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Review

Cystoviral RNA-directed RNA polymerases: Regulation of RNA synthesis on multiple time and length scales

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ABSTRACT

P2, an RNA-directed RNA polymerase (RdRP), is encoded on the largest of the three segments of the double-stranded RNA genome of cystoviruses. P2 performs the dual tasks of replication and transcription *de novo* on single-stranded RNA templates, and plays a critical role in the viral life-cycle. Work over the last few decades has yielded a wealth of biochemical and structural information on the functional regulation of P2, on its role in the spatiotemporal regulation of RNA synthesis and its variability across the *Cystoviridae* family. These range from atomic resolution snapshots of P2 trapped in functionally significant states, in complex with catalytic/structural metal ions, polynucleotide templates and substrate nucleoside triphosphates, to P2 in the context of viral capsids providing structural insight into the assembly of supramolecular complexes and regulatory interactions therein. They include *in vitro* biochemical studies using P2 purified to homogeneity and *in vivo* studies utilizing infectious core particles. Recent advances in experimental techniques have also allowed access to the temporal dimension and enabled the characterization of dynamics of P2 on the sub-nanosecond to millisecond timescale through measurements of nuclear spin relaxation in solution and single molecule studies of transcription from seconds to minutes. Below we summarize the most significant results that provide critical insight into the role of P2 in regulating RNA synthesis in cystoviruses.

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Abbreviations: ATP, adenosine triphosphate; BCS, binding-competent state; BOS, binding-occluded state; BVDV, bovine viral diarrhea virus; CTD, C-terminal domain; DdRP, DNA-directed RNA polymerase; DdDP, DNA-directed DNA polymerase; dsRNA, double-stranded RNA; DTD, dwell-time distribution; FMDV, foot-and-mouth disease virus; GTP, guanosine triphosphate; HCV, hepatitis C virus; HFC, high-fidelity conformation; HIV, human immunodeficiency virus; LFC, low-fidelity conformation; MD, molecular dynamics; NMR, nuclear magnetic resonance; NTP, nucleoside triphosphate; NTPase, nucleoside triphosphatase; PC, procapsid; PX, polymerase complex; RdRP, RNA-directed RNA polymerase; RT, reverse transcriptase; ssRNA, single-stranded RNA; TMC, terminal-base mismatched conformation; TNTase, terminal nucleotidyltransferase.

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1. Introduction

The *Cystoviridae* (Φ6 – Φ14, Φ2954 and ΦNN) comprise a family of lipid-enveloped viruses that contain double-stranded RNA (dsRNA) genomes. All members of this family exclusively infect Gram-negative bacteria, notably many strains of the plant pathogen *Pseudomonas syringae*. Some members of the family are also capable of targeting *Escherichia coli* or *Salmonella* (Mindich et al., 1999). The archetypal cystovirus, Φ6, was first isolated from bean straw infested with *Pseudomonas syringae* pv. *phaseolicola* in 1973 by Vidaver et al. (Vidaver et al., 1973), following which, eight additional members, Φ7-14, were identified and characterized by Mindich et al. (Mindich et al., 1999). In addition, Φ2954 that showed significant similarities to Φ12 was isolated and characterized by Qiao et al. (2010b). While all members (though not officially classified as such by the International Committee on Taxonomy of Viruses; Φ6 remains the only official member) of the *Cystoviridae* family share similar overall genetic organization and structural morphology, the level of sequence conservation within their genomes and encoded proteins, are weak at best. Based on the phylogenetic closeness to Φ6, the first discovered and most extensively studied cystovirus, the family may be broadly classified into two sub-groups. The first sub-group, comprising Φ7, Φ9, Φ10 and Φ11, are closely related to Φ6, while the second sub-group comprising, Φ8, Φ12, Φ13, Φ14 and Φ2954 are more distant from Φ6 (Mindich et al., 1999; Qiao et al., 2010b). Recently, a new member of the *Cystoviridae* family ΦNN with strong genomic similarity to Φ6 was isolated from a Finnish lake (Mäntynen et al., 2015).

Φ6 has been a key player in the development of many aspects of molecular virology including elucidation of detailed mechanisms of capsid assembly (Poranen et al., 2001; Poranen and Tuma, 2004; Poranen et al., 2005; Poranen and Bamford, 2012) and genome packaging (Frilander and Bamford, 1995; Qiao et al., 1997b; Mindich, 1999a; Mindich, 2004; Mindich, 2012). It was also the first RNA virus for which reverse genetics was developed (Olkkonen et al., 1990; Mindich, 1999b). Additionally, Φ6 is still considered to be an ideal model system to study the mechanisms of replication in RNA viruses, most notably the *de novo* initiation (see below) of viral RNA synthesis (Butcher et al., 2001; Laurila et al., 2002). Since Φ6 remains the prototypical and most thoroughly investigated cystovirus in terms of structure, biochemistry and function, we will use it as our point of reference for most discussions in this review, occasionally referring to other cystoviruses wherever additional information is available.

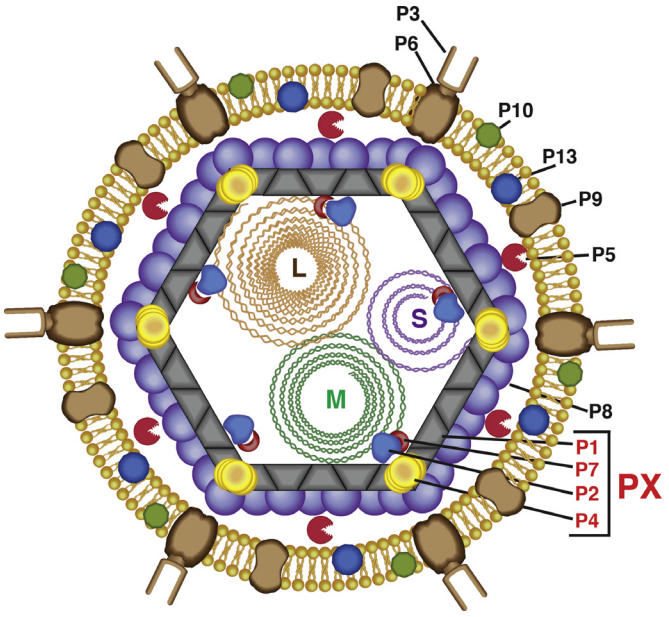

Fig. 1. Schematic representation of the structural organization of a mature cystovirus virion. The membrane proteins that include the major envelope protein, P9 (90 residues in Φ6), an envelope protein, P10 (42 residues), and a minor membrane protein, P13 (72 residues) are associated with the outer lipid envelope. Exposed to the extracellular medium, is the receptor-binding spike protein, P3 (648 residues) that forms a complex with the fusogenic membrane protein, P6 (167 residues), is able to interact specifically with the type IV pili of the bacterial host in the case of Φ6 (and Φ2954). In some cystoviruses, e.g. in Φ8, P3 comprises of two separate proteins P3a and P3b. The outer protein capsid is formed by 200 trimers of the major outer capsid protein, P8 (149 residues) assembled on a T = 13 lattice. Not all of all the cystoviruses contain a P8 shell e.g. Φ8. The lytic protein P5 (220 residues) that is involved in the degradation of the peptidoglycan layer during internalization into the host cytoplasm is found in the space between the lipid and outer protein layers. The inner protein capsid is formed from asymmetric dimers of the inner capsid protein, P1 (769 residues) arranged on a T = 1 lattice. Associated with the P1 shell are three other proteins all encoded on the genomic L-segment: an RNA-directed RNA polymerase, P2 (664 residues), a hexameric packaging NTPase, P4 (332 residues) and an accessory protein, P7 (161 residues). The proteins P1, P2, P4 and P7 assemble to form the polymerase complex (PX). The three segments, large (L, 6.4 kbp), medium (M, 4.1 kbp) and small (S, 2.9 kbp) of the double-stranded RNA genome are enclosed within the P1 shell. The L-segment encodes for the P1, P2, P4 and P7 proteins, the M-segment encodes for the P3, P6, P10 and P13 proteins; the S-segment encodes P5, P8 and P9 proteins.

2. Organization of the cystovirus virion

All cystoviruses possess similar overall organization with three structural layers (Fig. 1). The outermost layer that is comprised of a combination of phospholipids, extracted from the host (Laurinavičius et al., 2004), houses several integral membrane proteins (P9, P10 and P13) (Gottlieb et al., 1988). This layer also contains the receptor binding spike protein P3, that is critical for interactions with the host and P6, another membrane protein, that mediates fusion of the viral envelope with the bacteria outer membrane (Bamford et al., 1987). Located inside this membrane vesicle,

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