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Structure of Fusarium poae virus 1 shows conserved and variable elements of partitivirus capsids and evolutionary relationships to picobirnavirus

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ABSTRACT

Filamentous fungus *Fusarium poae* is a worldwide cause of the economically important disease Fusarium head blight of cereal grains. The fungus is itself commonly infected with a bisegmented dsRNA virus from the family *Partitiviridae*. For this study, we determined the structure of partitivirus Fusarium poae virus 1 (FpV1) to a resolution of 5.6 Å or better by electron cryomicroscopy and three-dimensional image reconstruction. The main structural features of FpV1 are consistent with those of two other fungal partitiviruses for which high-resolution structures have been recently reported. These shared features include a 120-subunit $T = 1$ capsid comprising 60 quasisymmetrical capsid protein dimers with both shell and protruding domains. Distinguishing features are evident throughout the FpV1 capsid, however, consistent with its more massive subunits and its greater phylogenetic divergence relative to the other two structurally characterized partitiviruses. These results broaden our understanding of conserved and variable elements of fungal partitivirus structure, as well as that of vertebrate picobirnavirus, and support the suggestion that a phylogenetic subcluster of partitiviruses closely related to FpV1 should constitute a separate taxonomic genus.

1. Introduction

Viruses in the family *Partitiviridae* have bisegmented dsRNA genomes, with each segment packaged in a separate virus particle. They persistently infect their plant, fungus, or protozoan hosts, and routinely transmit by intracellular means such as cell division and cell-cell fusion (Ghabrial et al., 2005, 2008). In plants, they generally are not associated with disease and hence are also called cryptoviruses. Plant partitiviruses are grouped in two genera, *Alphacryptovirus* and *Betacryptovirus*, though genome sequences indicate that members of the genus *Alphacryptovirus* are phylogenetically diverse (Boccardo and Candresse, 2005; Crawford et al., 2006; Ghabrial et al., 2008; Willenborg et al., 2009). No sequences are available for the genus *Betacryptovirus*. Fungal partitiviruses are grouped in one genus, *Partitivirus*, though genome sequences indicate that members of this genus, too, are phylogenetically diverse (Boccardo and Candresse, 2005; Crawford et al., 2006; Ghabrial et al., 2008; Willenborg et al., 2009). Protozoan partitiviruses are so far restricted to the genus *Cryspovirus* (Nibert et al., 2009) and infect species of the apicomplexan genus *Cryptosporidium*, including the human pathogens *Cryptosporidium parvum* and *Cryptosporidium hominis* (Khramtsov et al., 1997; Green et al., 1999; Leoni et al., 2003).

There are four recognized families of dsRNA viruses that have mono- or bisegmented genomes enclosed by icosahedral protein capsids: *Totiviridae* (monosegmented; $T = 1$ capsid; fungus, protozoan, and possibly arthropod hosts), *Birnaviridae* (bisegmented; $T = 13$ capsid; vertebrate and arthropod hosts), *Partitiviridae* (see above; $T = 1$ capsid), and *Picobirnaviridae* (bisegmented; T = 1 capsid; vertebrate hosts). Partitiviruses encompass the smallest of these genomes, ranging in total lengths from 3.2 to 4.4 kbp (Ghabrial et al., 2005, 2008). The only picobirnavirus genome for which a full-length sequence has been reported falls within this same range, at 4.3 kbp (Wakuda et al., 2005). In comparison, the smallest reported toti- and birnavirus genomes are 4.6 and 5.9 kbp, respectively (Fauquet et al., 2005). The two genome segments of partitiviruses respectively encode the viral RNA-dependent RNA

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polymerase (RdRp) and the viral capsid protein (CP). Some partitiviruses additionally package one or more satellite segments of dsRNA that are likely not essential for replication (Oh and Hillman, 1995; Compel et al., 1998; Kim et al., 2005).

The first three-dimensional (3D) structures of partitivirus particles have been recently determined by transmission electron cryomicroscopy (cryoTEM) and 3D image reconstruction as well as by X-ray crystallography (Ochoa et al., 2008; Pan et al., 2009; Tang et al., 2010). These structures are for two distantly related members of the genus *Partitivirus* that can co-infect the saprophytic, filamentous fungus *Penicillium stoloniferum*: Penicillium stoloniferum viruses F and S (PsV-F and -S) (Bozarth et al., 1971; Buck and Kempson-Jones, 1973, 1974; Kim et al., 2003, 2005). The structures have revealed a number of distinctive features, including quasisymmetrical CP dimers, 60 of which together form the $T = 1$ capsid; domain swapping between the two subunits within the shell domain of each CP dimer; prominent surface arches, one from each dimer, formed by protruding domains; and the occurrence of diamond-shaped CP tetramers (dimers of dimers) as probable assembly intermediates. Most of these features have also been recently observed in the X-ray crystal structure of the rabbit picobirnavirus (raPBV) capsid (Duquerroy et al., 2009). Thus, given their structural similarities and despite their different host ranges, partiti- and picobirnaviruses appear likely to share a distinct evolutionary lineage relative to other dsRNA viruses.

In the current study, we advanced structural characterizations of the family *Partitiviridae* by determining the cryoTEM structure of another fungus-infecting member, Fusarium poae virus 1 (FpV1), from the genus *Partitivirus* (Fekete et al., 1995; Compel et al., 1998). This virus was originally named FUPO-1 (Compel et al., 1998), but was renamed FpV1 for better consistency with existing nomenclature for other partitiviruses (Ghabrial et al., 2005). The phytopathogenic fungal host *Fusarium poae* is a causal agent of Fusarium head blight, or scab, of cereal grains worldwide and is of considerable importance because it not only reduces crop yields but also produces mycotoxins that pose threats to humans and other animals that consume the grains from infected plants (Stenglein, 2009).

PsV-F and -S are distantly related within the same phylogenetic subcluster, whereas FpV1 belongs to a distinct subcluster and is therefore more distantly related to PsV-F and -S than those are to each other (Boccardo and Candresse, 2005; Crawford et al., 2006; Ghabrial et al., 2008; Willenborg et al., 2009) (Table 1). The fact that the level of sequence identity among the CPs of PsV-F, PsV-S, and FpV1 is quite low (pairwise identity scores between FpV1 and PsV-F or -S are respectively 13% or 14%, as compared to 19% between PsV-F and -S, in alignments with T-Coffee (Poirot et al., 2003) generated for this study) led us to expect that a structure determination for FpV1 virions may reveal unique variations on the recently described architectural designs of PsV-F and -S (Ochoa et al., 2008; Pan et al., 2009; Tang et al., 2010). In addition, the CP of FpV1 is substantially (almost 50%) larger than those of PsV-F and -S (Table 1), suggesting that its structure may include other distinctive features with specific functions.

Table 1
Recognized viruses in the genus *Partitivirus* with sequenced genomic RNA(s).[a]

Subcluster[b]	Virus name (abbrev.)[c]	dsRNA1 (bp)	dsRNA2 (bp)	RdRp (aa, kDa)	CP (aa, kDa)
1	AoV	1754	1555	539, 62	433, 47
	DdV1	1787	1585	539, 62	436, 48
	DdV2	1781	1611	539, 62	442, 50
	FsV1	1645	1445	519, 60	413, 44
	GaV-MS1	1782	1586	539, 61	443, 47
	OPV1	1744	1567	539, 63	430, 46
	PsV-F	**1677**	**1500**	**538, 62**	**420, 47**
	PsV-S	**1754**	**1582**	**539, 62**	**434, 47**
2	AhV	2180	2135	665, 78	652, 74
	CrV1	2207	2305	663, 77	661, 73
	FpV1	**2203**	**2185**	**673, 78**	**637, 70**
	HaV	2325	nd	734, 87	nd
	HmV	2247	nd	706, 83	nd
	PoV1	2296	2223	706, 82	636, 71
	RhsV-717	2363	2206	730, 86	683, 76
	RnV1	2299	2279	709, 84	686, 77

[a] Viruses recognized as species by the International Committee on Taxonomy of Viruses.
[b] As defined in previous papers (see Section 1. Introduction).
[c] Virus names and Genbank numbers: AoV, Aspergillus ochraceous virus (EU118277 and EU118278); DdV1, Discula destructiva virus 1 (AF316992 and AF316993); DdV2, Discula destructiva virus 2 (AY033436 and AY033437); FsV1, Fusarium solani virus 1 (D55668 and D55669); GaRV-MS1, Gremmeniella abietina RNA virus MS1 (AY089993 and AY089994); OPV1, Ophiostoma partitivirus 1 (AM087202 and AM087203); PsV-F, Penicillium stoloniferum virus F (AY738336 and AY738337); PsV-S, Penicillium stoloniferm virus S (AY156521 and AY156522); AhV, Atkinsonella hypoxylon virus (L39125 and L39126); CrV1, Ceratocystis resinifera virus 1 (AY603051 and AY603052); FpV1, Fusarium poae virus 1 (AF015924 and AF047013); HaV, Heterobasidion annosum virus (AAL79540); HmV, Helicobasidium mompa virus (BAD32677); PoV1, Pleurotus ostreatus virus 1 (AY533036 and AY533038); RhsV-717, Rhizoctonia solani virus 717 (AF133290 and AF133291); and RnV1, Rosellinia necatrix virus 1 (AB113347 and AB113348). Viruses for which 3D structures have been determined are bolded.

2. Materials and methods

2.1. Fungal culture and purification of FpV1 virions

A culture of *F. poae* strain A-11 (Compel et al., 1998) was kindly supplied by László Hornok (University of Agricultural Sciences, Gödöllo, Hungary). This fungus was then maintained in the lab on slants of potato dextrose agar containing 0.5% yeast extract. For purification of FpV1 virions, a protocol was devised from one previously reported for Penicillium chrysogenum virus (Jiang and Ghabrial, 2004). *F. poae* was grown in a stationary culture containing potato dextrose broth supplemented with 0.5% yeast extract. Mycelium was harvested from a 7-day culture by straining through miracloth in a Buchner funnel and homogenized in 0.1 M sodium phosphate, pH 7.4, containing 0.2 M KCl and 0.5% mercaptoethanol in a Waring blender. The buffer was used at a rate of 3 ml/g of wet mycelium. The homogenate was clarified by emulsification with an equal volume of chloroform and the emulsion was broken by centrifugation at 5000 rpm in a Beckman JA-14 rotor. The upper aqueous layer was then subjected to two cycles of differential centrifugation with all high-speed pellets suspended in the 0.1 M phosphate buffer, pH 7.4. The first cycle consisted of ultracentrifugation for 2.5 h at 27,000 rpm in a Beckman Type-30 rotor followed by centrifugation at 10,000 rpm for 10 min in a Beckman JA-20 rotor. The resultant supernatant was then centrifuged at 40,000 rpm for 1.5 h in a Beckman 50Ti rotor. These second high-speed pellets were resuspended in the same phosphate buffer and centrifuged at 10,000 rpm for 10 min in a Beckman JA-20 rotor. Final purification was performed by rate zonal centrifugation in sucrose gradients (100–400 mg/ml) made in 0.1 M sodium phosphate, pH 7.4. The gradients were centrifuged at 24,000 rpm for 2.5 h in a Beckman SW28 rotor and the UV-absorbing band was collected by puncturing the tube with a syringe. This harvested sample was diluted 1:1 with the sodium phosphate buffer and centrifuged at 40,000 rpm in a Beckman 50Ti rotor. The final pellet was suspended in buffer A (0.05 M Tris–HCl, pH 7.6, containing 150 mM NaCl and 5 mM EDTA), and stored at 4 °C for short periods before analysis.

2.2. CryoTEM and 3D image reconstruction of FpV1

Small aliquots (~2–3 μl) of purified FpV1 virions were vitrified, and cryoTEM image data were recorded essentially as described for