Structure, Vol. 11, 605–614, June, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0969-2126(03)00099-6

Previews

CPV, a Stable and Symmetrical Machine for mRNA Synthesis

The structure of cytoplasmic polyhedrosis virus (CPV), an insect pathogen from the *Reoviridae* family of double-strand RNA viruses, has been determined at 8 Å by electron cryomicroscopy and image reconstruction. It provides new information about the functions of these viral particles as stable machines for mRNA synthesis.

Viral particles are widely understood to contain the "machinery" needed to invade cells. Polymerases are machine-like as well, and many RNA viruses must incorporate their RNA-dependent RNA polymerases (RdRps) into infectious virions so that the viral genomic RNA can be transcribed into mRNA early in infection. This is true for viruses with dsRNA genomes in the *Reoviridae* family, which infect a wide range of hosts, from plants to people. A notable detail is that the RdRps of these viruses, as well as their accessory enzymes for adding a dimethylated cap ($^{7N^m}GpppN^{2'O^m}$) to the 5′ end of each mRNA, are regularly arranged within particles through associations with the icosahedrally symmetrical viral capsids. Moreover, the transcription mechanisms of these viruses dictate that the icosahedral particles remain intact throughout repeated transcription cycles: the genomic dsRNA templates, RdRps, and other enzymes remain inside the particles, while the mRNA products are cotranscriptionally capped and released (Figure 1). Efforts to understand the structures and functions of these particles therefore promise to inform us about mRNA synthesis and transport mechanisms in general.

The structure of cytoplasmic polyhedrosis virus (CPV), an insect pathogen from the *Reoviridae* family, is reported in this issue of *Structure* (Zhou et al., 2003). This is the most recent in a series of informative articles by Zhou and colleagues describing CPV at progressively higher resolutions by electron cryomicroscopy and image reconstruction (cryoEM). The current structure is reported at 8 Å, up from 13 Å earlier this year (Xia et al., 2003) and 25 Å in 1999 (Zhang et al., 1999). A 25 Å cryoEM reconstruction was also reported in 1999 by Stuart and colleagues (Hill et al., 1999). This progress with CPV, as with many other viruses being studied by this approach, reflects recent improvements in electron cryomicroscopes as well as in data collection and processing methods (e.g., Liang et al., 2002). Increasing automation, allowing larger and larger numbers of particle images to be added to each reconstruction, is one crucial factor.

At a resolution of 8 Å, α helices and β sheets become distinguishable in the CPV structure and allow Zhou et al. to make deductions about subunit boundaries, domain organization, and folding patterns. They also use published X-ray crystal structures of homologous proteins from two other *Reoviridae* members, mammalian orthoreovirus (Reinisch et al., 2000) and bluetongue orbivirus (Grimes et al., 1998), to fit into the CPV cryoEM map, which allows them to draw further conclusions about important features of CPV. Such combinations of results from cryoEM and X-ray crystallography have gained increasing acceptance in recent years and hold continued promise for providing new information about the structures and functions of large macromolecular complexes, including, but not restricted to (e.g., Frank, 2001), viral particles.

Zhou et al. emphasize points about the structure and stability of the CPV capsid and the pentameric turrets that project above it in the particle (Figure 1). Using the crystal-derived model of the bluetongue orbivirus inner capsid protein (Grimes et al., 1998), they are able to dissect subunit boundaries in the CPV shell and to propose a believable model for formation of a complete T = 1 capsid from 120 copies of the CPV shell protein. This unusual arrangement, with each asymmetric unit comprising two shell subunits, is common to all *Reoviridae* members examined to date (e.g., Reinisch et al., 2000; Grimes et al., 1998) as well as to dsRNA viruses from the *Cystoviridae* (bacteriophage ϕ6) and *Totiviridae* (L-A virus of yeast) families (Butcher et al., 1997; Naitow et al., 2002). The authors attribute the marked stability of CPV particles to several factors, including not only the turret and nodule proteins, but also a unique small protrusion domain within the shell protein itself, which contributes extra contacts between these subunits, which are not found in other *Reoviridae* members (e.g., Reinisch et al., 2000; Grimes et al., 1998). Using the crystal-derived model of mammalian orthoreovirus turret protein (Reinisch et al., 2000), they are able to identify three domains in the CPV turret protein that probably correspond to the mRNA capping enzyme domains—guanylyltransferase, *7N*-methyltransferase, and *2′O*-methyltransferase—of the orthoreovirus protein. Thus, placement of these enzymes within external turret structures, so that nascent mRNAs can be capped as they exit particles, may be common to many (though clearly not all [e.g., Grimes et al., 1998]) *Reoviridae* members.

The most speculative discussion by Zhou et al. concerns the constricted protein diaphragms, each similar to a camera iris, which surround each 5-fold axis of the CPV particle. One constriction is formed by the five surrounding shell subunits. Two more constrictions are formed by the five turret subunits, one constriction near the bottom and another near the middle of each turret. The authors propose that these diaphragms may open and close during the course of mRNA synthesis to regulate the flow and processing of each mRNA as it exits the particle along that path. This proposal is reasonable and similar to propsals made for other *Reoviridae* members (e.g., Reinisch et al., 2000; Grimes et al., 1998), but it requires further experimental support to be taken as gospel.

Despite the recent progress in understanding CPV structure and functions, several key questions remain

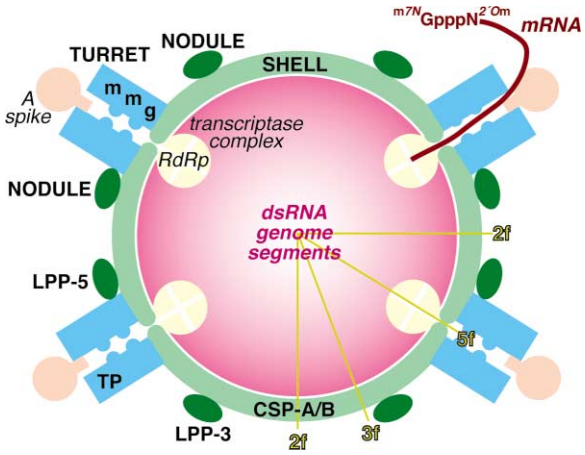

Figure 1. Schematic of the Cytoplasmic Polyhedrosis Virus (CPV) Particle, an mRNA Synthesis Machine

The CPV shell, turret, and nodule proteins were visualized at 8 Å by Zhou et al. (labels in bold). The abbreviations for these proteins used by Zhou et al. are included at bottom left. The shell protein (light green; 60 dimers per particle) forms a complete T = 1 capsid that surrounds the viral genome (ten linear segments of dsRNA). The turret protein (cyan; 12 pentamers per particle) and nodule protein (dark green; 120 monomers per particle at two different symmetry positions) bind to the outer surface of the shell. The A spike and transcriptase complexes were not visualized at 8 Å (labels in italics) but have been localized to these approximate positions by previous, lower-resolution studies. The A spike (light pink; probably 12 per particle) binds atop each turret. The viral transcriptase complexes (light yellow; probably 10–12 per particle), including the RNA-dependent RNA polymerase (RdRp), are thought to bind to the inner surface of the shell, at or near each icosahedral 5-fold axis (5f). Positions of icosahedral 2-fold (2f) and 3-fold (3f) axes are also shown. Nascent viral mRNA molecules, as they are synthesized by each transcriptase complex (only one mRNA is illustrated, with a completed cap at its 5′ end [dark red]), are proposed to be exported through a series of diaphragm-like channels, which can open and close, along each 5-fold axis through the shell and turret. Domains in the turret protein are proposed to mediate the last three reactions in capping—guanylyltransferase (g) and two methyltransferases (m)—before the 5′ end of the mRNA is released through the top of the turret.

unanswered. One obvious question is whether the turret domains indeed mediate the mRNA capping activities proposed by Zhou et al. More important is the question of exactly where and in which orientation the CPV RdRps are positioned within particles. In fact, the RdRps have yet to be visualized at high resolution in particles of any *Reoviridae* member, although, in several previous reports (including reports on CPV), the transcriptase complexes appear to be anchored near each of the 5-fold axes, on the inner side of the T = 1 shell (e.g., Xia et al., 2003; Zhang et al., 1999; Hill et al., 1999; Reinisch et al., 2000; Grimes et al., 1998) (Figure 1). To extend our understanding of these mRNA synthesis machines, we need to fit their most pivotal components, the RdRps, into the particle structures. This should allow us to define, with greater confidence, the paths traversed by the template and product RNAs as they move within, and exit from, the particles during mRNA synthesis. Since mammalian orthoreovirus is the only *Reoviridae* member for which X-ray crystal structures of the RdRp, the T = 1 shell, and the mRNA capping turrets have all been reported (Reinisch et al., 2000; Tao et al., 2002), it would seem to be an especially good virus with which to strive for this advance. Other unanswered questions include (1) whether the particles of some or all *Reoviridae* members contain RNA helicase-like proteins (e.g., Bisaillon et al., 1997; Stauber et al., 1997), which might play one or more roles in transcription and (2) how CPV and other *Reoviridae* members may manage the supercoiling of dsRNA templates that accompanies transcription.

Another intriguing feature of CPV and other cypoviruses is that they lack an icosahedral outer capsid, which other *Reoviridae* members contain. Instead, during assembly in infected cells, the single-shelled CPV particles are embedded within an inclusion body, or polyhedrin. This body, which is largely composed of one protein (polyhedrin protein), is thought to protect and stabilize the particles once they are released from the host or once the host dies. After ingestion by the next victim, the polyhedrin is disrupted by alkaline conditions in the insect midgut, and the embedded particles are freed to infect intestinal cells. But here a question arises. Since the outer capsids of other *Reoviridae* members contain proteins that mediate key steps in cell entry (e.g., attachment and membrane penetration), how does CPV survive without an outer capsid? In fact, CPV has a protein, forming the A spikes, that sits atop the turrets (Xia et al., 2003; Hill et al., 1999) and is suggested by Zhou et al. to fulfill a cell attachment function. Transcription by CPV may require loss of the A spikes to open the top of the mRNA exit channels (Hill et al., 1999). Like the CPV transcriptase complexes, the A spikes are not visualized at 8 Å in the reported structure, perhaps because the protein subunits are flexible or otherwise positioned asymmetrically. Thus, studies of the A spikes at higher resolutions should be another lucrative avenue for future work. We speculate that the A spikes may also play a role in membrane penetration, allowing a CPV particle to cross the membrane barrier during cell invasion.

Given the diversity of insects in the natural world, it seems likely that we have only begun to identify the diversity of viruses that infect them. CPV provides an excellent example of how an arguably obscure biological system can offer new opportunities to study problems of general interest.

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Structure, Vol. 11, June, 2003, DOI 10.1016/S0969-2126(03)00101-1

Complementing Thymidylate Synthase

The structure of thymidylate synthase complementing protein with substrates dUMP and FAD, presented in this issue of *Structure*, sheds light on a fascinating new catalytic mechanism, suggests a strategy for the design of new antimicrobial compounds, and highlights the promise of proteomics in medicine.

Thymidylate synthase (TS) has a rich history in the biochemical and biomedical worlds. Researchers have pursued TS for over 40 years to gain an understanding of many topics, including folate metabolism, enzyme mechanism, protein evolution, ligand-induced conformational changes, and the magic of enzyme-based chemistry. The medical community has long turned to TS inhibitors as a possible source of drugs, primarily for treating cancer, and TS-targeted drugs are a staple of current chemotherapeutic regimes and an active area of research.

TS catalyzes the conversion of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (dTMP), which entails the exchange of the 5-hydrogen on the dUMP pyrimidine ring for a methyl group contributed by cofactor 5,10-methylenetetrahydrofolate (MTF). Most articles discussing TS contain a statement in the opening paragraph along the lines of "thymidylate synthase provides the sole de novo means for synthesizing dTMP." However, the recent explosion in genomic and proteomic data has revealed that TS is not essential for life: numerous bacterial and archeal species, and at least one eukaryotic specie (slime mold), do not have a gene for TS and instead make thymidine with a newly discovered protein called thymidylate synthase complementing protein (TSCP), or Thy1 or ThyX (Myllykallio et al., 2002). Like TS, TSCP makes use of MTF as a one-carbon donor. However, where TS uses MTF for both carbon transfer and reduction, TSCP makes use of a tightly bound molecule of flavin adenine dinucleotide (FAD) and a reduced pyridine nucleotide (NADH or NADPH) for reduction.

The initial characterization of TSCP was through the confluence of parallel studies in microbial genomics, where analyses revealed that the gene for TS was often missing and apparently replaced by TSCP (Myllykallio et al., 2002), and in structural genomics, where efforts devoted to uncovering all of the protein folds in the model organism *T. maritima* (Lesley et al., 2002) led to the initial structure of TSCP (Kuhn et al., 2002). These studies made clear that TSCP was structurally and evolutionarily unrelated to TS, but nonetheless used dUMP and MTF as substrates. The new report by Kuhn and coworkers begins to uncover the mechanism by which TSCP catalysis occurs, through crystallographic studies with substrates and cofactors (Mathews et al., 2003).

The chemistry of one-carbon donation is difficult to achieve nonenzymatically. In TS, this is accomplished through a multistep mechanism whereby the homodimeric protein wraps around both substrates, covalently links to dUMP through an active site cysteine, and induces first methylene transfer and then hydride reduction from MTF, ultimately producing dihydrofolate and thymidylate. Structures of homotetrameric TSCP now exist for complexes with FAD and dUMP and provide insight into the reductive portion of the reaction. The FAD is well bound by the protein, with contacts for each FAD moiety by three of the four subunits. Binding of dUMP positions the pyrimidine ring such that it stacks with the flavin ring of FAD in an arrangement reminiscent of the presumed reductive complex between tetrahydrofolate and the exocyclic methylene intermediate of dUMP in TS (Fritz et al., 2002; Hyatt et al., 1997). Earlier steps in the reaction remain unclear, however. The means by which MTF is brought into the binding pocket is not yet known, but may require the flavin moiety to move out of the binding pocket. Likewise, reduction of FAD by NAD(P)H may also require such movement. Furthermore, a potential active site nucleophile, SER 88, has been identified, but a change in dUMP orientation would be required for nucleophilic addition to occur.