gel electrophoresis was in 12.5% SDS-polyacrylamide gels, as described in Materials and Methods.*

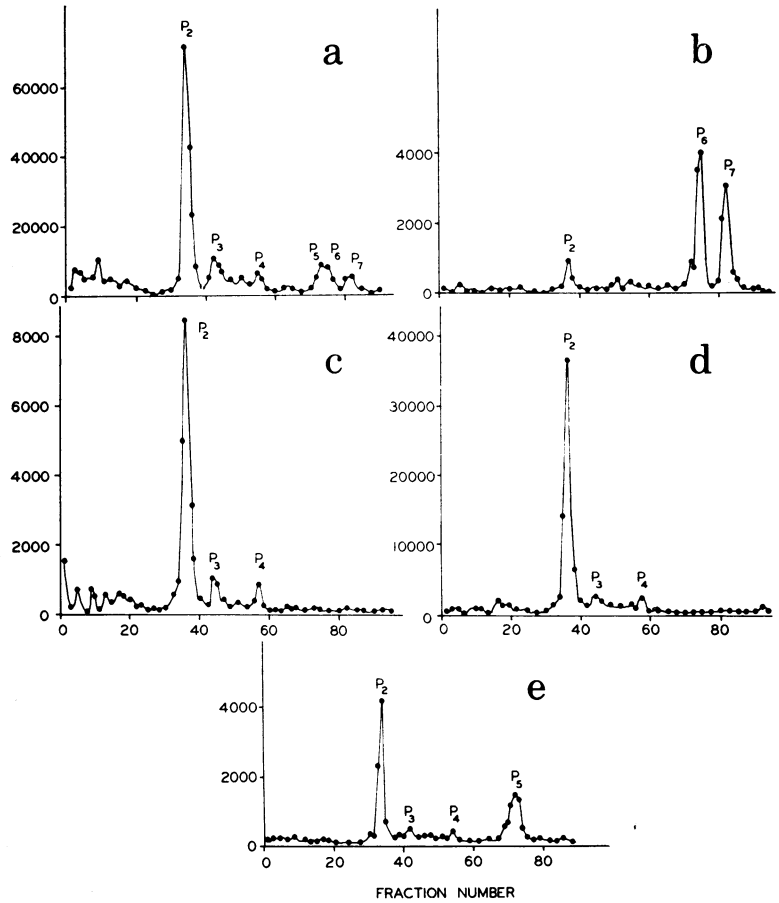

FIG. 5. *SDS-polyacrylamide gels of native virus and of disruption products after alkaline disruption (see Fig. 1). a, Native virus; b, 25S DNA-protein complex; c, 10S capsomeres; d, 7S capsomeres; e, top of gradient, region E in Fig. 1b. Samples were precipitated with cold 5% trichloroacetic acid, washed with acetone, and air dried. Electrophoresis, fractionation, and scintillation counting were as described in Materials and Methods.*

ing virus are shown in Fig. 6. Native virions and reassembled spherical 140*S* particles contained similar amounts of P_1, P_2, P_4, and P_7, but reassembled particles had somewhat reduced P_3 and virtually absent P_5 and probably P_6 when compared with starting virus. Similar results were obtained with material from 180*S* and CsCl shelf region of the gradient. In all reassembled samples, a small amount of protein label was found to be migrating faster than P_7.

DISCUSSION

Some of the proteins identified by Roblin et al. (21) in polyoma virions can be shown to participate in protein-protein and protein-DNA interactions. The major capsid protein, P_2, along with P_3 and P_4, is found in two classes of capsomeres after alkaline disruption of virus. There are approximately 5 or 6 copies of P_2, 1 copy of P_3, and 1 copy of P_4 in both kinds of capsomeres. Although the similar protein composition of the two capsomere regions and sedimentation relationship suggests that the 10*S* material may be composed of dimers of 7*S* capsomers, 10*S* capsomeres rerun in a second gradient sediment at 10*S* and do not generate any 7*S* forms (T. Friedmann, *unpublished results*). Likewise, 7*S* capsomeres do not generate 10*S* material when rerun. Although this implies structural differences between these two classes of capsomeres, it is not clear whether this is due to irreversible denaturation of a proportion of 7*S* capsomeres or to a real difference in composition or construction of these capsomeres. However, the relatively constant proportion of 10*S* capsomeres to 7*S* capsomeres suggests that there are real differences in structure or composition between these two kinds of capsomeres. It is clear that no proteins other than P_2, P_3, and P_4

TABLE 1. *Molecular weights and relative proportion of major polyoma proteins*

Component	Molecular weight × 1,000[a]	% of total virion protein[b]	Estimate of number of polypeptides per virion[c]
P_1	86	2.5 to 3.9	6 to 9
P_2	48	76.1 to 76.9	380 to 384
P_3	35	2.3 to 4.2	16 to 29
P_4	23	2.2 to 4.3	24 to 39
P_5	19	12.2 to 13.3	92 to 97
P_6	17		
P_7	15	3.5 to 4.5	40 to 62

[a] Molecular weights determined by Roblin et al. (21).

[b] Determined from acrylamide gel electrophoresis of purified polyoma labeled with ^{3}H-lysine or with ^{14}C-leucine, lysine, and valine.

[c] Corrected for amino acid composition of proteins (Murakami, *personal communication*). The virion protein weight was calculated, assuming that viral DNA has a molecular weight of 3×10^6 and constitutes 12% of the virion by weight (11).

are structural requirements for most capsomeres, since P_1 and the histone-like proteins P_5, P_6, and P_7 are not found in 10*S* and 7*S* capsomeres. We of course cannot exclude the possibility that capsomeres consist only of P_2, and that P_3 and P_4 are contaminants which are tightly bound to native virions and to capsomeres even after virus disruption.

Recent studies by Walter et al. (23) have suggested that in the closely related virus SV40, hexons may be composed of the major capsid protein and pentons of a second major virion protein. Although their suggestion is in keeping with the known structure of adenovirus, which has penton and penton fiber proteins different from the major hexon protein, our results do not yet clearly identify a unique penton structure.

Of the several kinds of DNA encapsidated within polyoma capsids (superhelical viral, nicked viral, host) (5, 18, 22, 24), apparently only the superhelical viral DNA interacts to a great extent with virion proteins. Most of the virion copies of the histone-like proteins P_6 and P_7, and to a variable extent P_5, remain bound to component 1 in a 25*S* complex after disruption, whereas noncomponent I DNA sediments at 17*S* and is essentially protein-free. Gels also containing viral proteins labeled with ^{3}H-lysine as internal markers have, on some occasions, shown that the DNA-protein complex at times contains all of the recovered P_5 as well as P_6 and P_7. In these cases, P_5 was not found in the 1 to 3*S* region. The amount of P_5 in the complex seems to be, at least, partly a function of the age of the complex preparation, and may indicate that this protein is bound less tightly to DNA than the other two histone-like proteins.

Only one of the minor histone-like proteins, namely P_7, is reincorporated efficiently into the new DNA-free, shell-like particles found during in vitro reassembly. Although reassembled particles contain some bound material labeled with ^{3}H-thymidine, this radioactive material is extensively degraded and does not represent macromolecular DNA (T. Friedmann, *unpublished results*). The reincorporated P_7 is therefore likely to be bound to protein in the capsid, and thereby may constitute a structural component of the capsid and provide sites on the capsid for interaction with viral superhelical DNA looping up toward the surface from the interior of the virion. The origin of the protein counts smaller than P_7 in reassembled particles is not clear, and we cannot exclude the possibility that they represent some cleavage products of P_5 and P_6.

The significance of P_1 is unclear. Murakami has shown that P_1 and P_2 have very similar or identical amino acid composition (*personal communica-*

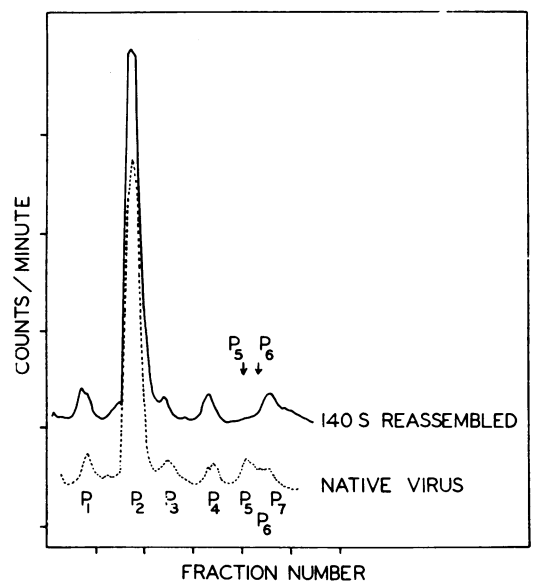

FIG. 6. *SDS-polyacrylamide gel of native virus (----) and 140S reassembled particles (——). Purified virus labeled with ^{14}C-leucine, lysine, and valine, and with ^{3}H-thymidine was disrupted and reassembled as in Materials and Methods. The region of hemagglutination activity corresponding to 140S (10) was collected and analyzed by gel electrophoresis as in Materials and Methods. The peaks were located by crushing and counting gels as described in Materials and Methods. Only ^{14}C-protein counts are shown. The peak height of P_2 of native virus was 12,400 counts/min, whereas the peak height of P_2 in reassembled particles was 4,800 counts/min.*

tion), and the additional fact that the molecular weight of P_1 is about twice that of P_2 suggests that P_1 might be a dimer of P_2 which is incompletely cleaved during preparation for electrophoresis. SV40 has a similar large protein which can be removed by purification of the virus (7).

The relationship of the virion 25*S* protein-DNA complex to the complexes found by Green et al. (12) and Bourgaux and Bourgaux-Ramoisy (24) in cells infected with polyoma, by White and Easson in cells infected with SV40 (25), and by Doerfler et al. in cells infected with adenovirus (6) is unclear, since the proteins of these complexes have not been identified, and since it is not known if they are incorporated into mature virions. There is a DNA-protein complex in SV40 which results in the restricted in vitro transcription of the viral DNA (7, 13). There is so far no firm evidence for a function of this complex, whether in the regulation of gene expression during infection or transformation, or in the encapsidation of the viral DNA into precursor particles. There is some evidence for a precursor role for empty shells in the maturation of SV40 (19), and it is likely that both polyoma and SV40 empty shells lack most or all of the virion histone-like proteins (7; T. Friedmann, *unpublished data*). This suggests that binding to histone-like proteins might be a requirement for DNA encapsidation. However, since polyoma assembly may involve a preliminary interaction of DNA with capsid proteins (10), the participation of histone-like proteins in virus maturation is likely to be more complex than solely to induce conformational changes in DNA during encapsidation of shells.

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LITERATURE CITED

1. Anderer, F. A., M. A. Koch, and H. D. Schlumberger. 1968. Structure of simian virus 40. III. Alkaline degradation of the virus particle. Virology **34**:452–458.
2. Anderer, F. A., H. D. Schlumberger, M. A. Koch, H. Frank, and H. J. Eggers. 1967. Structure of simian virus 40. II. Symmetry and components of the virus particle. Virology **32**:511–523.
3. Barban, S., and R. Goor. 1971. Structural proteins of simian virus 40. J. Virol. **7**:198–203.
4. Bourgaux, P., and D. Bourgaux-Ramoisy. 1972. Is a specific protein responsible for the supercoiling of polyoma DNA? Nature (London) **235**:105–107.
5. Crawford, L. V. 1969. Nucleic acids of tumor viruses, Advan. Virus Res. **14**:89–152.
6. Doerfler, W., A. Lundholm, and M. Hirsch-Kauffmann. 1972. Intracellular forms of adenovirus deoxyribonucleic acid. I. Evidence for a deoxyribonucleic acid-protein complex in baby hamster kidney cells infected with adenovirus type 12. J. Virol. **9**:297–308.
7. Estes, M., E. -S. Huang, and J. S. Pagano. 1971. Structural polypeptides of simian virus 40. J. Virol. **7**:635–641.
8. Everitt, E., B. Sundquist, and L. Philipson. 1971. Mechanism of arginine requirement for adenovirus synthesis. I. Synthesis of structural proteins. J. Virol. **8**:742–753.
9. Frearson, D. M., and L. V. Crawford. 1972. Polyoma virus basic proteins. J. Gen. Virol. **14**:141.
10. Friedmann, T. 1971. In vitro reassembly of shell-like particles from disrupted polyoma virus. Proc. Nat. Acad. Sci. U.S.A. **68**:2574–2578.
11. Green, M. 1970. The oncogenic viruses. Ann. Rev. Biochem. **39**:701–756.
12. Green, M. H., H. Miller, and S. Hendler 1971. Isolation of a polyoma-nucleoprotein complex from infected mouse cell culture. Proc. Nat. Acad. Sci. U.S.A. **68**:1032–1036.
13. Huang, B.-S., M. Nonoyama, and J. S. Pagano. 1972. Structure and function of the polypeptides in simian virus 40. II. Transcription of subviral deoxynucleoprotein complexes in vitro. J. Virol. **9**:930–937.
14. Koch, M. A., H. Becht, and F. A. Anderer. 1971. Structure of simian virus 40. V. Virology **43**:235–242.
15. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
16. Maizel, J. V., D. O. White, and M. D. Scharff. 1968. The polypeptides of adenovirus I. Evidence for multiple protein components in the virion and a comparison of types 2, 7A and 12. Virology **36**:115–125.
17. Maizel, J. V., D. O. White, and M. D. Scharff. 1968. The polypeptides of adenoviruses II. Soluble proteins, cores, top components and the structure of the virion. Virology **36**:126–136.
18. Michel, M. R., B. Hirt, and R. Weil. 1967. Mouse cellular DNA enclosed in polyoma viral capsid (pseudovirions). Proc. Nat. Acad. Sci. U.S.A. **58**:1381–1388.
19. Ozer, H., and P. Tegtmeyer. 1972. Synthesis and assembly of simian virus 40. II. Synthesis of the major capsid protein and its incorporation into viral particles. J. Virol. **9**:52–60.
20. Prage, L., and U. Pettersson. 1971. Structural proteins of adenoviruses. Virology **45**:364–373.
21. Roblin, R., E. Härle, and R. Dulbecco. 1971. Polyoma virus proteins. Virology **45**:555–566.
22. Vinograd, J., J. Lebowitz, R. Radloff, R. Watson, and P. Laipis. 1965. The twisted circular form of polyoma viral DNA Proc. Nat. Acad. Sci. U.S.A. **53**:1104–1111.
23. Walter, G., R. Roblin, and R. Dulbecco. 1972. Protein synthesis in simian virus 40-infected monkey cells. Proc. Nat. Acad. Sci. U.S.A. **69**:921–924.
24 Weil, R., and J. Vinograd. 1963. The cyclic helix and cyclic coil forms of polyoma viral DNA. Proc. Nat. Acad. Sci. U.S.A. **50**:730–738.
25. White, M., and R. Eason. 1971. Nucleoprotein complexes in simian virus 40-infected cells. J. Virol. **8**:363–371.