The Tailless Icosahedral Membrane Virus PRD1 Localizes the Proteins Involved in Genome Packaging and Injection at a Unique Vertex

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The double-stranded DNA (dsDNA) virus PRD1 carries its genome in a membrane surrounded by an icosahedral protein shell. The shell contains 240 copies of the trimeric P3 protein arranged with a pseudo T = 25 triangulation that is reminiscent of the mammalian adenovirus. DNA packaging and infection are believed to occur through the vertices of the particle. We have used immunolabeling to define the distribution of proteins on the virion surface. Antibodies to protein P3 labeled the entire surface of the virus. Most of the 12 vertices labeled with antibodies directed against proteins P5, P2, and P31. These proteins are known to function in virus binding to the cell surface. Proteins P6, P11, and P20 were found on a single vertex per virion. The P6 and P20 proteins are believed to function in DNA packaging. Protein P11 is a pilot protein that is involved in a complex that mediates the early stages of DNA entry to the host cell. Labeling with antibodies to P5 or P2 did not affect the labeling of P6, the unique vertex protein. Labeling with antibodies to the unique vertex protein P6 interfered with the labeling by antibodies to the unique vertex protein P20. We conclude that PRD1 utilizes 11 of its vertices for initial receptor binding. It utilizes a single, unique vertex for both DNA packing during assembly and DNA delivery during infection.

A crucial step in virus assembly is the recognition of virus-specific nucleic acid and its consequent encapsidation into the virus particle. Two principal mechanisms have been described: cocondensation of the viral genome with the assembling coat and formation of an empty polyhedral procapsid into which the virus genome is packaged.

The first mechanism was initially illustrated using tobacco mosaic virus (16) and is assumed to occur during the assembly of small RNA nonmembranous and membrane-containing viruses (15, 28, 30). The second mechanism is well described for tailed double-stranded DNA (dsDNA) bacteriophages (11, 37). It may be a common mechanism of genome packaging for complex icosahedral viruses such as herpes simplex virus (39).

Viral capsids of the second type are not simply static compartments that protect the genome but dynamic molecular machines that translocate the viral nucleic acid into the capsid so that the packing nears crystalline density. One of the icosahedral capsid vertices (31) is specialized for nucleic acid translocation. This portal vertex (54) contains a multimeric portal protein or connector, since it connects the phage head to the tail. The portal typically has a different symmetry than the vertex, which must respect the fivefold symmetry of its icosahedral position. The connectors of some tailed dsDNA phages are dodecamers (50) on the virus. The symmetry mismatch between the fivefold vertex and the packaging protein is be-

lieved to facilitate movement (50) or rotation (27) during genome translocation through the vertex. A separate ATPase (the terminase [17]) that is not part of the capsid is found in dsDNA phages. The terminase recognizes and binds to the phage DNA, associates with the portal complex, and provides the energy for the translocation event (17). A symmetry mismatch is often found at the site of packaging. For example, a hexameric protein, P4, has been found at the vertices of a membrane dsRNA virus, $\phi 6$ (18, 29; E. J. Mancini, D. H. Bamford, J. M. Grimes, and D. I. Stuart, unpublished data). The $\phi 6$ portal protein is an NTPase that provides the energy for translocation (41, 42).

The membrane-containing bacteriophage PRD1 has a linear 15-kbp genome enclosed in a membrane vesicle that is surrounded by a protein coat (Fig. 1). The genomic DNA packaging is believed to occur through a vertex, although this has not been directly demonstrated. The vertices are also responsible for receptor recognition (23, 44) through a spike-like structure containing proteins P2, P5, and P31. The vertices are also assumed to be the site for the initiation of DNA delivery through a complex involving the pilot protein P11 (53a). The combination of structural information obtained by cryo-electron microscopy (13, 46) and image processing with that obtained by X-ray crystallography (9, 10, 53) shows a surprising similarity to that obtained for adenovirus (1, 51, 52). This led to a proposal that these viruses share a common ancestor and that viruses may form lineages that have members infecting hosts in all domains of life (3).

The order of PRD1 assembly is unusual for a membrane-containing virus. PRD1 has an assembly intermediate that contains the capsid and the membrane but no DNA (34). These particles package DNA and mature to virions. This order is

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FIG. 1. Labeling of wt PRD1 with antibodies to P3, P5, and P2. PRD1 particles were labeled with antibodies and visualized using 10-nm gold coupled to protein A as described in the Materials and Methods. (A) The extensive labeling of wt PRD1 with anti-P3 is shown. (B) Labeling of a mixture of wt PRD1 and MMTV (arrows) with the same anti-P3 is shown. No labeling of MMTV is visible. (C and D) Labeling of a PRD1/MMTV mixture with antibodies against P5 (C) and P2 (D) is also shown. The labeling, while less extensive than that with P3, was associated with the PRD1 and not with the MMTV particles (arrows).

more similar to that described for tailed dsDNA phages than to that described for other membrane viruses (2, 30). The behavior of the packaging-deficient mutant, *susI*, provides support for this idea. This mutant has a defect in protein P9 that contains an ATP-bridging Walker motif (7). The mutation leads to the formation of membrane-containing particles that lack DNA. Two other mutant classes, lacking the integral membrane proteins P20 and P22, also show packaging-associated defects (33, 34). Intracellular particles of both mutants package DNA but release it upon particle isolation. Genetic evidence links protein P6 to a complex involved in packaging (53a). Attempts to demonstrate a special packaging vertex by structural methods have been less effective (47). The use of icosahedral symmetry (2, 14, 18, 20) in averaging blurs the density of the differing 1 in 12 vertices.

PRD1 injects DNA into its gram-negative hosts by a complex multistep mechanism. The vertex-associated spike complex, containing P2, P5, and P31, binds to a cell surface receptor via the elongated P2 protein. This interaction triggers a cascade of events that lead to the penetration of the cell envelope by a virally derived membrane tube (24, 25) and delivery of the DNA into the cytosol (24). This process involves the

viral proteins P7/P14 and P11, P15, P16, P18, and P32 (see references in reference 17) that interact with the host membranes and digest the peptidoglycan layer. The viral membrane is involved in the final DNA translocation process, and empty particles frequently display a tubular structure protruding from a vertex (4). The initial binding of P2 is reversible; however, the interaction that leads to penetration of the host cell wall is not. It had been proposed that any vertex can function in DNA delivery, since the receptor binding spike is found at the vertices and it is logical to associate binding with penetration (4, 24, 25, 32, 43, 44). This assumption has never been tested directly. Infection in other membrane-containing viruses is a multistep process that does not proceed directly from binding to productive infection (19, 30, 48, 49, 56).

We can test the possibility of separate functions and structures for the different vertices by forgoing the use of icosahedral averaging that would mask the differences between them. The availability of poly- and monoclonal antibodies (8, 23, 26, 44) against several PRD1 structural proteins allows an immunoelectron microscopy approach to the structure. The high contrast afforded by gold labeling allows differentiation of the composition of the sites, although the detailed structural in-

formation is lost. We have identified two types of vertices in PRD1. The first, or initial binding, vertex contained the receptor binding P2 protein as well as the associated vertex components P31 and P5. The second, or translocation, vertex contained proteins P6 (53a) and P20, which are involved in genome packaging, and protein P11, which serves as a pilot protein for the formation of the tube that conducts the genome into the target cell. Only 1 translocation vertex and 11 binding vertices were present per virion. Our labeling studies showed that these vertices were distinct; the translocation vertex did not contain the binding vertex components. Infection by PRD1 must therefore involve a two-step process similar to that in other membrane viruses in which reversible binding at multiple vertices leads to permanent attachment and tube formation at the unique translocation vertex.

MATERIALS AND METHODS

Bacteria and phages. Bacterial cells were grown in Luria-Bertani medium (45). Wild-type (wt) PRD1 was propagated on *Salmonella enterica* serovar Typhimurium strain DS88 (6). *sus539* is an amber mutant with a mutation in gene II encoding the receptor binding protein P2. Growth in nonsuppressor strain DS88 produces particles that do not have protein P2 but have all the other viral proteins that can be detected (23). These particles are thus described as P2⁻. *sus690* is an amber mutant with a mutation in gene V. The suppressor hosts were DB7156(pLM2) for *sus539* and PSA(pLM2) for *sus690* (33, 35). Particles lacking both proteins P5 and P2 (P2⁻ and P5⁻) are produced when *sus690* is propagated in the nonsuppressor strain DS88 host. This phenomenon does not reflect genetic polarity, since protein P2 is produced in normal amounts. It represents an assembly defect. The receptor binding protein P2 is associated with the viral particle via protein P5 (6).

All viruses were grown with strain DS88 and purified on 5 to 20% linear sucrose gradient (5) for particle production. The virus zone from the gradient was either collected by differential centrifugation (Sorval T865 rotor; 32,000 rpm, 3 h, +5°C) or applied for use in ion exchange chromatography (Sartorius). The *Sus539* particles were purified by sucrose gradient centrifugation followed by ion exchange chromatography on a MemSep filter and elution with 5 mM potassium phosphate (pH 7.2)–300 to 400 mM NaCl (55).

Protein concentrations of the virus preparations were determined using bovine serum albumin as a standard (12). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described previously (40). The proteins were either stained with Coomassie brilliant blue or transferred from the gel onto a polyvinylidene difluoride membrane (Millipore) and detected with antibodies (Western blotting).

Antibodies. Specific rabbit antisera recognizing phage PRD1 proteins P2 (23), P5 (26), and P31 (8, 44), as well as monoclonal antibodies 6A488 (recognizing protein P6), 7N41 (recognizing protein P7/P14), 11A401 (recognizing protein P11), and 16T56 (recognizing protein P16) (26), were used for labeling. The polyclonal serum against P20 was raised using a specific peptide as an antigen. The peptide CNHFGKNAVSTSE (linked to the keyhole limpet hemocyanin via the cysteine residue) was purchased from KJ Ross-Petersen AS, Holte, Denmark. For the antiserum production, a rabbit was immunized three times at 3-week intervals with 900 µg of the antigen emulsified with either Freund's complete (for the first immunization) or incomplete adjuvant. The chemiluminescent detection of peroxidase-conjugated secondary antibodies was performed using either an ECL Plus (Amersham) or a Super Signal R West Pico (Pierce) system.

Antibody labeling. All steps were performed in a humidified room-temperature chamber with Parafilm at its base (21, 22, 57). Glow-discharged, carbon-coated Formvar 300-mesh nickel grids were placed onto 10- to 15-µl virus aliquots for 1 min. All subsequent steps were performed by floating the grid with the specimen side down on aliquots of the solutions (25 to 30 µl). After two 1-min washes in phosphate-buffered saline (PBS) the grids were fixed in 0.5% glutaraldehyde in PBS for 15 min. After three 5-min washes in PBS, the grids were treated with 0.5% Triton X-100 in PBS for 15 min. After four 5-min washes in PBS, the potential residual-free glutaraldehyde was neutralized for 15 min in PBS containing 0.02 M glycine. After three 5-min washes in PBS, the grids were then treated with PBS containing 1.0% ovalbumin (albumin, chicken egg, catalogue no. A-5503; Sigma) for 10 min. The grids were then exposed for 1 h to the primary antibodies diluted in PBS–1.0% ovalbumin. The grids were then washed four times for 5 min each time in PBS–1.0% ovalbumin. For the polyclonal

TABLE 1. Qualitative summary of results for all antibodies and mutant strains tested Immunolabeling results^a

Antibody	PRD1	MMTV	Sus539 (2× purified)	Background	Localization on virions
P3	+++	–	+++	+	Many sites
P5	++	–	++	+/-	Most vertices
P2	++	–	* ^b	+/-	Most vertices
P6	+	–	+	+/-	One vertex
P7/P14	–	NT ^c	NT	+/-	Not detected
P11	+	NT	NT	+/-	One vertex
P16	–	NT	NT	+/-	Not detected
P31	++	NT	NT	+/-	Most vertices
P20	+	NT	NT	+/-	One vertex

^a +++, very high labeling; ++, high labeling; +, some labeling; –, no labeling.

^b *, a few (~5%) particles labeled at many vertices, as seen for wt PRD1; however, the rest had no label.

^c NT, not tested.

primary antibodies, the grids were exposed for 30 min to 5- or 10-nm protein A gold (Sigma) diluted in PBS–1.0% ovalbumin. Monoclonal primary antibodies were detected by exposure for 1 h to anti-mouse immunoglobulin G (IgG) (whole molecule) conjugated to 10-nm colloidal gold (Sigma, G-7652) diluted in PBS–1.0% ovalbumin. Double-labeling experiments were performed by washing the grids four times for 5 min each time in PBS–1.0% ovalbumin after the primary antibody and gold secondary treatment and then treating with the second antibody and the appropriate gold conjugate as in a single-labeling experiment. After all labeling steps, the grids were washed four times for 5 min each time in PBS and three times for 1 min each time in distilled water. The virus was then negatively stained with Nanovan (catalogue number 2011; Nanoprobes, Inc.) and air-dried. (Nanovan was used because it produces an intermediate contrast between glucose and heavy-metal stains. Using glucose would provide a good contrast with gold and poor contrast with the particle. Heavy-metal stains [e.g., uranyl acetate] would outline the particle well while almost matching the density of the gold.) The labeled samples were viewed on a 200-kV field emission gun transmission electron microscope (CM series; FEI, Eindhoven, The Netherlands) at a magnification of ×38,000 and photographed on S0163 film (Kodak). Images are represented here in reverse contrast after scanning with a UMAX3000 scanner at a pixel size of 8.33 µm.

Antibody labeling controls. Serial dilution tests were performed with each antibody to determine its optimal concentration for use under our conditions. Little or no labeling was seen when the antibody was too dilute. Too high a concentration of antibody resulted in a high background of gold on the carbon film. All primary antibodies were exposed to protein A gold, protein G gold, or anti-mouse IgG (whole molecule) conjugated to 10-nm colloidal gold solutions (all Sigma products) to ensure their proper secondary detection. In some experiments, mouse mammary tumor virus (MMTV) was mixed with the PRD1 sample as an internal experimental control. None of the antibodies tested labeled the MMTV significantly at the concentrations that produced labeling on the PRD1.

Omission of the primary antibodies resulted in no labeling of the samples or the background. PRD1 mutants lacking specific proteins *sus539* (P2⁻; 23) and *sus690* (P5⁻ and P2⁻; 6) were tested to ensure that the appropriate proteins were absent (Table 1). Two antibodies with presumed internal epitope locations (P7/P14 and P16) were also tested on wt PRD1. Neither antibody resulted in significant labeling of the virus.

By using a ×10 magnifier, gold particles were counted on the negatives. Areas in which virus was clumped were disregarded, as were background gold particles that were not associated with virions. Statistical analysis of the labeling data (including *t* tests, median calculation, and histogram representation) was performed with Microsoft Excel for Macintosh, version 1.0.

Cryo-transmission electron microscopy. All of the virus samples (wt PRD1, *sus539*, and *sus690*) were prepared and imaged under standard holey carbon cryo-grid conditions with a 200-kV field emission gun transmission electron microscope (CM Series, FEI, Eindhoven, Netherlands) (13, 14).

RESULTS

Microscopy of the preparations. Cryo-electron microscopy (14) showed that most virions were intact and that the wt PRD1 and *sus539* were morphologically similar. Few virions in

FIG. 2. Labeling of the *sus539* mutant. The *sus539* mutant of PRD1 that expresses much reduced levels of P2 (23) was labeled with antibodies against protein P5 (A) and antibodies against P2 (B) that were visualized with protein A coupled to 10-nm gold. All particles of the *Sus539* mutant labeled with antibody against P5, while the antiP2 label was concentrated on a small fraction (~5%) of the particles.

these preparations showed a single membranous tube associated with the ejection of the DNA. Many virions in the *Sus690* preparation displayed a single tube indicative of DNA loss.

The cryo-electron micrographs and the negatively stained images of the labeled samples showed a consistent morphology for the virus. The Nanovan-negative stain often penetrated the membrane so that internal features were revealed (Fig. 1). All of the virus preparations contained small, particulate material that was not associated with the virus. A gold marker was often attached to this particulate material in the images of the anti-P3-labeled samples (Fig. 1). We believe that this reflects the fact that our purification procedure did not preserve the intactness of the particle perfectly, although it isolated particles efficiently. In addition, particles may have deteriorated after isolation and shed proteins. This material was present on the grids in the labeling experiments and was what often contributed to the “background labeling” observed in these images. This background labeling was only observed with anti-P3. The antibody concentration selected for the other antigens (P2, P5, P6, P11, P20, and P31) resulted in a consistent background that was lower than the corresponding specific label.

Several controls showed the specificity of the labeling. Omitting the primary antibody always resulted in a clean background. MMTV was mixed with the PRD1 to act as an internal control (Fig. 1B to D, 2A, and 3A) for the specificity of labeling. None of the antibodies used showed any significant labeling of the MMTV particles. Labeling was restricted to PRD1 particles (Fig. 1, 2, and 3).

The labeling results are summarized qualitatively in Table 1, and representative images are shown in Fig. 2, 3, 4, and 5. The observation that different antibodies labeled the virion in distinct ways (i.e., anti-P3 labeled the whole of the virion surface, anti-P5 labeling was consistent with its labeling of most vertices, anti-P6 labeled only a single vertex, and no labeling was observed with antibody to P16; Table 1) was a further indicator of the specificity of our immunolabeling procedures. All spe-

cific labeling values exceeded the density of gold attachment to the film between particles and the density of the nonspecific labeling of MMTV.

The *sus539* (P2-deficient) mutant gave an unexpected result when labeled with an anti-P2 antibody (Table 1). Most of the virions had no label at all, as expected. However, a few of the virions had a labeling pattern similar to that of wt PRD1 labeled with anti-P2, with gold markers at most vertices (Fig. 2). As described below, we believe this reflects leakage of the mutant in a fraction of the cells.

Single labeling showed a marked difference between the antibodies that labeled multiple vertices, such as those against proteins P5 (Fig. 1C), P2 (Fig. 1D), and P31 (Fig. 5A), and the antibodies that labeled a single vertex, such as those against proteins P6 (Fig. 3A), P20 (Fig. 3B), and P11 (Fig. 5B).

We used double labeling (Fig. 4) to test whether the single-labeling results reflected the alterations in labeling efficiency or a variation in protein localization. The availability of antibodies from different species allowed us to visualize separate antigens with differently sized gold labels. We believe that the fact that we did not see multiple labels at a single vertex indicates that there was not enough room either for two primary Igs or for two gold secondary markers (Fig. 4). Single-labeling experiments with these antibodies resulted in only one gold marker per vertex (Fig. 1C and D, 2, 3A and B, and 5). Steric hindrance can prevent the occurrence of two gold markers at a single vertex but, of course, cannot prevent different labels binding to different vertices.

Double labeling of wt PRD1 with antibodies to P2 or P5 that labeled multiple vertices when applied singly did not prevent the subsequent labeling with antibodies to P6. This indicates that P6 is at a different vertex from that labeled with P5 and P2 (Fig. 4). In contrast, we did not observe labeling with antibodies to P6 and P20 in the same wt PRD1 virion (Fig. 3C) under double-labeling conditions. If the P20 and P6 were at different vertices, we would have expected to see two gold labels (at

different vertices) per virion with the frequency expected from single labeling. Labeling with antibodies to P20 appeared to block subsequent labeling with antibodies to P6. We concluded that P20 and P6 must be located at the same vertex.

Quantitation of the labeling results. Our labeling conditions did not result in quantitative labeling of all of the vertices. Nevertheless, quantitation of the labeling results (Fig. 6) reinforced the impression given by the individual images. It also allowed us to define the limits for the reliability of our results and to address the effect of differing efficiencies of labeling on our results. The variation in labeling appears less dramatic when one considers that the orientation of the virion on the grid can shield a potential binding site and that this effect is more pronounced in the case of the unique vertex antigens.

Figure 6A documents the labeling of multiple sites on individual particles by antibodies against P31, P5, and P2. Antibodies to P31 produced an average of 4.6 gold particles (median, 4.5; standard deviation, 1.7) per virion. Antibodies to P5 produced similar labeling (mean of 5.7 gold particles; median, 5; standard deviation, 2.0) per virion. Both of these antibodies labeled all of the particles. The antibody against P2 produced somewhat lower labeling, with an average of 2.4 gold particles (median, 2; standard deviation, 1.0) per virion. Only 94.6% of the particles showed labeling with anti-P2. No more than 10 sites were labeled with any of the antibodies on any particle. This presumably reflects the lack of exposure of all vertices when the particle lies on the grid.

Antibodies against P6, P20, and P11 showed a completely different labeling pattern. The extent of labeling was much lower with these antibodies. None of the virions were labeled at multiple sites (Fig. 6B). Antibodies against P6 labeled 19.2% of the particles ($n = 297$). Less labeling was seen with antibodies against P20 (7.8%; $n = 488$) and P11 (10.3%; $n = 341$). If labeling of a second site were independent of the labeling at the first, one would expect to have seen two or more labels on a fraction of the particles in the case of P6 (8.8 double labels expected in 297 particles) since the frequency of single labeling is relatively high. The lower frequency of labeling with antibodies against P11 and P20 would still have allowed the detection of multiple labeling if it were present (3.2/341 expected for P11 and 2.4/488 expected for P20) and the labeling of the sites were independent. We conclude that only a single site was labeled in the particle by any of these antibodies.

The result with the *sus539* mutant was unexpected. Since this mutant should be deficient in P2, we expected no labeling. We saw labeling in 4.5% of the particles ($n = 852$); however, the distribution of the gold found on this labeled fraction showed characteristics similar to those of the wt (mean = 3.0; median = 2; standard deviation = 2.0 gold labels per particle). One

FIG. 3. Labeling of wt PRD1 with anti-P6 and anti-P20. (A) Mixture of wt PRD1 and MMTV that labeled with antibodies against P6 that was visualized with anti-mouse IgG coupled to 10-nm gold. No labeling is seen on the large, irregular MMTV particles (arrow), and a single site of labeling is seen on individual PRD1 particles. (B) Labeling of wt PRD1 with anti-P20 visualized with protein A coupled to 5-nm gold. (C) A double-labeling experiment in which wt PRD1 was labeled with antibody to P20 and visualized with protein A coupled to 5-nm gold and then with antibody to P6 visualized with 10-nm gold coupled to anti-mouse IgG.

FIG. 4. Double labeling of PRD1. (A) wt PRD1 was labeled with antibodies against P5 visualized with protein A coupled to 5-nm gold and then washed and labeled with antibody to P2 that was visualized with protein A coupled to 10-nm colloidal gold. Panel B shows the corresponding experiment in which wt PRD1 was labeled with antibodies to P5 and visualized with protein A coupled to 5-nm gold, washed, and then labeled with antibodies to P6 and visualized with 10-nm gold coupled to anti-mouse IgG. (C and D) Labeling of wt PRD1 (C) and the *Sus539* mutant (D) with antibody to P2 visualized with protein A coupled to 5-nm gold and then with antibody to P6 visualized with 10 nm of gold coupled to anti-mouse IgG.

explanation of this effect is that the amber mutation responsible for the lack of synthesis of P2 in the mutant cells is suppressed in a small fraction of the cells, which then give rise to wt virus. In addition, amber mutants such as *sus539* are known to be leaky. The ion exchange chromatography may enhance this phenomenon by selecting the P2-containing virions always present in the virus stocks. This observation gives insight into the mechanism of expression, since it suggests that different cells in the population react differently. This phenomenon could only be observed with a method that can examine the composition of individual particles.

The use of double labeling indicates that antibodies to P5 and P2 label independently of P6. Antibody to P5 labeled the virion at 4.8 ± 0.4 sites in the double-labeling experiment and

shows an efficiency similar to that obtained in the single-labeling experiment. Antibody to P6 labeled 23% of the virions in the double-labeling experiment, giving efficiency similar to that shown in single labeling. Antibody to P2 showed efficiency similar to that seen in the single-label experiment (P2, 2.4 labels/virion; P6 labels 17% of the virions). A very different picture was seen when double labeling was performed with antibodies to P20 and P6. P6 and P20 labeling were decreased in the double-label situation to 13.3 and 9.6% of the virions, respectively. The sum of P20 and P6 labeling (22.9%) approached that of single labeling with antibodies to P6. We concluded that antibodies against P5 and P2 label at an independent site from that labeled by antibodies to P20 and P6 and that labeling with antibodies to P20 interferes with labeling of P6.

FIG. 5. The binding and translocation vertices. The labeling of wt PRD1 with antibodies directed against a binding vertex component (A) (anti-P31) contrasted with labeling of translocation vertex components (B) (anti-P11). The anti-P31 in panel A was visualized with protein A coupled to 5-nm gold. P31 is known to be involved in the receptor binding complex. The anti-P11 in panel B was visualized with anti-mouse IgG coupled to 10-nm gold. P11 is known to be involved in the DNA delivery that leads to infection and translocation of the genome into the target cell.

DISCUSSION

Our labeling results with PRD1 are simply summarized. An antibody to P3 labeled many sites on the virion surface. This is consistent with the biochemical and structural results that show it to be the major structural protein forming the virion shell (8, 13, 14, 46). The labeling with antibodies directed against proteins P2, P5, and P31 was consistent with their presence at most of the vertices. The labeling with antibodies to P6, P11, and P20 supported their localization to the same unique vertex. Double labeling showed the unique nature of this vertex in that labeling with antibodies to P20 inhibited subsequent labeling with P6. Labeling with antibodies to P2, P5, or P31 did not inhibit labeling with antibodies to the unique vertex component P6.

We are able to interpret our labeling results functionally because of the wealth of biochemical and genetic data that are available for this membrane virus. Our data and recent genetic evidence (53a) support a division of the 12 vertices into two classes. Of the 12 vertices, 11 correspond to the location of the three proteins in the receptor binding complex. We refer to this class as the initial binding vertex. The later events in infection that result in penetration of the DNA through the target cell wall occur through the twelfth vertex that is also responsible for the initial packaging of the genome. We refer to this as the translocation vertex. Whether any of the unique vertex proteins are involved in the initiation of particle assembly to assure the presence of the single vertex is an intriguing question. P11- and P20-deficient mutants are not compromised for particle assembly. This question will remain open until a P6-deficient mutant becomes available.

How does this arrangement of initial binding and translocation vertices function? This work, in combination with recent genetic evidence (53a), shows that P6, P9, P20, and P22 are all

FIG. 6. Quantitation of labeling results. The percentages of virions are plotted against the number of sites labeled on each particle. Panel A shows the distribution for antibodies that label the initial binding vertices (anti-P2, anti-P5, and anti-P31). Panel B shows the distribution for the antibodies that label the translocation vertex (anti-P6, anti-P11, and anti-P20).

involved in packaging and reside at the same vertex. The presence of P11 indicates that it is also the site of DNA exit (24). It may seem paradoxical that the major receptor binding protein, P2, is found at the other 11 vertices of the particle; however, it reveals the nature of the interaction of this membrane virus with the host.

The fact that the initial binding is reversible (23) and that a further transition must occur prior to permanent binding and infection is consistent with the infection pathways of other membrane-containing viruses such as influenza virus, Semliki Forest virus, and human immunodeficiency virus (19, 30, 48, 49). In these mammalian viruses, the process of infection is multistep. A weak initial binding concentrates the virus near the target cell and allows it to search the cell surface for a productive binding site (36, 38, 53a). Only at the correct and productive binding site does the virus trigger its permanent binding and infection. The distinct multiple vertices of PRD1 accomplish searching followed by triggering similar to that seen in other membrane viruses.

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