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The Unique Vertex of Bacterial Virus PRD1 Is Connected to the Viral Internal Membrane

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Icosahedral double-stranded DNA (dsDNA) bacterial viruses are known to package their genomes into preformed procapsids via a unique portal vertex. Bacteriophage PRD1 differs from the more commonly known icosahedral dsDNA phages in that it contains an internal lipid membrane. The packaging of PRD1 is known to proceed via preformed empty capsids. Now, a unique vertex has been shown to exist in PRD1. We show in this study that this unique vertex extends to the virus internal membrane via two integral membrane proteins, P20 and P22. These small membrane proteins are necessary for the binding of the putative packaging ATPase P9, via another capsid protein, P6, to the virus particle.

The genome packaging of icosahedral double-stranded DNA (dsDNA) bacteriophages occurs by translocation of the genome into preformed procapsids. This translocation is performed by a specific enzyme, a terminase or a packaging ATPase, and is powered by ATP hydrolysis. The packaging of DNA occurs at a unique portal vertex, which also functions as the head-to-tail connector as well as the channel through which DNA is injected into the host cell during infection. The portals characterized thus far are ring-like structures of portal protein multimers with a central channel correctly sized for the dsDNA to be threaded through. Packaging is believed to occur by winding the DNA up through this central channel, involving movement of the portal structure (16, 20, 35, 42, 43, 64, 66). A symmetry mismatch between the packaging machinery and the rest of the capsid has been proposed to assist the movement of the packaging machinery with respect to the capsid during nucleic acid transport (35, 42, 64).

The most detailed description of a packaging system exists for bacterial virus ϕ29, a short-tailed icosahedral prolate virus with a linear dsDNA genome, infecting the gram-positive *Bacillus subtilis* (2). The ϕ29 portal occupying a pentagonal vertex is a dodecamer of portal protein gp10, forming a propeller-shaped structure with a central channel (35, 64). In addition to the portal, which is attached to the prohead, the packaging machinery consists of five or six copies of the viral ATPase (gp16) and six copies of a ϕ29-encoded packaging RNA (pRNA) (36–39, 74). The DNA, connector, and prohead-pRNA-ATPase complex form a set of concentric structures with 10-, 12-, and 5- or 6-fold symmetry, respectively, embedded in the 5-fold vertex of the prohead (35, 64).

A portal assembly complex has also been identified in a eukaryotic virus. The portal of herpes simplex virus type 1 (HSV-1), identified by immunogold labeling, is located at a single vertex of the icosahedral HSV-1 capsid and contains a ring-shaped multimer of the UL6 protein (52). So far, other portal vertices of icosahedral eukaryotic viruses have not been described. It is conceivable, however, that many more complex icosahedral viruses assemble through packaging of empty precursor particles, which would require a unique portal complex.

PRD1 is the type organism for the family *Tectiviridae* (7). It is a bacterial virus that infects a variety of gram-negative hosts harboring an N-, P-, or W-type conjugative plasmid (54). The PRD1 virion consists of an icosahedral protein capsid surrounding an internal membrane that encloses a 14,927-bp linear dsDNA genome with inverted terminal repeat sequences at both termini and covalently linked 5′-terminal proteins (5, 6, 12, 62). DNA replication of PRD1 is initiated by protein priming from the terminal proteins (27, 28, 63). The PRD1 membrane is comprised of approximately half lipid and half protein (12, 31). The lipids are derived from the plasma membrane of the host cell, and the proteins are encoded by the viral genome (12, 31). During infection, the spherical membrane vesicle undergoes a structural transformation into a tubular tail-like structure that is thought to inject the genome into the host cell (4, 34, 46, 56).

Structural information from cryoelectron microscopy and image processing as well as X-ray crystallography has shown that PRD1 is surprisingly similar to adenovirus (17–19, 26, 60, 61). Similar to the organization of the adenovirus capsid (24), the capsid of PRD1 is organized in a pseudo-$T=25$ lattice with 240 copies of the trimeric coat protein P3, an arrangement that has not been observed in any other virus (26). In addition, the fold of the PRD1 major capsid protein P3 closely resembles that of the adenovirus coat protein (18, 19). The PRD1 capsid is stabilized by a glue protein, P30 (57), and is further stabilized by the N and C termini of the major coat protein (61), analogous to the four different species of cementing proteins stabilizing the capsid of adenovirus (25). The vertex structures of the two viruses are also very similar. Analogous to the penton-spike complex of adenovirus, the PRD1 vertex structure is comprised of the pentameric penton base protein P31 and the trimeric spike protein P5 (13, 29, 55, 65). In PRD1, a third protein species, the receptor-binding protein P2, is the functional analogue of the knob domain of the adenovirus spike (32, 55, 71, 72). The PRD1 P31$^-$ mutant lacks the entire spike complex and the peripentonal coat protein trimers, and large openings can be seen at the vertices of the virus particle (55).

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TABLE 1. Bacterial strains used in this study

Strain	Description	Relevant property	Reference(s)
S. enterica serovar Typhimurium LT2			
DS88	SL5676 ΔH2 H1-i::Tn*10* (Tc^s) *non rev* (pLM2)	Nonsuppressor host	10
PSA(pLM2)	*supE*	Suppressor host for *sus400*	47
DB7154(pLM2)	DB700 *leuA141*(Am) *hisC527*(Am) *supD10*	Suppressor host for *sus526*	70
DB7156(pLM2)	DB700 *leuA141*(Am) *hisC527*(Am) *supF30*	Suppressor host	70
E. coli K-12			
HMS174	*recA1 hsdR* Rif^r		30
HB101	*supE44 hsdS20*(r_B^- m_B) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*	Cloning host	21, 22
DH5α	*supE44* Δ*lacU169* (ϕ80 *lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Cloning host	40, 59

Translocation of the PRD1 genome into the capsid probably is performed by the putative packaging ATPase P9 (12), since in its absence only empty virus particles are produced (49). Unlike packaging ATPases of most other icosahedral dsDNA bacteriophages, P9 is a structural protein (49). In addition, PRD1 has many other structural proteins, which are either located within or attached to the internal membrane (14). P6 is a minor capsid protein, the function of which is still unclear (48). Small integral membrane proteins P20 and P22 are known to be involved in DNA packaging or in the stable maintenance of the DNA within the particle (49). Proteins P7, P14, P11, P16, P18, and P32 are all part of the DNA delivery apparatus (33, 34). Protein P11 has been proposed to be necessary for penetrating the bacterial outer membrane and making the peptidoglycan layer accessible for P7, the viral transglycosylase (34, 56). The delivery process is then continued by formation of the membrane tail tube structure in which at least proteins P14, P16, P18, and P32 are involved (4, 33, 34). Lysis of host cells is performed by P15, a lytic muramidase (58).

Although most icosahedral dsDNA bacterial viruses contain a unique vertex, which is used for DNA packaging and injection (20, 43), no such vertex has yet been shown to exist in PRD1. The structural methods used in analysis of PRD1 architecture are based on icosahedral averaging. This excludes any asymmetrically located structures, e.g., at a single vertex. Very recently, immunogold labeling has provided the first evidence of a unique vertex in PRD1 (B. Gowen, J. K. H. Bamford, D. H. Bamford, and S. D. Fuller, submitted for publication). Gold-conjugated P6- and P20-specific antibodies were shown to bind to a single vertex of PRD1. This suggested that at least the minor capsid protein P6, which has no previously appointed function, and the membrane protein P20 are located at a unique vertex.

During the years of research on PRD1, mutants with amber mutations in different PRD1 genes have been isolated (13, 32, 33, 48, 55, 56, 61). A thorough analysis of all available PRD1 mutant particles with all available antibodies against structural proteins was performed in this study. Special emphasis was given to mutants with mutations in the spike complex proteins P31, P5, and P2 and to mutants with defects in DNA packaging or stabilization of packaged particles.

MATERIALS AND METHODS

Bacteria and phages. Bacterial strains and viruses used in this study are listed in Tables 1 and 2, respectively. Cells were grown at 37°C in Luria-Bertani (LB) medium (59), and when appropriate, chloramphenicol (25 μg/ml) was added. Wild-type (wt) PRD1 was propagated on *Salmonella enterica* serovar Typhimurium LT2 DS88 and its mutant derivatives on suppressor strains PSA(pLM2), DB7154(pLM2), and DB7156(pLM2). PRD1 amber mutants were induced with *N*-methyl-*N*′-nitrosoguanidine and isolated as described previously (48).

For production of wt and mutant PRD1 virus particles, DS88 cells were infected at a multiplicity of infection of 6 and 8, respectively. After lysis of the cells, phage particles were purified by polyethylene glycol-NaCl precipitation and 5 to 20% rate-zonal sucrose gradient centrifugation, as previously described (10). The virus was concentrated by either differential centrifugation or further purified by ion-exchange chromatography on Sartorius D100 anion-exchange cartridges, essentially by the method of Walin et al. (69).

DNA techniques. Plasmids used in this study are listed in Table 2. DNA manipulations were done using standard molecular biology techniques (59). For the complementation analysis of the PRD1 mutants, PRD1 genes *IX* and *XXII*, together with their preceding ribosome binding site sequences, were amplified by PCR using primers specific to the corresponding genes in the PRD1 genome, and the resulting fragments were inserted between the *Eco*RI and *Hin*dIII sites of

TABLE 2. Bacteriophages and plasmids used in this study

Bacteriophage or plasmid	Description	Reference(s) or source
PRD1		
wt	wt	54
sus1	Amber mutation in gene *IX*, two missense mutations in gene *VI*	48
sus42	Amber mutation in gene *XXII*	48
sus148	Amber mutation in gene *XVIII*	48
sus234	Amber mutation in genes *VII* and *XIV*	48
sus400	Amber mutation in gene *XX*	This study
sus471	Amber mutation in gene *II*, opal mutation in gene *VII* (P2$^-$/P7$^-$ when propagated in nonsuppressor host, P7$^-$ in suppressor host)	56
sus525	Amber mutation in gene *XXXI*	55
sus526	Amber mutation in gene *XX*	This study
sus539	Amber mutation in gene *II*	13, 55
sus607	Amber mutation in gene *XI*	61
sus690	Amber mutation in gene *V*	13
[*lacZ*α]-9	Amber mutation in gene *XXXII*	33
Plasmids		
pSU18	Cloning vector, p15A replicon	15
pNS1	PRD1 gene *IX* (nt 7624–8320)[a] in pSU18	This study
pMG119	PRD1 gene *XX* (nt 8445–8588) in pSU18	Marika Grahn
pMV11	PRD1 gene *XXII* (nt 9784–9944) in pSU18	This study
pLM2	Encodes the PRD1 receptor	47

[a] Numbers refer to the PRD1 genome sequence (GenBank accession number M69077).

plasmid pSU18. The constructs were transformed into *Escherichia coli* HB101 or DH5α cells. The DNA sequences of the inserts were determined, and the constructs were transformed into *E. coli* HMS174(pLM2). The exact locations of the amber mutations in the PRD1 mutants were determined by sequencing the region from nucleotides (nt) 6784 to 9944 from each of the mutants. Overlapping PCR fragments amplified with primers specific to the PRD1 genome were used as templates in the DNA sequencing reaction. Purified virus DNA (in the case of *sus1* and *sus42*) or viral DNA from plaques (45) (in the case of *sus400* and *sus526*) was used as a template in the PCRs. Sequencing was performed using an automated sequencer (at the DNA Synthesis and Sequencing Laboratory, Institute of Biotechnology, University of Helsinki).

Electron microscopy. For thin-section electron microscopy, DS88 cells were grown in LB to a density of 10^9 CFU/ml and infected with *sus400* and *sus526* PRD1 mutants using a multiplicity of infection of 8. Samples were taken 40, 60, and 70 min postinfection and fixed with 3% glutaraldehyde (vol/vol) in 20 mM potassium phosphate buffer (pH 7.2) for 20 min at room temperature. The cells were collected by centrifugation, washed twice with 20 mM potassium phosphate buffer (pH 7.2), and prepared for transmission electron microscopy as described previously (3). Electron micrographs were taken with a JEOL 1200 CX microscope (at the electron microscopy unit, Institute of Biotechnology, University of Helsinki) operating at 60 kV.

Polyclonal sera. Polyclonal sera against proteins P9 and P22 were raised in rabbits using specific peptides as antigens. The peptides CTADAVLARFDLG KKR, corresponding to residues 209 to 223 of P9, and MQLITDMAEWSSKPC, corresponding to residues 1 to 14 of P22, were purchased from KJ Ross-Petersen AS, Copenhagen, Denmark. The peptides were linked to keyhole limpet hemocyanin via the cysteine residue. Rabbits were immunized three times at 3-week intervals using the protein-conjugated specific peptides emulsified with either Freund's complete adjuvant (in the first immunization) or incomplete adjuvant (in the subsequent immunizations). One milligram of the conjugated peptide was used per immunization. The specificity of the serum obtained was determined by Western blotting.

Analytical methods. The protein concentration of purified virus preparations was determined by the Coomassie brilliant blue method using bovine serum albumin as a standard (23). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described (53). Western blotting was performed by transferring the proteins from SDS-polyacrylamide gels (17% polyacrylamide) onto polyvinylidene difluoride membranes (Millipore). Polyclonal antisera against PRD1 proteins P2 (32), P5 (41), P31 (55), P9 and P22, together with monoclonal antibodies 6T58, 7A5, 7N41 (41), and 11A401 (Gowen et al., submitted) against proteins P6, P7/P14, P7/P14, P16, and P11, respectively, were used as primary antibodies. Proteins were visualized using the Supersignal West Pico chemiluminescent substrate (Pierce) with horseradish peroxidase-conjugated swine anti-rabbit immunoglobulin G (IgG) antibodies (Dako) or peroxidase-labeled horse anti-mouse IgG antibodies (Vector) as secondary antibodies.

RESULTS

Isolation and identification of PRD1 mutants with amber mutations in gene *XX*. Two suppressor-sensitive gene XX^- mutants of PRD1, *sus30* and *sus78*, have been described previously (48). However, when analyzed by DNA sequencing, these mutants did not contain mutations in gene *XX,* but instead, the mutations were in several other genes (data not shown). To obtain genuine gene *XX* mutants, *N*-methyl-*N*′-nitrosoguanidine mutagenesis was performed on PRD1 and new amber mutants were selected. Mutations were provisionally mapped to particular regions of the genome by marker rescue analysis (12, 50). Two new amber mutants were obtained and subsequently named *sus400* and *sus526*.

DNA sequencing reveals amber mutations in genes *IX*, *XXII*, and *XX*. The exact defects in PRD1 packaging mutants, first preliminarily mapped by marker rescue analysis to the region of the genome containing genes *VI*, *X*, *IX*, *XX*, and *XXII* were identified by DNA sequencing of the genome region from nt 6784 to 9944 of the mutants (Fig. 1). The mutations in *sus400* and *sus526* were located at nt 8470 (G→A) and 8497(G→A),

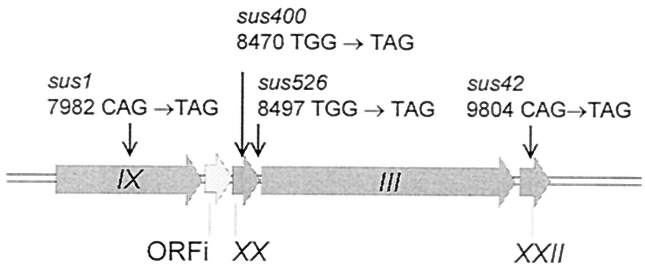

FIG. 1. Sequencing of the region from nt 6784 to 9944 of the PRD1 genome that contains genes *IX*, *XX*, and *XXII*. Gene *III* encodes the major capsid protein. The function of open reading frame ORFi is unknown. Defects found in different suppressor-sensitive mutants are marked with arrows.

respectively. Both mutations result in amber codons and fall within the sequence of gene *XX*, yielding 3- and 12-amino-acid-long amber fragments for *sus400* and *sus526*, respectively. *sus42* contained a transversion at nt 9804 (C→T), resulting in introduction of an amber mutation at the second codon of gene *XXII*. *sus1* contained an amber mutation at nt 7982 (CAG→TAG) within gene *IX*, yielding a 115-amino-acid-long amber fragment instead of the 227-amino-acid-long wt P9. Thus, we confirm that *sus1* and *sus42* contain amber mutations in genes *IX* and *XXII*, respectively, as had been proposed previously (48).

The mutations can be complemented by a single gene. To ensure that the amber mutations identified by DNA sequencing were the only relevant mutations affecting the mutant viruses, complementation analysis was performed. In the complementation assay, the titers of PRD1 mutants were determined on *E. coli* K-12 HMS174(pLM2) strain harboring either vector pSU18 or recombinant plasmid pNS1, pMV11, or pMG119 containing a single wt virus gene in pSU18. Overexpressed wt proteins P9 (pNS1) and P22 (pMV11) were found to fully complement the defects in *sus1* and *sus42*, respectively (Table 3). The defects in both *sus400* and *sus526* were corrected by P20, produced from pMG119 (Table 3), further confirming that *sus400* and *sus526* have mutations in gene *XX*. The titers of the mutant viruses on the complementing strains were, in all cases, equivalent to the titers obtained with wt virus. This confirms that these mutants do not have other defects affecting the viability of the viruses.

$P9^-$, $P20^-$, and $P22^-$ PRD1 mutants produce empty virus particles. PRD1 mutant and wt particles were grown in DS88 cells and purified by rate-zonal centrifugation. In the case of wt virus, two light-scattering zones, corresponding to packaged (80%) and empty (20%) virus particles, are observed in sucrose gradients as determined by using radioactively labeled virus particles and fractionation (11, 49). When *sus1*, *sus42*, *sus400*, and *sus526* mutants were purified by sucrose gradients, a clear light-scattering zone, corresponding to empty particles, could be seen in all cases. Another, albeit weak, zone corresponding to packaged particles, was visible in the case of *sus42*. This mutant has previously been demonstrated to package DNA, as determined by thin-section electron micrographs of infected cells (49). However, the DNA was lost upon subsequent purification. When *sus400* and *sus526* PRD1 mutants were analyzed by thin-section electron microscopy, only empty

TABLE 3. Complementation titers of gene *IX*$^-$, *XX*$^-$, and *XXII*$^-$ mutants

Strain	Property	Titer (PFU/ml)			
		sus1 (*IX*$^-$)	*sus400* (*XX*$^-$)	*sus526* (*XX*$^-$)	*sus42* (*XXII*$^-$)
S. enterica					
DS88	Nonsuppressor	4.4×10^4	4.5×10^5	4.0×10^7	3.4×10^6
PSA	Suppressor	3.5×10^{11}	8.0×10^{11}		1.5×10^{12}
DB7154	Suppressor			1.0×10^{12}	
E. coli					
HMS174(pLM2)					
HMS174(pSU18)	Negative control	1.0×10^3	1.2×10^5	3.8×10^6	3.3×10^5
HMS174(pNS1)	Gene *IX* in pSU18	1.0×10^{11}			
HMS174(pMG119)	Gene *XX* in pSU18		3.3×10^{11}	2.4×10^{11}	
HMS174(pMV11)	Gene *XXII* in pSU18				3.4×10^{11}

virus particles were seen in samples of infected cells taken 40, 60, and 70 min postinfection (Fig. 2). This was also the case for *sus1* (49).

Western blot analysis of the purified mutant particles. To analyze the phenotypic effects caused by different nonsense mutations, the protein content of different PRD1 mutant particles (P2$^-$, P5$^-$, P7$^-$, P7$^-$/P14$^-$, P9$^-$, P11$^-$, P18$^-$, P20$^-$, P22$^-$, P31$^-$, and P32$^-$) was assayed using Western blotting with all available PRD1-specific antibodies against structural proteins (see Materials and Methods). Unfortunately, the previously obtained anti-P20 serum (Gowen et al., submitted) did not give any signal on Western blots. Polyclonal sera against the putative packaging ATPase P9 and the membrane protein P22 were obtained by immunization of rabbits with keyhole limpet hemocyanin-conjugated P9- and P22-specific peptides.

It has been previously shown that mutant particles lacking the vertex protein P31 (*sus525*) are also devoid of the spike proteins P5 and P2 and of the peripentonal trimers, thus displaying large openings at the vertices (55). However, we show here by Western blotting that these particles contain proteins P6 and P22. P6 and P20 have been shown by electron microscopy immunolabeling to be located at a single vertex (Gowen et al., submitted), indicating that 1 of the 12 vertices is different. Western blotting also revealed that the putative packaging ATPase P9 and the small membrane protein P22 were present in P31$^-$ particles (*sus525*) (Table 4 and Fig. 3). Further, when mutant particles lacking spike protein P2 or P5 were examined, stoichiometric amounts of proteins P9, P6, and P22 were present (Table 4).

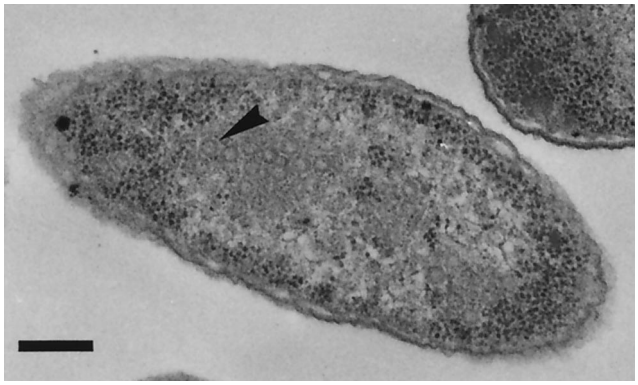

FIG. 2. PRD1 P20$^-$ mutants produce only empty virus particles. As an example, an electron micrograph of thin-sectioned DS88 cells infected with mutant *sus400* collected 60 min postinfection is shown. Bar, 500 nm.

Interestingly, all of the packaging mutant particles (P9$^-$, P20$^-$, or P22$^-$) were shown to contain a full complement of spike complex proteins P31, P5, and P2 (Table 4). There was no mutant available for protein P6, but intriguingly P20$^-$ and P22$^-$ mutant particles were found to lack both proteins P6 and P9 (Table 4 and Fig. 3, which contains representative Western blots.) The expression levels of proteins P6 and P9 in cells infected with P20$^-$ and P22$^-$ mutants were analyzed by Western blotting, and their levels were equivalent to those found in wt virus infection (Fig. 4). Thus, the absence of P6 and P9 in the purified virus particles was not due to reduced production of these proteins in infected cells. None of the other PRD1 mutant particles analyzed (P7$^-$, P7$^-$/P14$^-$, P11$^-$, P18$^-$, P31$^-$, and P32$^-$) was found to lack any of the spike complex or packaging proteins. Furthermore, none of the other proteins analyzed (P11, P7, P14, and P16) by specific antibodies was linked to the loss of P6, P9, P20, P22, or the spike complex proteins (Table 4). In *sus1* particles (defect in gene *IX*), the only protein missing was P9.

The unique vertex is linked to the membrane. All mutants lacking either one of the small membrane proteins P20 and P22 were devoid of P6. Furthermore, in the absence of P6, none of the mutants tested contained P9. This suggests that the small integral membrane proteins P20 and P22 are attached directly or indirectly, probably via protein-protein interactions, to protein P6, which in turn is associated with protein P9. This series of associations, together with the fact that both the integral membrane protein P20 and the minor capsid protein P6 have been localized at a single vertex (Gowen et al., submitted), suggests that P9 and P22 are located at the same vertex. Thus, we can conclude that PRD1 has a unique vertex, which contains the proteins involved in DNA packaging and/or maintenance within the particle and which extends to the phage internal membrane.

DISCUSSION

In all icosahedral dsDNA bacteriophages studied so far, packaging occurs by translocation of the genome into preformed procapsids via a unique portal vertex that is also the

TABLE 4. Analysis of the protein composition of different PRD1 mutant particles by Western blotting

Genotype	Mutant	Protein composition[a]									
		P9 (DNA-packaging ATPase)	P6 (minor capsid protein, packaging)	P22 (DNA packaging) (M)	P2 (receptor-binding protein)	P5 (spike protein)	P31 (penton protein)	P7 (Transglycosylase) (M)	P14 (DNA delivery) (M)	P11 (DNA delivery)	P16 (infectivity) (M)
wt		+	+	+	+	+	+	+	+	+	+
wt (E)[b]		−	+	+	+	+	+	+	+	+	+
IX$^-$	*sus1*	−	+	+	+	+	+	+	+	+	+
XXII$^-$	*sus42*	−	−	−	+	+	+	+	+	+	+
II$^-$	*sus539*	+	+	+	−	+	+	+	+	+	+
V$^-$	*sus690*	+	+	+	−	−	+	+	+	+	+
XXXI$^-$	*sus525*	+	+	+	−	−	−	+	+	+	+
VII/XIV$^-$	*sus234*	+	+	+	+	+	+	−	−	+	+
VII$^-$	*sus471*	+	+	+	+	+	+	−	+	+	+
XI$^-$	*sus607*	+	+	+	+	+	+	+	+	−	+
XVIII$^-$	*sus148*	+	+	+	+	+	+	+	+	+	+
XX$^-$	*sus400*	−[c]	−	+	+	+	+	+	+	+	+
XX$^-$	*sus526*	−	−	+	+	+	+	+	+	+	+
XXXII$^-$	[*lac*Zα]-9	+	+	+	+	+	+	+	+	+	+

[a] The presence (+) or absence (−) of the indicated proteins in PRD1 mutant particles is given. (M), integral membrane protein based on transmembrane helix prediction and location in the viral membrane.
[b] (E), empty particles.
[c] (−), a small amount could be detected.

site of tail assembly (and thus is easily visualized). A unique vertex has also been found in a complex dsDNA animal virus, HSV-1, which may be evidence for the conservation of a basic mechanism of DNA packaging in viruses. In addition, we have shown here that the internal membrane-containing icosahedral dsDNA bacteriophage PRD1 has a unique vertex.

The unique vertex of PRD1 comprises at least the putative packaging ATPase P9, minor capsid protein P6, and two small membrane proteins P20 and P22, which are all sequentially associated, probably via protein-protein interactions, with each other. The location of P6 and P20 at the unique vertex has been confirmed by immunogold labeling with specific antibodies (Gowen et al., submitted). Interestingly, the unique vertex of PRD1 extends to the phage membrane via the integral membrane proteins P20 and P22. How P20 and P22 are located with respect to each other in the virus is not clear: P20$^-$ mutant particles do not lack the protein P22, so association of P22 with the virus capsid cannot be dependent, at least solely, on protein P20. The need for P22 for the association of P20 with the virus could not be proven, as a specific antibody against P20 on Western blots was not available. P6 was missing in both P20$^-$ and P22$^-$ mutants, suggesting that both of the proteins are necessary for P6 binding, either directly or via some other protein species.

The PRD1 spike complex, comprised of the penton base P31, the spike P5, and the receptor-binding P2 proteins, is probably located at all vertices (55). The fact that all of the packaging proteins identified thus far can be found in the mutant virus particle (*sus525*) missing the spike complexes and the peripentonal P3 trimers suggests that the unique vertex is functionally and structurally distinct from the spike complex. It is not known if the unique vertex of PRD1 contains the spike complex proteins in addition to the packaging machinery or if the spike complex actually occupies only 11 of the 12 vertices, the twelfth vertex being reserved for the packaging machinery and possibly for other unique vertex proteins.

The identification of a unique vertex in PRD1 will be a valuable tool for solving the structure of the whole virus in more detail, thereby revealing more of the functional structures of PRD1. Current methods used for structural analysis, such as X-ray diffraction and cryoelectron microscopy combined with image reconstruction, are based on averaging of the icosahedral data, which will, of course, average out the density from asymmetrically located protein species. On the basis of structural data, only a few of the 18 or more PRD1 structural proteins have been allocated exact positions in the virion. We believe that many of these proteins might be found at the unique vertex. The DNA delivery protein P11 could not be located by difference imaging of quasi-atomic models of cryoelectron microscopy reconstructions of P11$^-$, P9$^-$, and wt particles, suggesting that the distribution of P11 does not follow icosahedral symmetry (61). If P11 were located at the unique vertex, it would seem logical for the other proteins involved in DNA entry (P7 and P14) also to be situated at the same vertex. It has been proposed that association of the lysis protein P15 with the virus capsid is dependent on the small membrane protein P22 (58). Importantly, P22 was shown in this study to be part of the unique vertex, leading to the hypothesis that P15 is yet another unique vertex protein.

The striking structural and functional similarities found between adenovirus (which infects members of the domain *Eucarya*) and PRD1 (which infects members of the domain *Bacteria*) have led to the hypothesis that these viruses share a common ancestor and further that viruses may form lineages that have members infecting hosts in different domains of life (8, 9, 18, 44). This lineage may contain viruses of other evolutionarily distant hosts as well. For example, the same coat protein fold found in PRD1 and adenovirus can also be observed in Paramecium bursaria chlorella virus 1 (PBCV-1) (51), another internal-membrane-containing virus which infects unicellular, eukaryotic, chlorella-like green algae (67, 68, 73). Also, the genome organization and predicted coat protein

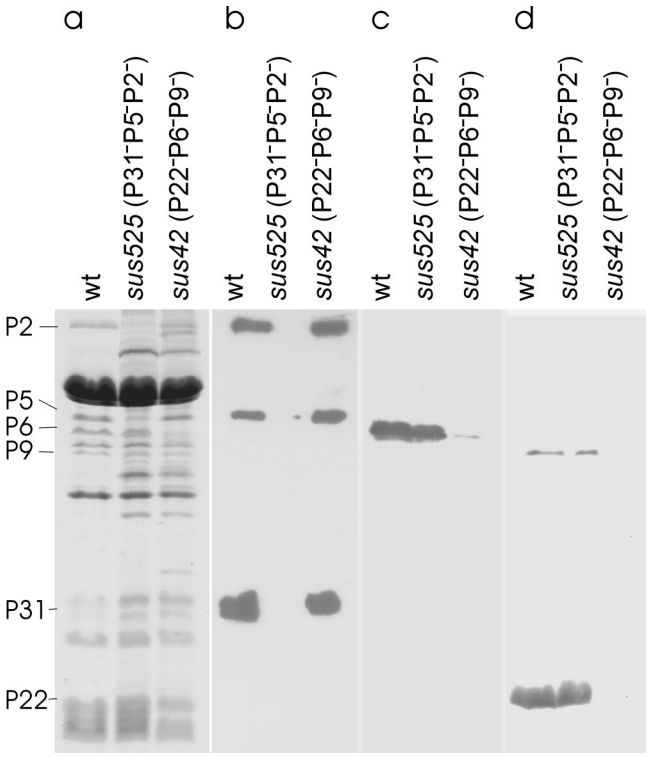

FIG. 3. Protein composition of purified virus particles of wt PRD1, spike complex mutant *sus525* (*XXXI*$^-$), and the small membrane protein mutant *sus42* (*XXII*$^-$) analyzed by Coomassie brilliant blue-stained SDS-PAGE (a) and Western blotting (b to d). For Western blotting, polyclonal sera against P2, P5, and P31 (b), monoclonal antibody against P6 (c), and polyclonal sera against P9 and P22 (d) were used for detection. The positions of proteins of interest are indicated to the left of the gel.

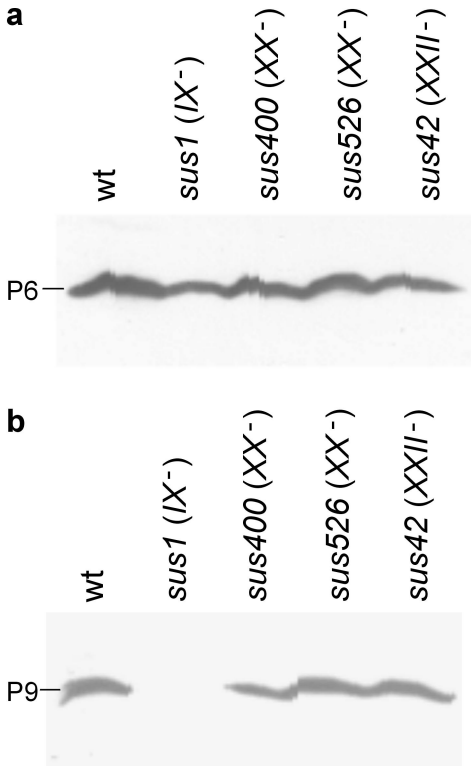

FIG. 4. Western blotting of cells infected with wt PRD1 (positive control), packaging mutant *sus1* (*IX*$^-$, negative control for P9 expression), and membrane protein mutants *sus400* (*XX*$^-$), *sus526* (*XX*$^-$), and *sus42* (*XXII*$^-$). (a) P6. (b) P9.

fold of bacteriophage *B*am35, which infects the gram-positive bacterium *Bacillus thuringiensis* (1, 7) are similar to those of PRD1 (54a).

So far, a unique vertex has been identified in only one icosahedral dsDNA virus infecting higher organisms, HSV-1 (52). As DNA packaging generally is regarded as a more conserved function and not as susceptible to evolutionary pressure as DNA entry-related mechanisms (9), it is enticing to hypothesize that although the hosts of PRD1, adenovirus, PBCV-1, and *B*am35, are evolutionarily far apart, their DNA packaging mechanisms may resemble each other. PBCV-1 has been observed to form empty precursor capsids and to inject its DNA into the host cell while leaving the empty capsid bound to the cell surface (68), in a fashion similar to PRD1. Both adenovirus and PBCV-1 contain structural proteins whose location and function in the virion are still poorly characterized (25, 68). It is conceivable that these viruses also possess a unique vertex for packaging, and thus studies on a bacterial virus are paving the way to understanding such properties in the families *Adenoviridae* and *Phycodnaviridae*.

It has been proposed that PRD1 could use any of its vertices for binding to the host cell and for DNA injection (4, 34, 46, 56). This is in contrast to the tailed dsDNA bacteriophages in which the attachment and injection machinery are found only at one vertex, attached to the portal, or connector, through which the DNA has been packaged. Even though the PRD1 receptor-binding protein P2 can be found at all or at least the majority of vertices (32, 55), it has not been shown directly whether DNA injection occurs in vivo through any of the vertices or if a certain vertex is preferred. The finding of a unique vertex in PRD1 points to the possibility that its DNA injection might actually occur through a single vertex. We propose a mechanism whereby PRD1 would make primary contact with the cell and bind reversibly to the host via P2 but then roll over so that the unique vertex could be reached, leading to irreversible binding and DNA injection. This model is supported by the flexibility of the spike protein P5 (45), which would enable the proposed rolling movement.

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