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Use of the baculovirus system to assemble polyomavirus capsid-like particles with different polyomavirus structural proteins: analysis of the recombinant assembled capsid-like particles

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The genes encoding the structural proteins (VP1, VP2 and VP3) of murine polyomavirus were cloned into the p2Bac dual multiple cloning site vector, individually or jointly, and the corresponding proteins were expressed in *Spodoptera frugiperda* (Sf9) insect cells by cotransfecting Sf9 cells with the constructed vector and the linear DNA of *Autographa californica* multiple nuclear polyhedrosis virus (Ac*M*NPV). Recombinant capsid-like particles could be purified 5 days post-infection from Sf9 cells infected with Ac*M*NPV–VP1, with or without the involvement of minor protein (VP2 or VP3). Although VP2 and VP3 alone could not generate recombinant particles, they became incorporated into these particles when expressed with VP1 in Sf9 cells. Recombinant particles with different polyomavirus structural protein(s) were obtained by using different combined expression of these proteins in Sf9 cells. Cellular DNA of 5 kbp in size was packaged in all of the recombinant particles, which showed the same diameter as that of native virions. Agarose gel electrophoresis indicated that DNA packaged in these recombinant particles had a different pattern than that of native virions. Two-dimensional gel electrophoresis of the VP1 species of recombinant particles showed more VP1 species than those of the native virions from mouse cells, and an additional species of VP1 when VP2 was co-expressed with VP1. The recombinant particles were also compared for their ability to compete for polyomavirus infection. The competition assay indicated that the recombinant particles containing VP2 were the most efficient in inhibiting the native polyomavirus infection of 3T6 cells.

Introduction

Murine polyomavirus is a small, nonenveloped DNA virus with a diameter of 45–50 nm and a genome of about 5·3 kbp, which encodes six proteins. Three of these proteins, the early T antigens, are responsible for the induction and maintenance of tumorigenic state and for the lysis of cells infected by polyomavirus virions. The other three proteins are structural proteins, VP1, VP2 and VP3, which form the icosahedral capsid protecting its circular supercoiled covalently closed double-stranded DNA genome, which associates with host histones. In the native, wild-type polyomavirus virion, VP1 is the major capsid protein, with a molecular mass of 45 kDa, and makes up about 80% of the proteins found in the capsid. VP2 and VP3 are minor capsid proteins, with molecular masses of 35 and 25 kDa, respectively, and each of them makes up about 10% of the capsid protein. The capsid of polyomavirus has been shown to be built of 72 pentamers of VP1 (Belnap *et al.*, 1996; Rayment *et al.*, 1982), with VP2 and VP3 in the centre of the pentamers (Liddington *et al.*, 1991).

Besides its structural function, VP1 plays other important roles in the polyomavirus life-cycle. This protein possesses the receptor-binding domain (Anders & Consigli, 1983*a*; Stehle *et al.*, 1994), a nuclear localization signal domain, a DNA-binding domain and a calcium-ion-binding domain (Chang *et al.*, 1992*a*, 1993; Haynes *et al.*, 1993; Moreland *et al.*, 1991), which makes it crucial in virus attachment and virion assembly. When expressed in the bacterial system, polyomavirus VP1 could assemble into empty capsid-like structures spontaneously in the presence of calcium ions, with a size and morphology similar to those of the native polyomavirus capsids (Leavitt *et*

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al., 1985; Salunke *et al.*, 1986; Haynes *et al.*, 1993). The bacterial expression systems have proven to be an invaluable resource for the production and purification of large quantities of all the polyomavirus structural proteins (Cai *et al.*, 1994; Chang *et al.*, 1992*a*, *b*, 1993; Delos *et al.*, 1993; Haynes *et al.*, 1993; Moreland *et al.*, 1991). It is important to consider the feature that bacterially expressed proteins may lack many of the sophisticated posttranslational modifications associated with proteins produced in eukaryotic cells, many of which have been shown to occur in the native polyomavirus structural protein components. For example, the VP1 protein of native polyomavirus can be further separated into several species by two-dimensional gel electrophoresis, indicating its posttranslational modifications, such as phosphorylation, sulfation, methylation, acetylation and hydroxylation (Anders & Consigli, 1983 *b*; Bolen *et al.*, 1981; Burton & Consigli, 1996; Fattaey & Consigli, 1989; Ludlow & Consigli, 1987 *b*; Ponder *et al.*, 1977; Yuen & Consigli, 1985).

The eukaryotic baculovirus–insect system has been applied to produce the structural proteins of polyomavirus. These proteins have been used to probe essential questions dealing with the polyomavirus life-cycle, such as the cellular localization of these structural proteins and their transport to the nucleus, protein–protein interactions involving the structural proteins, posttranslational modifications of the structural proteins, and the essential nature of calcium ions in VP1 capsid assembly (Delos *et al.*, 1993; Forstová *et al.*, 1993; Li *et al.*, 1995; Montross *et al.*, 1991).

Recently, our laboratory has shown that polyomavirus VP1 protein can assemble into capsid-like particles which are capable of packaging cellular DNA and histones when expressed in the baculovirus system after 5 days of infection (Gillock *et al.*, 1997). We have also cloned the genes encoding the polyomavirus minor structural proteins, VP2 and VP3, into the p2Bac dual multiple cloning site transfer vector. Although these minor capsid proteins could not generate capsid-like particles when expressed alone in the baculovirus system, they became incorporated into the recombinant capsid-like particles when co-expressed with *Autographa californica* multiple nuclear polyhedrosis virus (Ac*M*NPV)–VP1 DNA in *Spodoptera frugiperda* (Sf9) insect cells. The current investigation is the initial demonstration that by expressing polyomavirus structural proteins in Sf9 cells, individually or jointly, we have purified recombinant capsid-like particles with different components, namely, VP1 alone, VP1/VP2, VP1/VP3 and VP1/VP2/VP3. We examined these recombinant particles by electron microscopy and characterized them by SDS–PAGE, Western blotting, two-dimensional gel electrophoresis and their ability to compete for polyomavirus infection.

Methods

■ **Polyomavirus structural protein gene constructs.** The cloning of individual polyomavirus structural protein genes was performed as previously described (Gillock *et al.*, 1997). The VP1, VP2 or VP3 gene was amplified by PCR and inserted into the polyhedrin promoter site of the p2Bac (Invitrogen) dual multiple cloning site transfer vector using the *Bgl*II restriction endonuclease. For the dual gene cloning, VP1 was put in the polyhedrin promoter site as mentioned above, while one of the minor structural protein genes (VP2 or VP3) was inserted into the P10 promoter site using the *Xba*I restriction endonuclease, and the resultant recombinant is denoted as VP1/2 or VP1/3. The constructed transfer vector and the linear Ac*M*NPV DNA were used to cotransfect Sf9 cells. Recombinant Ac*M*NPVs were selected and amplified as previously described (Gillock *et al.*, 1997).

■ **Capsid-like particle purification.** Sf9 cells were grown in 150 cm² flasks containing Grace's medium with 5% foetal calf serum and antibiotics. Confluent cell cultures were then infected with different combinations of recombinant Ac*M*NPVs containing one or two polyomavirus structural protein genes, i.e. VP1, VP1/2, VP1/3, VP1/3+VP2 or VP1/2+VP3. Five days post-infection (p.i.), the recombinant particles composed of different polyomavirus structural proteins were purified by using the same procedure as previously described (Gillock *et al.*, 1997). This procedure entailed putting the recombinant particle cell-free lysate through a 20% sucrose shelf. The pelleted particles were resuspended and purified through a preset CsCl density gradient. The bottom band of the capsid-like particles was then centrifuged through a second CsCl gradient. In order to obtain recombinant particles with high purity, the bottom band from the second CsCl gradient ultracentrifugation was incubated with 0·1% deoxycholic acid at 37 °C for 1 h and subjected to a third CsCl gradient purification.

■ **DNA analysis.** Native polyomavirus virions and the recombinant particles purified by one or three CsCl gradient ultracentrifugations were analysed to determine the size and components of the DNA within the particles. The purified preparations were treated with proteinase K (final concentration, 200 μg), SDS (final concentration, 1%) and EDTA (final concentration, 25 mM) and allowed to react at 37 °C for 30 min. DNA was extracted with an equal volume of phenol–chloroform, precipitated with 3 vols of cold ethanol and pelleted by centrifugation (Gillock *et al.*, 1997). The DNA pellet was resuspended in a minimal amount of distilled water and resolved in a 0·8% agarose gel in TBE buffer (0·09 M Tris, 0·09 M boric acid and 0·05 M EDTA, pH 8·0) as described previously (Ausubel *et al.*, 1988).

■ **SDS–PAGE and immunoblotting.** The proteins of the CsCl-purified recombinant particles were analysed on an SDS–PAGE minigel (Serva) with 12·5% acrylamide and 0·33% *bis*-acrylamide cross-linker, and visualized by Coomassie blue staining as previously described (Haynes & Consigli, 1992).

The proteins of these purified recombinant particles were also analysed by immunoblotting as previously described (Gillock *et al.*, 1997). Briefly, the proteins were resolved by using an SDS–PAGE minigel as mentioned above, and then transferred to a nitrocellulose membrane by using a semi-dry system. Membranes were blocked in a solution of 3% dried milk powder in TBST [Tris-buffered saline (50 mM Tris, 150 mM NaCl), 0·05% Tween 20, pH 7·4] and then probed with an antibody cocktail consisting of rabbit anti-polyomavirus VP1 polyclonal antibody (1:500) and rabbit anti-VP2/3 polyclonal antibody (1:500), which was raised against bacterially expressed recombinant polyomavirus VP2 (Cai *et al.*, 1994). After probing with goat anti-rabbit antiserum conjugated to horseradish peroxidase (1:10000), the corresponding protein bands were visualized by developing the membrane with an enhanced chemiluminescence kit in conjunction with exposure to X-ray film.

■ **Two-dimensional gel electrophoresis.** In order to separate different species of the VP1 protein, two-dimensional gel electrophoresis

was performed by following the method of O'Farrell, essentially as previously described (Bolen *et al.*, 1981; Forstová *et al.*, 1993; Haynes & Consigli, 1992; O'Farrell, 1975; O'Farrell & Goodman, 1976). The protein concentrations of CsCl-purified native polyomavirus virions and recombinant particles were determined by using the Coomassie Plus protein assay reagent kit (Pierce) with BSA as the standard. The virions and recombinant particles (5 μg protein for immunoblotting and 10 μg for Coomassie blue stained SDS–PAGE) were dried and resuspended in 20 μl of sample buffer (9·5 M urea, 5% β-mercaptoethanol, 2% Nonidet P-40 and 2% ampholines). After being incubated at 45 °C for 45 min, samples were loaded on the urea–acrylamide tube gels (150 mm by 1 mm, internal diameter) containing 9·5 M urea, 4% acrylamide, 2% Nonidet P-40 and 2% ampholines. The ampholine mixture consisted of equal volumes of pH 3–5, pH 4–6, pH 5–7, pH 6–8 and pH 3–10 ampholines (all from Bio-Rad Laboratories). Isoelectrofocusing was performed at 400 V for 6 h and 800 V for 45 min, and the gels were then loaded onto second dimension SDS–PAGE minigels as mentioned above. Proteins were visualized either by Coomassie blue staining or by immunoblotting analysis.

■ **Competition assay.** Purified polyomavirus virions having a haemagglutination (HA) titre of 100 were mixed with the respective recombinant capsid-like particles (VP1, VP1/2, VP1/3, VP1/3 + VP2 and VP1/2 + VP3) having a HA titre of 1000. The mixture of native virion and capsid-like particles was used to infect 3T6 mouse cells, which were grown on glass coverslips. After adsorption for 1 h on ice, the infecting solution was removed, the cells were washed three times with cold PBS, then fresh medium was added, and the cells were incubated at 37 °C. At 32 hours p.i., an indirect immunofluorescence assay using anti-polyomavirus antiserum was performed (McMillen & Consigli, 1977) to determine the percentage of infection. The assay was quantified by counting multiple fields of cells and scoring for positive fluorescent nuclei.

■ **Electron microscopy of recombinant capsid-like particles.** CsCl-purified native polyomavirus virions and the recombinant particles were placed on pioloform-coated grids, and the particles were allowed to adsorb to the grid for 5 min. After a distilled water rinse, the samples were stained with a 1% aqueous uranyl acetate solution and examined with a Philips 201 electron microscope operating at 60 kV.

Results

The polyomavirus structural genes (VP1, VP2 and VP3) were successfully cloned, individually or jointly, into the p2Bac dual multiple cloning site transfer vector and the corresponding proteins were expressed in the Sf9 insect cells. Recombinant capsid-like particles could be purified by CsCl density gradient centrifugation from the cells infected with Ac*M*NPVs carrying at least the VP1 gene, with or without a minor protein gene(s). Electron micrographs of the bottom bands from the third CsCl gradient showed that all of the capsid-like particles were full and their morphology was similar to that of the native polyomavirus virions (Fig. 1). These capsid-like particles showed a diameter of 45–50 nm, the same as that of native polyomavirus virions (Fig. 1A), although they were composed of different polyomavirus structural protein(s). The recombinant particles shown in Fig. 1(B) consisted of VP1 alone; other particles were purified from the Sf9 cells infected with Ac*M*NPVs containing dual polyomavirus structural protein genes, so that they were composed of VP1 and minor proteins (VP2 and/or VP3), namely VP1/2 (Fig. 1C), VP1/3 (Fig. 1D) and VP1/3 + VP2 (Fig. 1E). The presence of VP2 and/or VP3 in the recombinant particles did not show an obvious effect on the morphology and size of these particles in comparison with those of the native polyomavirus virions (Fig. 1A) or the recombinant particles composed of VP1 alone (Fig. 1B).

Western blot analyses of the recombinant particles purified by one CsCl gradient ultracentrifugation demonstrated that the corresponding VP1, VP2 and VP3 proteins were expressed in Sf9 cells (Fig. 2). The molecular masses of these expressed proteins were in good agreement with those of the native polyomavirus virions, indicated by Western blots (Fig. 2). Since no recombinant particles could be purified from Sf9 cells infected with Ac*M*NPV–VP2 or Ac*M*NPV–VP3 alone, Western blotting of these two proteins was performed by using the lysates of infected Sf9 cells (Fig. 2, lanes 4 and 5). Although VP2 or VP3 alone could not generate recombinant capsid-like particles, they became components of these particles when expressed with VP1 in the Sf9 cells (Fig. 2, lanes 6–9; Fig. 3, lanes 4–6).

SDS–PAGE analysis of the recombinant particles purified by three CsCl gradient centrifugations demonstrated that their protein composition was appropriate when compared to the purified native polyomavirus virions (Fig. 3, lane 2). It is important to note that Coomassie blue-stained SDS–PAGE gels demonstrated the association of cellular histones in all the recombinant capsid-like particles (Fig. 3), implying that cellular DNA was packaged inside as previously demonstrated for VP1 particles (Gillock *et al.*, 1997). The capsid-like particles with various combinations of structural protein(s) shown in Fig. 2 (excluding VP1/2 + VP3) were then treated with phenol–chloroform for the extraction of DNA, which was analysed by agarose gel electrophoresis. It was found that all of these recombinant particles had packaged cellular DNA of about 5 kbp in size, similar to that of native polyomavirus virions (Fig. 4A). In order to further investigate the form of DNA packaged in these particles, we extracted DNA from native polyomavirus virions and recombinant particles (composed of VP1) after three CsCl gradient purifications and analysed them by agarose gel electrophoresis as mentioned above (Fig. 4B). The predominant lower band (I) is circular supercoiled viral DNA and the minor upper band (II) is probably mouse cell DNA from pseudovirions (Türler & Beard, 1985). The DNA packaged in the recombinant particles showed only one band (Fig. 4A, lanes 4–6; B, lane 3), which was similar to the upper band found in the virion preparation.

It was previously shown that the VP1 protein found in native virions of polyomavirus can be separated into multiple species by two-dimensional gel electrophoresis, based on the different isoelectric points of the VP1 species (Bolen *et al.*, 1981 and references therein). Posttranslational modifications found on the VP1 isospecies, i.e. phosphorylation, sulfation, acetylation, hydroxylation and methylation have been shown to be

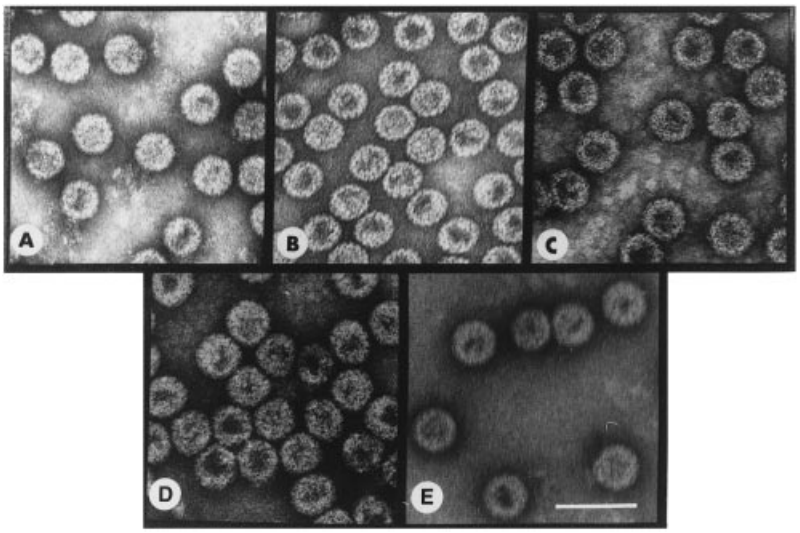

Fig. 1. Electron micrographs of native polyomavirus virions and recombinant capsid-like particles. The particles were purified by three CsCl density gradient centrifugations from the Sf9 insect cells, which were infected or coinfected with Ac*M*NPVs carrying different polyomavirus structural protein gene(s), after 5 days of infection. (A) Native polyomavirus virions produced and purified from mouse cells. (B) Ac*M*NPV–VP1. (C) Ac*M*NPV–VP1/2. (D) Ac*M*NPV–VP1/3. (E) Ac*M*NPV–VP1/3 + Ac*M*NPV–VP2. Bar, 100 nm.

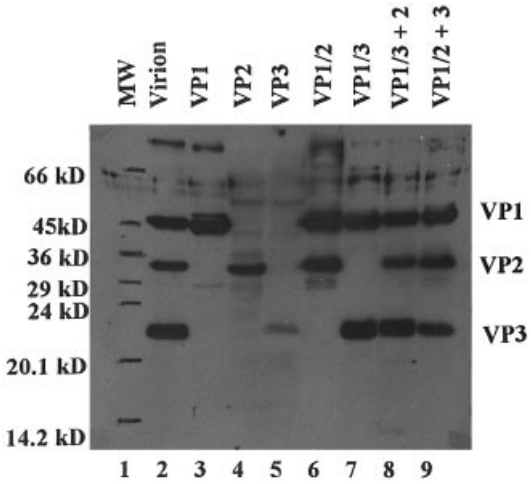

Fig. 2. Western blot analysis of polyomavirus structural protein expression in Sf9 cells. The recombinant capsid-like particles were purified by one CsCl density gradient centrifugation and used in the analysis, except that VP2 and VP3 were from lysates of Sf9 cells infected with Ac*M*NPV–VP2 or Ac*M*NPV–VP3, since they did not form particles. Lanes 1, molecular mass markers; 2, native virions; 3, Ac*M*NPV–VP1; 4, Ac*M*NPV–VP2; 5, Ac*M*NPV–VP3; 6, Ac*M*NPV–VP1/2; 7, Ac*M*NPV–VP1/3; 8, Ac*M*NPV–VP1/3 + Ac*M*NPV–VP2; 9, Ac*M*NPV–VP1/2 + Ac*M*NPV–VP3.

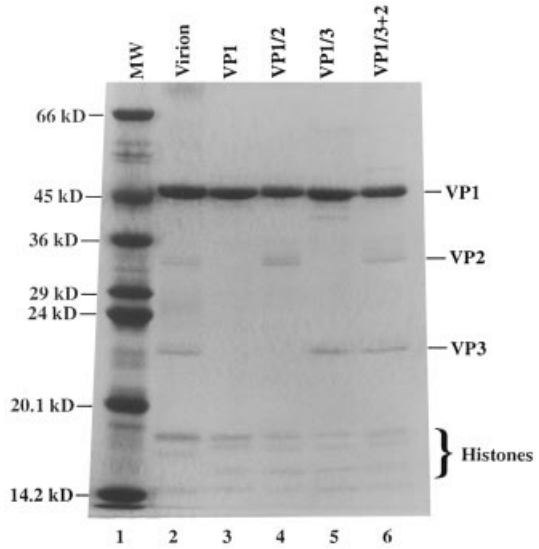

Fig. 3. SDS–PAGE analysis of recombinant capsid-like particles. The particles were purified in the same way as those shown in Fig. 1. Lanes 1, molecular mass markers; 2, native virions; 3, Ac*M*NPV–VP1; 4, Ac*M*NPV–VP1/2; 5, Ac*M*NPV–VP1/3; 6, Ac*M*NPV–VP1/3 + Ac*M*NPV–VP2.

responsible for these differences (Burton & Consigli, 1996 and references therein). Recent studies using the eukaryotic baculovirus system have demonstrated that the interaction of VP1 protein with VP2 was responsible for VP1 changes in posttranslational modifications, as demonstrated by two-dimensional gel analyses of the infected Sf9 cell lysates (Forstová *et al.,* 1993) and by immunoprecipitation of cell lysates with specific antipeptide sera (Li *et al.,* 1995). Since we have purified the recombinant particles with various combinations of polyomavirus structural proteins, we analysed these highly purified intact particles by two-dimensional gel electrophoresis to determine if the inclusion of the minor proteins in the capsid-like particles caused visible changes in VP1 isoelectric focusing species. The two-dimensional gel analyses of the native polyomavirus virions and the various recombinant particles purified by three CsCl gradient centrifugations were performed using equal amounts of protein. The findings of Coomassie blue-stained gels (Fig. 5 A) and Western blots (Fig. 5 B) of the two-dimensional gel electrophoresis analysis of the capsid-like particles are shown. The VP1 protein of native polyomavirus virions was separated into six distinct isospecies designated A to F, with 'A' being the most basic species, as previously described (Bolen *et al.,* 1981). All of the recombinant particles showed a similar pattern, but were

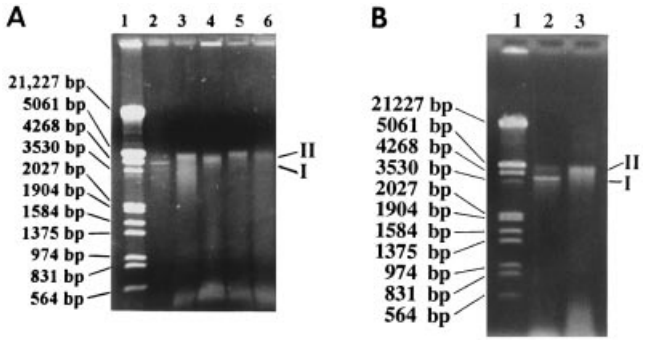

Fig. 4. Agarose gel analysis of DNA isolated from native polyomavirus virions and the recombinant capsid-like particles. (A) DNA was extracted from native polyomavirus virions and recombinant particles purified by one CsCl density gradient centrifugation. Lanes 1, DNA molecular mass markers (λ phage DNA cut with *Eco*RI and *Hin*dIII); 2, DNA isolated from native virions; 3, DNA from VP1 recombinant particles; 4, DNA from VP1/2 recombinant particles; 5, DNA from VP1/3 recombinant particles; 6, DNA from VP1/3 + VP2 recombinant particles. (B) DNA was extracted from native polyomavirus virions and VP1 recombinant particles purified by three CsCl gradient ultracentrifugations in the same way as those in Figs 1 and 3. Lanes 1, DNA molecular mass markers, as in (A); 2, DNA isolated from native virions; 3, DNA isolated from VP1 recombinant particles. A lower band (I) corresponding to the circular supercoiled viral DNA and an upper band (II), which is probably linear mouse cell DNA from pseudovirions, can be seen in (A) and (B) in the virion lanes.

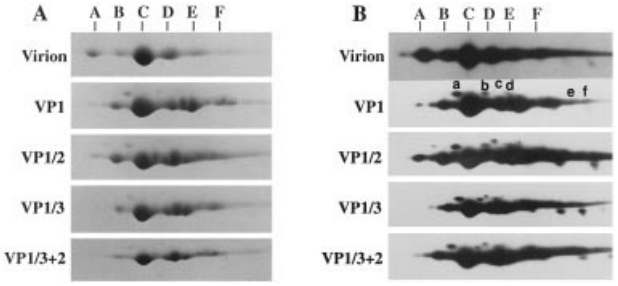

Fig. 5. Two-dimensional gel analysis of recombinant capsid-like particles. The native polyomavirus virions and recombinant particles were purified in the same way as those in Figs 1 and 3. VP1 species were separated by two-dimensional gel electrophoresis, with species 'A' being the most basic. The native polyomavirus virions and recombinant particles were fractionated in the first dimension by isoelectric focusing (pH of 3–10) and in the second dimension by SDS–PAGE. (A) Coomassie blue-stained gels with 10 μg of protein used for each sample. (B) Western blots of the same components as (A), except that 5 μg of protein was used for each sample.

slightly different from that of native polyomavirus virions, no matter whether the particles consisted of VP1 alone or VP1 with one or two minor structural proteins. In comparison with the VP1 of native polyomavirus virions, the recombinant particles showed more VP1 species (Fig. 5). In addition to the main species of VP1, A to F, there were several minor species, denoted a to f, detected by immunoblotting in these recombinant particles (Fig. 5B). It seems that the amount of species 'A' of the recombinant particles was much less than that of the native polyomavirus VP1 (Fig. 5). Among the recombinant particles, the co-expression of VP1 and VP2 (VP1/2) showed

Table 1. Effect of recombinant capsid-like particles on polyomavirus infection

	% Infection*	% Inhibition
Virion	31·1 ± 2·9	–
VP1 + virion	27·5 ± 3·7	11·6
VP1/2 + virion	15·9 ± 0·6	48·9
VP1/3 + virion	26·6 ± 4·4	14·5
VP1/3 + VP2 + virion	20·9 ± 1·5	32·8
VP1/2 + VP3 + virion	22·1 ± 1·0	28·9

* Infection was determined by indirect immunofluorescence of positive nuclei and counting 600 cells in multiple fields. The average of three separate experiments ± the standard error is shown.

an additional minor species seen above the main species 'B' (Fig. 5 B, see arrow); and the relative amount of its 'A' species was higher than those in other particles (Fig. 5B).

The various purified recombinant capsid-like particles were also compared for their ability to compete with polyomavirus for infection of 3T6 mouse cells, as determined by specific nuclear immunofluorescence. As seen in Table 1, the VP1 recombinant particles were found to have the least ability (VP1, 11·6%) to compete and prevent infection of the cells, followed by the particles composed of the VP1 and VP3 proteins (VP1/3, 14·5%). However, the capsid-like particles containing VP1 and VP2 proteins were the most efficient (VP1/2, 48·9%) in competing with the infectious virions for infection. The particles which contained a combination of all three polyomavirus structural proteins had a slightly lower ability to compete for infection, 28·9% for VP1/2 + VP3 and 32·8% for VP1/3 + VP2, although they were still quite effective. The finding that the VP1/2 capsid-like particles were the most effective in competing for polyomavirus infection indicates that the VP2 minor protein might play a role in the early events of virus entry.

Discussion

It has been demonstrated that bacterially expressed recombinant VP1 can self-assemble into capsid-like particles *in vitro* in the presence of calcium ions (Haynes *et al.*, 1993; Salunke *et al.*, 1986, 1989) and that eukaryotic baculovirus-expressed polyomavirus VP1 can also produce capsid-like particles in the nuclei of insect cells (Delos *et al.*, 1993; Forstová *et al.*, 1993; Li *et al.*, 1995; Montross *et al.*, 1991). Our current report demonstrates for the first time that recombinant particles with different combinations of polyomavirus structural protein(s) can be purified from Sf9 cells infected with Ac*M*NPVs containing the genes encoding these proteins and that the VP1 protein is critical for the generation of these capsid-like particles. The Western blots and SDS–PAGE of the recombinant particles purified from the coinfections showed that the

minor protein(s) became components of the particles (Figs 2 and 3). The electron micrographs showed that the presence of minor protein(s) in the recombinant particles did not visibly affect their morphology and size in comparison with the native polyomavirus virions (Fig. 1). These recombinant particles will be useful in elucidating the characteristics and functions of polyomavirus structural proteins alone since they are produced in the baculovirus system where neither early T antigens nor infectious polyomavirus DNA is involved.

The fidelity of the recombinant capsid-like particles produced by the co-expression of VP1 and a minor protein (VP2 or VP3) using Ac*M*NPVs carrying dual polyomavirus structural protein genes was more accurate than using Ac*M*NPVs carrying individual genes, since both the polyhedrin and P10 promoters of the p2Bac vector drive high expression of inserted cDNAs and the two genes in the same Ac*M*NPV should assure their simultaneous expression in the same infected cell. On the contrary, the expression of the same proteins by combined infections of Sf9 cells with Ac*M*NPVs containing individual polyomavirus structural protein genes could generate a population of recombinant particles with different components (Forstová *et al*., 1993). For example, the co-expression of VP1 and VP2 by combined infection of Sf9 cells with Ac*M*NPV–VP1 and Ac*M*NPV–VP2 could produce the recombinant particles composed of VP1 alone or VP1 + VP2. This might explain the findings (Table 1) in the capsid-like particle competition for polyomavirus infection of 3T6 cells where the VP1/2 particles were more effective than the VP1/2 + VP3 or VP1/3 + VP2 particles.

In the present investigation, all of the recombinant particles, no matter whether they were made of VP1 alone, or VP1 with one or two minor proteins, showed the capability of packaging cellular histones and DNA of about the same size as that found in the native polyomavirus virions (Fig. 4A). Electrophoresis analysis indicated that the DNA packaged in the recombinant particles consists of only one form, while the DNA from native polyomavirus was found to have two bands – a predominant lower circular supercoiled DNA band (I) and a minor upper band (II), which is probably linear mouse cell DNA from pseudovirions (Fig. 4). Nicked circular viral DNA, which migrates much slower than the other two forms in the agarose gel, was not observed in these virion preparations (Türler & Beard, 1985). This finding indicates that the DNA packaged in these recombinant particles is cellular DNA in a linear form. It was previously shown that [^{3}H]thymidine-prelabelled Sf9 cellular DNA was degraded after 3 days of infection with Ac*M*NPV–VP1 and fragments of about 5 kbp were packaged into recombinant VP1 particles (Gillock *et al*., 1997).

Two-dimensional gel analysis of the native polyomavirus virions has previously demonstrated that the VP1 protein is not a single protein but can be separated into six or more species, based on posttranslational modifications (Bolen *et al*., 1981; Burton & Consigli, 1996 and references therein; Hewick *et al*., 1977). Our present findings demonstrate that VP1 species of the recombinant particles purified from the infected Sf9 cells are slightly different from those of native polyomavirus virions purified from infected mouse cells. Two-dimensional gel electrophoresis showed that the major species 'A' (the most basic) of VP1 from the recombinant particles was in relatively lower ratios in comparison with that of the native polyomavirus virions, although the same amounts of protein were used in the analysis. On the other hand, there were about six additional minor VP1 species (a to f) in the recombinant particles seen by immunoblotting analysis (Fig. 5B). The difference seen in VP1 species between native polyomavirus virions and the recombinant particles might be due to the fact that the native virions and the recombinant particles were generated from different host cells, since VP1 modification is a host-dependent process (Ludlow & Consigli, 1987*a*). Polyomavirus does not code for any modification enzymes of its own, so the VP1 modifications are the results of host cytoplasmic enzymatic activity (Fattaey & Consigli, 1989).

It was reported that co-expression of polyomavirus VP1 with VP2 in the baculovirus insect cell system might cause changes in VP1 modification(s) when Sf9 cell lysates were used for two-dimensional gel analysis (Forstová *et al*., 1993). In our present investigation, all of the purified recombinant particles showed similar VP1 species patterns, although the coinfection of VP1/VP2 generated one more minor species (Fig. 5B, arrow), and its main species 'A' was more pronounced than that in the other capsid-like particles (Fig. 5B). These findings are analogous to those previously reported by Forstová *et al*. (1993), indicating a possible involvement of the VP2 protein in the VP1 isoelectric focusing profiles. This was probably caused by posttranslational modification changes of the VP1 protein. In addition, it was also reported that the bacterially expressed VP2 or VP3 induced a conformational change in VP1 capsomeres when assayed *in vitro* (Delos *et al*., 1995). The different folding of VP1 protein, caused by interacting with VP2 or VP3, might result in changes of exposed amino acid residues and sites of posttranslational modifications, such as phosphorylation (Li *et al*., 1995). Furthermore, there is also some evidence that in the native infection, the middle T antigen may be involved in determining the proper modifications of the VP1 protein (Garcea & Benjamin, 1983; Garcea *et al*., 1985). It is likely that in the native infection both the middle T antigen and the VP2 protein may play a specific role in VP1 modification and function. However, in the present study using the baculovirus system to express the polyomavirus structural proteins, T antigen influence on VP1 modification is not involved.

The recombinant capsid-like particle competition presented in this report (Table 1) indicates that the VP2 minor protein seems to play a role in the early events of polyomavirus infection. It was found that the VP1/2 particles were the most effective at competing with infectious virus for productive infection, while the VP1 and VP1/3 recombinant particles were quite ineffective in competing for infection. It is well

established that the VP1 protein possesses the virion attachment domain (Anders & Consigli, 1983*a*; Marriott & Consigli, 1985), which reacts with the sialic-acid-containing cell receptor (Stehle *et al*., 1994; Herrmann *et al*., 1997). However, the presence of the VP2 protein in the virion was shown to be critical for productive infection. It was previously reported that the N terminus of polyomavirus VP2 protein was found to be modified by myristylation (Streuli & Griffin, 1987) and studies of VP2 myristylation-defective mutants of polyomavirus demonstrated that VP2 plays a role in the early events of virus entry (Krauzewicz *et al*., 1990; Sahli *et al*., 1993). In addition, a recent study, utilizing a prokaryotic-expressed truncated VP2 protein interaction with VP1 protein, has revealed that the N terminus of VP2 is flexible and possibly exposed at the virion surface, and may be involved in virus entry (Chen *et al*., 1998). Whether the VP2 protein has the ability to cause a conformational change in the VP1 protein structure, exposing the proper VP1 epitope for cell attachment, or whether the exposed N terminus of VP2 protein is directly involved in virus entry is still to be determined.

These recombinant capsid-like particles containing various combinations of the polyomavirus structural proteins can be used in future studies to dissect the biological and biochemical functions of these structural proteins, particularly the minor proteins VP2 and VP3, in the life-cycle of polyomavirus.

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