

Chapter 16

Lipid-Containing Viruses: Bacteriophage PRD1 Assembly

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Abstract PRD1 is a tailless icosahedrally symmetric virus containing an internal lipid membrane beneath the protein capsid. Its linear dsDNA genome and covalently attached terminal proteins are delivered into the cell where replication occurs via a protein-primed mechanism. Extensive studies have been carried out to decipher the roles of the 37 viral proteins in PRD1 assembly, their association in virus particles and lately, especially the functioning of the unique packaging machinery that translocates the genome into the procapsid. These issues will be addressed in this chapter especially in the context of the structure of PRD1. We will also discuss the major challenges still to be addressed in PRD1 assembly.

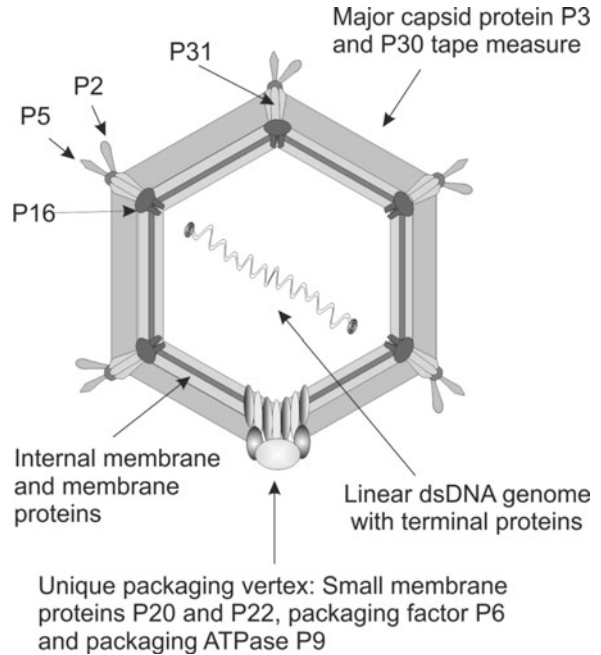
16.1 Introduction

The archetypal member of the Tectiviridae family is PRD1, shown schematically in Fig. 16.1. It infects Gram-negative bacteria that contain a conjugative IncP, IncN or IncW plasmid which codes for the receptor. Viruses of this family typically have a spike-decorated, tailless, icosahedrally symmetric capsid approximately 65 nm in diameter, surrounding a lipid membrane which in turn encapsulates the linear, double-stranded DNA genome (Bamford and Ackermann 2000; Saren et al. 2005). There are four major reasons why PRD1 is interesting: First, the genome is replicated using a protein-primed, sliding-back mechanism utilising inverted terminal repeats on the genome and a covalently bound terminal protein at the 5' ends (Bamford et al. 1983; Caldentey et al. 1992; Salas 1991; Savilahti and Bamford 1986; Savilahti et al. 1989, 1991). Second, it can be used to study viral membrane biogenesis in a well-characterised genetic background as it grows, for example, in *Escherichia coli* (Laurinavicius et al. 2004, 2007). Third, structure-based phylogeny of viruses has been shaken out of its slumber by the observation that the major capsid protein fold of PRD1 resembles that found in certain viruses infecting archaea and eukaryotes, possibly signifying descent from

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Fig. 16.1 Schematic representation of the PRD1 virion including a model for the unique vertex organisation



a common ancestor. This idea was originally formulated when the first picornavirus structures were determined and has recently been expanded to other capsid folds (Abad-Zapatero et al. 1980; Abrescia et al. 2010; Bamford 2000, 2003; Bamford et al. 2002a, 2005; Benson et al. 1999; Hogle et al. 1985; Nandhagopal et al. 2002; Rice et al. 2004; Rossmann et al. 1985; Rossmann and Johnson 1989; Simpson et al. 2003). Finally, and of most relevance to this work, the structure of the virion is known to atomic resolution; extensive genetic and biochemical analyses have been carried out on the system and an *in vitro* packaging system is available for PRD1, thus many of the steps in virus assembly have been characterised (Abrescia et al. 2004; Bamford et al. 1995; Caldentey et al. 1990; Cockburn et al. 2004; Huiskonen et al. 2007; Karhu et al. 2007; Merckel et al. 2005; Mindich et al. 1982b; Strömsten et al. 2005; Xu et al. 2003; Ziedaite et al. 2009).

16.2 Virion Structure and Properties

Extensive genetic and structural studies have been carried out on the virion and subviral particles of PRD1, using, for example, Raman spectroscopy, electron microscopy and X-ray crystallography. The constituents of the virion are approximately 70% protein, 15% lipid and 15% DNA, with a total mass of about 66 MDa. The virion contains about 18 protein species split roughly evenly between the capsid and the membrane (Fig. 16.1 and Table 16.1). Some of the structural proteins have been characterised individually, some in the context of the capsid or subviral complexes. X-ray crystallography of the major capsid protein, P3, showed that it consists of two eight-stranded β -barrels or jelly rolls (Fig. 16.2). The topology in both barrels is similar even though there is little sequence conservation. Each jelly roll contains a short α -helix inserted between the F and G strands. The first helix (FG1- α) locks the two jelly rolls together, the “trimerisation” loop (the FG1 loop) following FG1- α forms interactions with two other subunits to make a stable trimer. The barrels thus give the trimer pseudo-hexagonal symmetry that is well suited to packing on a spherical surface (Benson et al. 1999).

Table 16.1 PRD1 genes, open reading frames (ORF) and corresponding proteins

Gene ^a	ORF ^a	Coordinates in PRD1 genome ^b	Protein	Mass kDa ^c	Description
<i>VIII</i>		233–1012	P8	29.5	Genome terminal protein
<i>I</i>		1,016–2,677	P1	63.3	DNA polymerase
	(ORF a)	2,415–2,495		3.1	
<i>XV</i>		2,679–3,128	P15	17.3	Muramidase
<i>II</i>		3,128–4,903	P2	63.7	Receptor binding
	(ORF b)	3,453–3,587		5.1	
<i>XXXI</i>	(ORF c)	4,907–5,287	P31	13.7	Pentameric base of spike
	(ORF d)	5,103–5,294		7.2	
<i>V</i>		5,287–6,309	P5	34.2	Trimeric spike protein
<i>XVII</i>		6,328–6,588	P17	9.5	Assembly
<i>XXXIII</i>	(ORF f)	6,578–6,784	P33	7.5	Assembly
<i>VI</i>		6,784–7,284	P6	17.6	Minor capsid protein. DNA packaging
<i>X</i>		7,029–7,640	P10	20.6	Assembly
<i>IX</i>		7,637–8,320	P9	25.8	Minor capsid protein. DNA packaging ATPase
	(ORF i)	8,332–8,460		4.5	
<i>XX</i>	(ORF j)	8,460–8,588	P20	4.7	DNA packaging
<i>III</i>		8,595–9,782	P3	43.1	Major capsid protein
	(ORF h)	9,427–9,681		9.2	
<i>XXII</i>	(ORF k)	9,801–9,944	P22	5.5	DNA packaging
	(ORF l)	10,044–10,166		4.4	
<i>XVIII</i>	(ORF m)	10168–10440	P18	9.8	DNA delivery
<i>XXXII</i>	(ORF n)	10,440–10,604	P32	5.4	DNA delivery
<i>XXXIV</i>	(ORF o)	10,617–10,823	P34	6.7	
<i>XXX</i>	(ORF p)	10,833–11,087	P30	9.0	Minor capsid protein
	(ORF q)	11,090–11,200		4.2	
<i>XI</i>		11,202–11,825	P11	22.2	DNA delivery
<i>XVI</i>	(ORF s)	11,836–12,189	P16	12.6	Infectivity
<i>VII</i>		12,190–12,987	P7	27.1	DNA delivery, transglycosylase
<i>XIV</i>		12,535–12,987	P14	15.0	DNA delivery
<i>XXXV</i>	(ORF t)	12,984–13,337	P35	12.8	Holin
<i>XXXVI</i>	(ORF u)	13,330–13,692	P36	12.7	Lysis protein, Rz1-like
<i>XXXVII</i>	(ORF v)	13,616–13,888	P37	10.2	Lysis protein, Rz-like
<i>XIX</i>		14,132–13,848 ^d	P19	10.5	ssDNA-binding protein
<i>XII</i>		14,687–14,205 ^d	P12	16.6	ssDNA-binding protein

^aORF shown to code for functional proteins are classified as genes and are given Roman numerals

^bGenBank accession AY848689

^cThe mass does only include the initial methionine if present in the mature protein

^dGene transcription in the opposite direction to that of the rest of the genes

Three-dimensional electron microscopy and X-ray crystallography of virions, and P2-deficient particles, respectively, have shown that the capsid is organised on a pseudo $T=25$ surface lattice. The major capsid protein is arranged in 240 trimers forming the facets, with pentamers of P31 at the vertices. There is an underlying framework of dimers of the minor coat protein P30 adjacent to the membrane, and the transmembrane protein P16 is found under the vertices as illustrated in Fig. 16.2 (Abrescia et al. 2004; Butcher et al. 1995; Cockburn et al. 2004). In the capsid, the termini of P3 face the membrane and have different conformations depending on the location in the surface lattice. In half of the P3 subunits, the first five amino-terminal residues are embedded in the outer leaflet of the

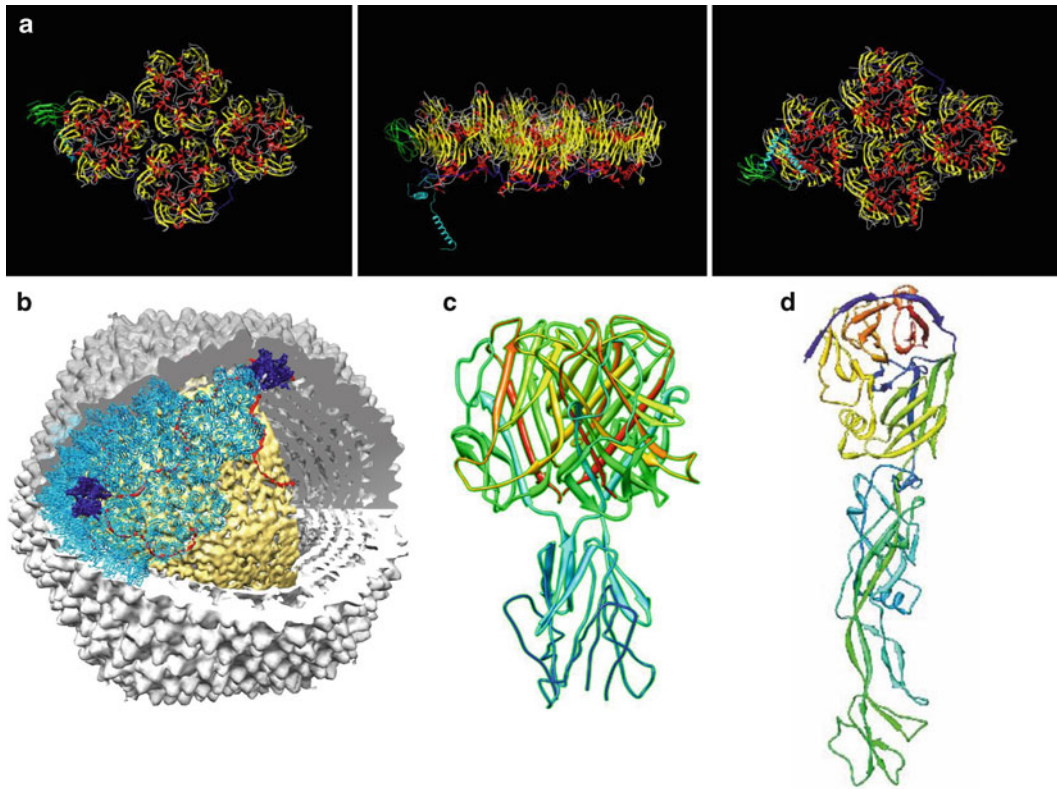


Fig. 16.2 Structure of PRD1. (a) Ribbon model of the asymmetric unit: P3 sheet in *yellow*, helices in *red*, loops in *grey*. P16 in *cyan*, P30 in *blue* and P31 in *green*, from the top, side and bottom. The polarity of the structure is very obvious, the helices control the interaction with the membrane, both in P3 and in P16, with the majority interacting with the phospholipid head groups, and only one transmembrane helix in P16 locking the membrane in place, at the position of greatest curvature of the membrane (Abrescia et al. 2004). (b) Isosurface representation of an icosahedral reconstruction of the particle (*grey*) with a segment cut away to reveal the underlying outer leaflet of the membrane (*yellow*), the inner leaflet and the organised DNA. Several copies of the atomic models of P3 (*blue ribbon*), P30 (*red ribbon*) and P31 (*navy blue ribbon*), are shown superposed on top of the outer leaflet showing how P30 stretches along the facet edges (San Martín et al. 2002; Abrescia et al. 2004). (c) Ribbon model of P5, rainbow coloured from the N terminus in *blue* to the C terminus in *red* (Merckel et al. 2005). (d) Ribbon model of P2, rainbow coloured from the N terminus in *blue* to the C terminus in *red* (Xu et al. 2003)

membrane, making P3 a peripheral membrane protein. These are the P3 subunits arranged along the edge of the facet, and may help to stabilise the membrane curvature at these points. The other termini switch the subunit conformation depending on the position in the lattice (Abrescia et al. 2004; Cockburn et al. 2004).

P31 is a single eight-stranded β -barrel which also shares the same topology as the P3 β -barrels (Abrescia et al. 2004). P31 pentamers are stabilised by β -strand A interacting with β -strand B of the adjacent P31 monomer and by side-to-side interactions between the BIDG sheet of one subunit and the neighbour's strands G and F. P31 pentamers are intricately linked to the peripentonal P3 trimers, to the C terminus of P30 and to the minor integral membrane protein, P16. P30 is an intrinsically disordered protein in solution, but in the capsid it plays an essential role in a very extended, yet distinct conformation, forming dimers along the facet edges, stabilising the junction of the facets and anchoring the vertices in place through interaction with the integral membrane protein P16 (Abrescia et al. 2004; Cockburn et al. 2004; Jaatinen et al. 2004; Rydman et al. 2001). The P30 dimers are

formed by amino-terminal hooks. Together, 30 such dimers form a P30 network over the membrane (Fig. 16.2). The structures of the other membrane proteins are unresolved in the atomic model. Raman spectroscopy has shown that the lipids are in a liquid crystalline state, with the head groups tightly interacting with the DNA (Cockburn et al. 2004; Tuma et al. 1996b). The effective DNA concentration has been estimated to be about 515 mg/ml and the apparent pressure of the DNA within the capsid has been estimated to be around 45 atm. The spacing between the outermost DNA layer and the inner leaflet is about 2 nm, the spacing between the second, third and fourth layers is about 2.2 nm. However, the spacing between the first and second layers is about 2.6 nm (Cockburn et al. 2004).

Two types of spike proteins, the adsorption protein, P2, and a second trimeric spike protein, P5, are attached to P31 as shown at low resolution by averaging vertices from electron micrographs of virions, P2-deficient and P5-deficient particles (Grahn et al. 1999; Huiskonen et al. 2007; Rydman et al. 1999). The exact interaction is averaged out in the electron density of the P2-deficient virus particle and thus it is still not clear how the symmetry mismatches between P31, P5 and P2 are accommodated in the particle (Abrescia et al. 2004). P2 is an extended monomer with a pseudo β -propeller at the distal end to the capsid which most likely contains the receptor-binding site (Xu et al. 2003). P5 is a trimeric protein with the N terminus apparently inserted into the P31 pentamer (Caldentey et al. 2000; Huiskonen et al. 2007; Sokolova et al. 2001). The P5 stalk resembles the fold of the adenovirus fibre (Merckel et al. 2005; van Raaij et al. 1999) and the head is a ten-stranded β -barrel with a TNF-like fold (Huiskonen et al. 2007; Merckel et al. 2005) (Fig. 16.2).

16.3 Assembly

After the viral genome has entered the cell, transcription begins (Fig. 16.3). The genome of 14,927 bp with 37 identified genes (GenBank accession number AY848689) is organised into five operons as illustrated in Fig. 16.4. (Bamford et al. 1991, 2002b; Grahn et al. 1994; Saren et al. 2005). The properties of the identified ORFs in the genome are summarised in Table 16.1. The early operons, OE1 and OE2, express the terminal protein, the DNA polymerase and the two single-stranded DNA-binding proteins that take part in the replication reaction that is illustrated in the overall life cycle of PRD1 in Fig. 16.3. The three late operons mainly contain the genes for the structural components of the virion as shown in Fig. 16.4. PRD1 uses protein-primed replication to initiate DNA synthesis. Replication starts with the recognition of the terminal protein by the PRD1 DNA polymerase and free terminal protein. The covalently-linked terminal protein on each 5' end of the linear dsDNA molecule serves as a primer for initiation instead of the more usual RNA priming mechanism. The polymerase then catalyses covalent phosphodiester bond formation between the 5'-terminal nucleotide (dGMP) and Tyr190 in the terminal protein (Savilahti et al. 1991). DNA polymerisation is then initiated in the inverted terminal repeats from the complementary base to the fourth nucleotide in the 3'-terminal end of the genome (Caldentey et al. 1993). The polymerase's "sliding-back" mechanism maintains the 5' end of the sequence. Replication from both DNA ends is coupled to strand displacement, resulting in duplication of the parental strands (Salas 1999; Yoo and Ito 1989). Early on in this replication process, the terminal protein recruits the viral polymerase to the bacterial nucleoid, thus helping to organise the DNA replication machinery (Munoz-Espin et al. 2010). The single-stranded DNA-binding proteins P12 and P19 (Table 16.1) stimulate replication in a model system (Pakula et al. 1990, 1993).

The first DNA-containing viral particles are seen about 40 min into the infection, and cell lysis occurs after 60 min. Late protein synthesis starts about 15 min post infection in EM, the first precursors produced in the cytoplasm are a number of soluble capsid proteins including P3 trimers, P5 trimers and P31 pentamers, reflecting their final state in the assembled capsid. Phage-encoded

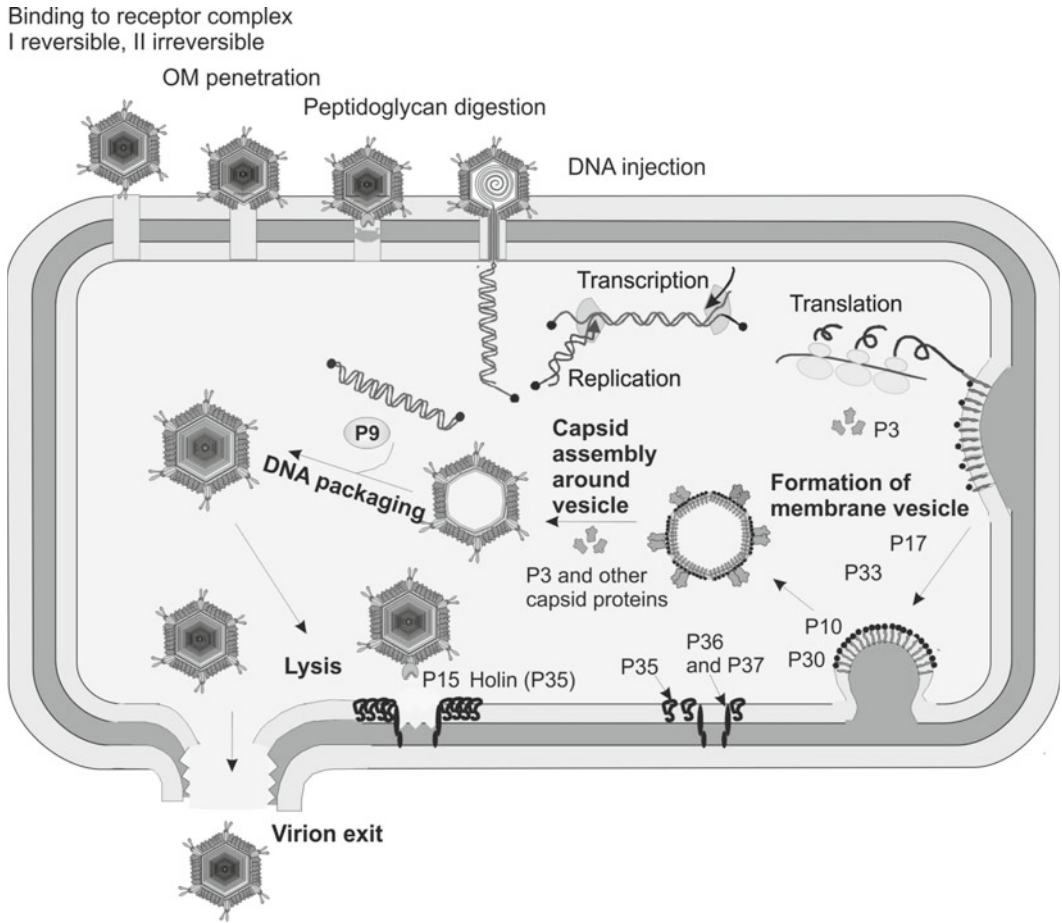


Fig. 16.3 Schematic representation of the life cycle of PRD1

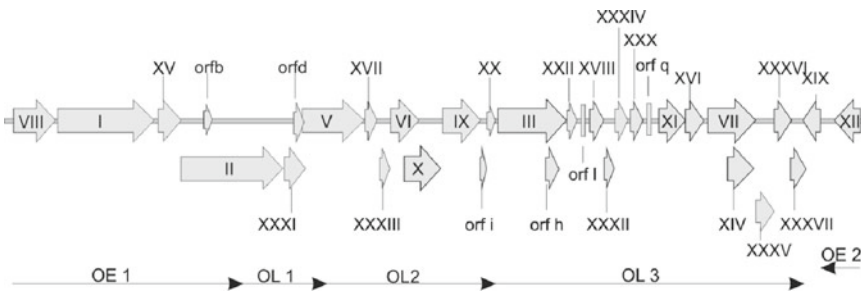


Fig. 16.4 Genome organisation of PRD1. ORFs have a roman numeral if they have been shown to encode a protein. The two early (OE) and the three late (OL) operons of PRD1 are shown at the *bottom*

membrane proteins such as P18, P7, P11 and P14 are found in the cytoplasmic membrane of the host (Mindich et al. 1982b; Rydman et al. 1999). The next observed intermediate is the procapsid which lacks the viral DNA. No other assembly intermediates have been directly observed. The procapsids are subsequently packaged with the linear dsDNA before the host cell is lysed. The cellular chaperones, GroEL and GroES, are required for the folding of the major capsid protein, although the viral

chaperone P33 can substitute for GroES (Bamford et al. 2002b; Hänninen et al. 1997). The membrane-bound P10 is also a viral chaperone aiding the formation of the procapsid, potentially by budding off phage-specific membrane patches from the cytoplasmic membrane (Rydman et al. 2001). This process could involve the interaction with the essential major and minor capsid proteins P3, P31 and P30, and the integral membrane proteins of the virion, like P16. Budding is probably thus driven by multivalent interactions and by the extensive lateral interactions of P3 (Butcher et al. 1995; Mindich et al. 1982b; Rydman et al. 2001). The soluble chaperone P17 is also known to play an essential role in particle assembly and can interact with positively charged lipids (Holopainen et al. 2000; Mindich et al. 1982b). Particle assembly coincides with additional folding of the P3 N- and C-termini from random coil into short α -helices and β -sheets, detected by Raman spectroscopy (Tuma et al. 1996a). This agrees with the comparison of the atomic models of the major capsid protein in solution (disordered termini) and in the assembled capsid where folding of the termini into different conformational states dictates the quasi-equivalent interactions in the capsid and interaction with proteins P30, P31, P16 and the membrane (Abrescia et al. 2004; Benson et al. 1999; Cockburn et al. 2004; San Martín et al. 2002). Curvature is induced into what otherwise might be a planar P3 lattice by the introduction of the vertex protein P31 and the membrane curvature is maintained at this point by the integral membrane protein P16 (Abrescia et al. 2004; Cockburn et al. 2004; Jaatinen et al. 2004). It is thought that the length of each facet is dictated by the length of the essential P30 dimer that runs down the edges of the facets, organising the P3 trimers (Abrescia et al. 2004).

What role(s) does the membrane have in assembly? One could consider it initially as an assembly platform, where the viral membrane proteins can congregate prior to capsid formation. Once the vesicle has formed, it can affect both the DNA packaging and the assembly of additional capsid proteins. Careful characterisation of the lipid content of both the host and the phage has shown that the virus membranes contain significantly more phosphatidylglycerol and less phosphatidylethanolamine than the host membranes. The distribution within the membrane leaflets is skewed as phosphatidylglycerol and cardiolipin are enriched in the outer membrane leaflet and the zwitterionic phosphatidylethanolamine is enriched in the inner one (Cockburn et al. 2004; Laurinavicius et al. 2004, 2007). The membrane also undergoes an approximately 5% expansion in the virion compared to empty, packaging ATPase P9-deficient particles (Butcher et al. 1995). It is likely that the shape and charge of the phospholipid molecules and specific lipid–viral protein interactions are the driving forces for this asymmetric distribution of phospholipids in the membrane. As there is a very close interaction with the viral DNA and the inner leaflet, this distribution may be important in condensing the DNA during packaging (Tuma et al. 1996b). The negative charge of the outer leaflet could help the assembly of P3 which has a positively charged base (Cockburn et al. 2004; San Martín et al. 2002).

The assembly of the spike proteins P5 and P2 onto the virion is dependent on the incorporation of P31 (Rydman et al. 1999). Genetically, P2 assembly is dependent on P5, as mutants in P5 lack P2 (Bamford and Bamford 2000). However, P5 and P2 do not interact in solution and image reconstruction of the vertices has shown that P5 and P2 both interact with the vertex protein P31 (Caldentey et al. 2000; Huiskonen et al. 2007; Sokolova et al. 2001). This assembly occurs prior to packaging the DNA into the procapsid.

There is evidence for a unique packaging vertex in PRD1 from antibody labelling studies and mutational studies (Gowen et al. 2003; Strömsten et al. 2003), probably lacking proteins P2, P5 and P31 (Gowen et al. 2003; Huiskonen et al. 2007). How could this anomaly occur? The unique vertex might either be the initiation point for assembly as in tailed phages, or, due to the incorporation of the membrane, it could also be the last vertex to be formed, where pinching off from the host membrane has occurred. As the unique vertex is intact in P16-, P2-, P5- and P31-deficient particles, the 11 adsorption vertices and the 1 portal vertex are functionally and structurally distinct. Hence, their assembly is apparently independent (Jaatinen et al. 2004; Strömsten et al. 2003). It has been shown that the packaging vertex includes proteins P6, P9 (the packaging ATPase) and the membrane proteins P20 and P22 (Gowen et al. 2003; Strömsten et al. 2003). The tractability of the PRD1 system

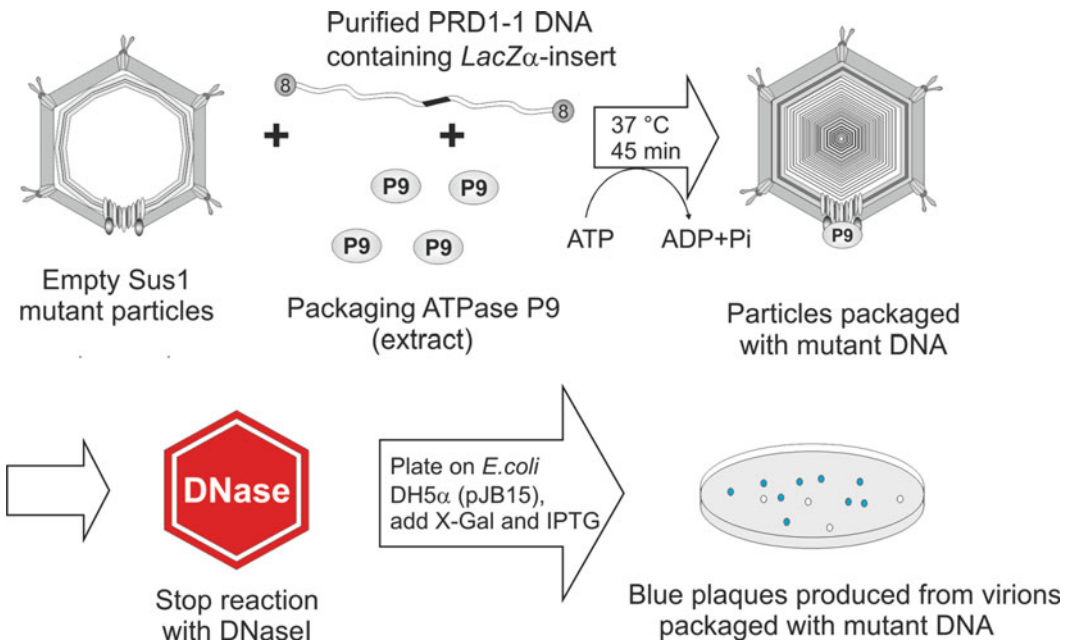


Fig. 16.5 PRD1 in vitro packaging assay

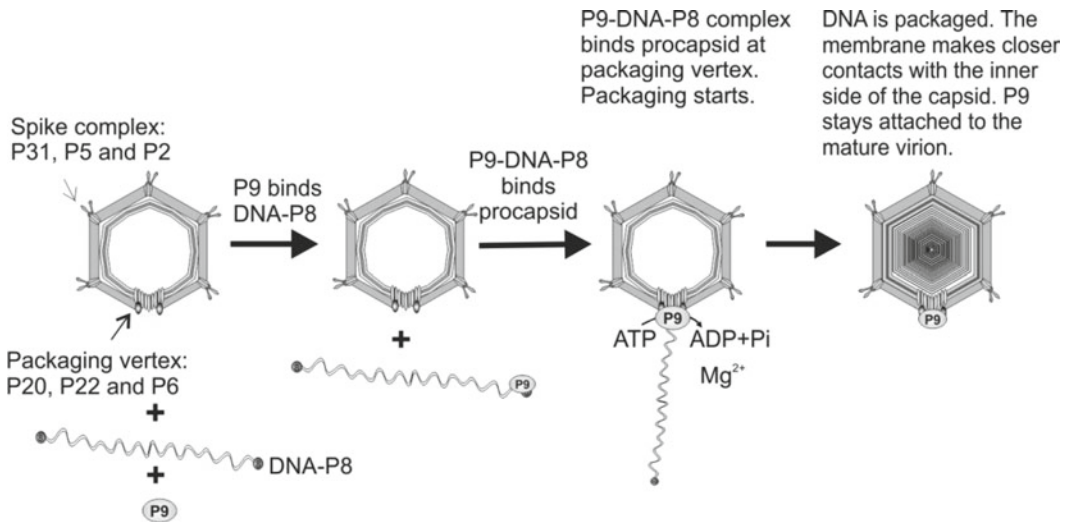


Fig. 16.6 A model for the events of PRD1 DNA packaging

as a molecular machine has been greatly aided by the setting up of an in vitro packaging system (Fig. 16.5) using purified mutant procapsids, a cell lysate containing recombinant P9 extract, and purified PRD1 DNA containing a *lacZ* marker and the covalently linked terminal proteins (Strömsten et al. 2005; Ziedaite et al. 2009). Using this system, it has been shown that the packaging ATPase can provide the energy required for DNA translocation from the breakdown of ATP, deoxy ATP and dideoxy ATP, and that it remains associated with the capsid after packaging has finished (Fig. 16.6) (Mindich et al. 1982b; Strömsten et al. 2005). In addition, sequence analysis of P9-type putative

ATPases in other viruses and mutation of conserved residues in P9 were used to identify a number of critical residues in three conserved motives that prevented packaging *in vitro* and *in vivo* complementation assays (Strömsten et al. 2003, 2005). Subsequently, it has been shown that P6 increases the packaging efficiency (Karhu et al. 2007). The genome is thought to pass through a pore in the membrane formed from the membrane proteins P20 and P22. The role of the terminal proteins in the packaging was elucidated using an optimised packaging reaction that allows the recovery of packaged particles (Ziedaite et al. 2009). PRD1 DNA with the covalently linked terminal proteins was shown to be essential for DNA packaging. Hence, it is likely that as in ϕ 29, the packaging ATPase recognises the terminal protein linked to the genome (Guo et al. 1987). One of the consequences of this packaging pathway in assembly is that the terminal protein P8 and the packaging ATPase P9 are the last proteins to be added to the assembled particle.

The first infectious particles were detected after only 45 s in the optimised packaging reaction, the minimum time that could be measured experimentally. This gives a lower estimate of the packaging rate as 340 bp/s (Ziedaite et al. 2009) in comparison to a rate of up to 170 bp/s measured for ϕ 29 using optical tweezers (Fuller et al. 2007). No large structural rearrangements are seen in the PRD1 procapsid after packaging, although there is an expansion and an increase in the order of the membrane (Butcher et al. 1995; Tuma et al. 1996b).

Like most dsDNA phages, PRD1 uses a two-component lysis (holin–endolysin) system (Mindich et al. 1982a) for host cell lysis (Fig. 16.3). The bacteriophage genome encodes a muraminidase, P15, which is virion-associated (Rydman and Bamford 2002), a holin P35 (Rydman and Bamford 2003) and two additional lysins, the proteins P36 and P37 (Krupovic et al. 2008). In concert, these proteins act on the host cytoplasmic membrane and peptidoglycans (Caldentey et al. 1994; Krupovic et al. 2008; Ziedaite et al. 2005). Monitoring of ion fluxes and the ATP content of the infected cells has revealed a sequence of lysis-related physiological changes. A decrease in the intracellular level of ATP is the earliest indicator of cell lysis, followed by the leakage of K⁺ from the cytosol approximately 20 min prior to the decrease in culture turbidity. However, the K⁺ efflux does not immediately lead to the depolarisation of the cytoplasmic membrane or leakage of intracellular ATP. These effects are only observed approximately 10 min prior to cell lysis (Ziedaite et al. 2005). The pair of accessory lysis proteins, P36 and P37, has been shown by complementation analysis to be functional analogues of the Rz and Rz1, respectively, of bacteriophage lambda. They ensure efficient disruption of the infected cell and consequent release of the phage progeny under less favourable growth conditions. A model has been proposed where complexes of P36 and P37 transform the mechanical stress caused by holin lesions at the cell membrane to the outer membrane leading to its disintegration (Krupovic et al. 2008).

16.4 Summary and Future Prospectives

There are still many open questions in the assembly of the PRD1 molecular machine. It is striking that so few intermediates in PRD1 assembly have been isolated. This really emphasises that in the future one should try to address how the capture of the viral membrane actually occurs. It is possible that there are host proteins involved in assembly, such as cell division proteins, and this area is as yet totally unexplored for PRD1. It is especially noteworthy that the particles seem to assemble in the centre of the cells, rather than at the periphery. It has become evident from extensive cryo-electron tomography work on many different bacterial cells (reviewed in Morris and Jensen 2008) that the organisation of the bacterial cytoplasm is much richer than previously thought, and that the cytoplasmic membrane can make extensive invaginations. Hence, in PRD1-infected cells perhaps the sites of DNA replication and the procapsid formation are actually juxtaposed. The role of some of the viral chaperones like P17 may actually be in remodelling the cytoplasmic membrane to allow this to

occur. One possibility to explore this would be to couple fluorescence light microscopy of green-fluorescent-protein-labelled proteins with whole cell cryo-electron tomography of infected cells, using different host cell and virus phenotypes to explore the interplay of viral and host factors in more detail.

Assembly of the particle is tightly linked to the function of delivering the genomic cargo to the next host cell. One must also bear in mind that a viral particle is a dynamic assembly, whose structure does not reveal the whole truth. Instead, many stages present in the life cycle need to be considered when linking structure to function. In PRD1, P5 probably helps to roll the virus around the surface of the cell so that the primary adsorption protein, P2, can find its target on the cell, and this is still a reversible interaction. Under laboratory conditions using laboratory hosts, P5 is not terribly significant, but in the wild P5 may be much more important. There is still a further uncharacterised interaction that leads to irreversible attachment of the particle to the host cell. Delivery of the genome probably utilises the packaging vertex, with sequential release of proteins allowing penetration of the outer membrane and peptidoglycan possibly involving the transformation of the viral membrane in to a tube (Butcher et al. 1995; Grahn et al. 2002). It has been proposed that the genome is under pressure, leading to a passive release of the genome in the final stages of entry (Cockburn et al. 2004). To understand genome release, one should look carefully at the roles of the many membrane proteins in PRD1, how they are ordered, how the tube is organised and what components are in it. One should also investigate if the translocation of DNA in to the cell is really passive like in ϕ 29 or is active like in T7 requiring transcription (Fuller et al. 2007; Garcia and Molineux 1995, 1996; Smith et al. 2001). In order to better understand both entry and packaging, the molecular organisation of the packaging vertex in capsids and the DNA should be explored, for instance, by orienting the capsids with respect to the receptor, or labelling the P9 in capsids to enable detailed structure determination without resorting to icosahedral symmetry, as has been done with, for example, MS2 and P-SSP7 (Liu et al. 2010; Toropova et al. 2011).

One can only really understand a car motor by looking under the hood. In order to really understand the detailed mechanism behind PRD1 packaging, one needs to move to single-particle experiments with laser tweezers as has been done so elegantly with ϕ 29 (Smith et al. 2001). Currently, the frequency of packaging is still probably so low that this will need a lot of patience and optimisation.

One reason that entry should be from the same vertex where packaging occurs is that we expect the linear genome has one end at least close to the entry point, topologically, it would seem most likely that the DNA would start to exit from the end that was packaged last. Hence, it may be that even the terminal protein has a crucial role in DNA entry into the cell. We would like to leave you with one last thought, how does the terminal protein P8 get into the capsid through a channel designed for dsDNA?

Acknowledgements We would like to thank all our colleagues in the greasy phage field for their useful discussions over the years. This work was supported by the Academy of Finland Centre of Excellence Programme in Virus Research (2006–2011; 1129684 to SJB). V.M. is a fellow of the VGSB.

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