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Review

Picornaviral polymerase structure, function, and fidelity modulation

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ABSTRACT

Like all positive strand RNA viruses, the picornaviruses replicate their genomes using a virally encoded RNA-dependent RNA polymerase enzyme known as 3D^{pol}. Over the past decade we have made tremendous advances in our understanding of 3D^{pol} structure and function, including the discovery of a novel mechanism for closing the active site that allows these viruses to easily fine tune replication fidelity and quasispecies distributions. This review summarizes current knowledge of picornaviral polymerase structure and how the enzyme interacts with RNA and other viral proteins to form stable and processive elongation complexes. The picornaviral RdRPs are among the smallest viral polymerases, but their fundamental molecular mechanism for catalysis appears to be generally applicable as a common feature of all positive strand RNA virus polymerases.

Contents

Abbreviations: 3D^{pol}, picornavirus RNA-dependent RNA polymerase; CTP, cytosine triphosphate; CV, coxsackievirus B3; EC, polymerase-RNA elongation complex; EMCV, encephalomyocarditis virus; EV71, enterovirus 71; FMDV, foot-and-mouth disease virus; HCV, hepatitis C virus; HRV, human rhinovirus; IRES, internal ribosome entry site; NMR, nuclear magnetic resonance; NTP, nucleotide triphosphate; PDB, protein databank; PV, poliovirus; RdRP, RNA-dependent RNA polymerase; UMP, uracil monophosphate; UTR, untranslated region.

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1. Introduction

Positive strand RNA viruses provide their own genome replication machinery *via* an RNA-dependent RNA polymerase (RdRP) protein, allowing them to replicate without a DNA intermediate. For the picornaviruses the RdRP is $3D^{pol}$, a ≈460 residue protein found at the very C-terminal end of the ~250 kDa viral polyprotein. The core function of $3D^{pol}$ is genome replication, but the protein also functions as part of the $3CD^{pro}$ precursor to modulate protease domain specificity and binding to RNA sequences that control virus replication. Notably, the picornaviral $3CD^{pro}$ proteins have no polymerase activity, which is elegantly activated only upon proteolytic processing to generate the mature $3D^{pol}$ enzyme.

The first positive strand RNA virus RdRP structures were solved in the late 1990′s with a partial poliovirus polymerase structure (Hansen et al., 1997) that was followed by a pair of hepatitis C virus polymerase structures (Bressanelli et al., 1999; Lesburg et al., 1999), the caliciviral rabbit hemorrhagic disease virus polymerase structure (Ng et al., 2002), and then the essentially simultaneous publication of complete picornaviral polymerase structures from foot and mouth disease virus, three rhinovirus strains, and poliovirus (Ferrer-Orta et al., 2004; Love et al., 2004; Thompson and Peersen, 2004). Since that time, a plethora of RdRP structures have been solved as isolated proteins, in proteolytic precursor forms, and in complexes with other viral proteins, RNA, and nucleotides. Together, these structures highlight the strong conservation of structural and functional elements needed for replicative polymerase activity. The picornaviral $3D^{pol}$s are the smallest positive strand RNA virus polymerases, yet their highly conserved structure is found at the core of many larger viral RdRPs. The picornaviral polymerases are also proving to be excellent systems for studying the molecular mechanisms underlying catalysis and nucleotide selection, providing insights that pertain to most (+) strand RNA virus polymerases.

2. Genome architecture and polymerase biochemistry

2.1. Genome structure

The picornaviruses currently encompass 54 species grouped into 31 genera (www.picornaviridae.com) and have 7–8 Kb long single stranded RNA genomes. Using the enteroviruses as an example (Fig. 1A), the viral genome begins with a 5′ UTR composed of a ~100 nt long RNA cloverleaf structure followed by a ~700 nt long IRES, the highly structured RNA that directly recruits ribosomes for viral protein translation (Fitzgerald and Semler, 2009). Next, a 6500–7200 nt open reading frame encodes for a single 240–260 kDa polyprotein that is ultimately cut into 11–13 different proteins by the viral $2A^{pro}$, $3C^{pro}$, and $3CD^{pro}$ proteases. Note that there are some differences in the genome organization and resulting proteomes among the different picornaviral genera (Palmenberg et al., 2010). The most common replaces the 2A protease with a leader protease (L) found at the very beginning of the polyprotein, and some viruses have multiple tandem copies of the 3B (*i.e.* VPg) peptide (Gorbalenya and Lauber, 2010). The pathways for polyprotein processing are temporally complex and generate multiple intermediate species that provide functions and interactions somewhat different from the fully processed proteins, expanding the biochemical diversity arising from the rather small genome. These processing pathways will not be discussed here except for their role in activating $3D^{pol}$ function upon cleavage of the $3CD^{pro}$ precursor protein. Finally, there is a short ~300 nt 3′ UTR that includes a pair of RNA stem-loops followed by a 25–100 nt long poly(A) tail.

The picornaviral genomes also contain an important internal regulatory element, the RNA stem-loop structure known as *cre* (oriI). *Cre* templates the addition of two uracils onto the hydroxyl group of Tyr3 on the viral 3B protein, resulting in the VPg-pUpU primer used for all RNA synthesis by $3D^{pol}$ *in vivo* (Paul et al., 2000). The *cre* secondary structure as an RNA stem with a 14-nt loop containing two key adenosines is conserved among picornaviruses, but its exact sequence and location within the genome vary greatly (Yang et al., 2002). Lastly, the poliovirus genome also contains a pair of RNA hairpin elements called α and β that are important for proper RNA synthesis during virus infection (Burrill et al., 2013; Song et al., 2012). These 75-nucleotide segments are located within the $3D^{pol}$ coding region, where α encompasses residues 341–356 within $3D^{pol}$ folding motif D and β encompasses residues 416–440 within the thumb domain. They are conserved among the group C enteroviruses, but their importance for replication beyond poliovirus is not yet known.

2.2. $3D^{pol}$ biochemistry

Initiation of RNA synthesis during normal virus replication always uses a tyrosine residue on the viral 3B (VPg) protein, but in an *in vitro* context with purified components $3D^{pol}$ is capable of initiation on RNA primer-template pairs and RNA hairpin structures (Fig. 1B). This ability to efficiently initiate *in vitro* has enabled detailed studies of RNA binding, initiation kinetics, and subsequent RNA elongation steps. Overall, the data from these experiments have shown that $3D^{pol}$ forms a very stable and highly processive elongation complex with a well defined catalytic cycle. We now have a very good biochemical and structural understanding of the steps involved in each cycle of nucleotide incorporation (Fig. 1B,C).

RNA binding takes on the order of a minute with a dissociation constant in the low micromolar range when studied as purified components in solution at moderate 75–100 mM monovalent salt concentrations (Arnold and Cameron, 2004; Gohara et al., 2000; Mestas et al., 2007). The primer can be either RNA or DNA *in vitro*, but the reaction is much less efficient with a DNA primer. This can be overcome by using terminal transferases to add 2–3 ribonucleotides to the 3′ end of the DNA primer (Svensen et al., 2016). Following initial RNA binding there is a conformational change in the $3D^{pol}$-RNA complex that is necessary before the first nucleotide can be added to the RNA primer. This change occurs with a time constant of ≈13 s and it thus takes about a minute for an entire population to convert to the catalytically competent state (Arnold and Cameron, 2000). Notably, the $3D^{pol}$-RNA complex becomes more stable as a result of this conformational change, resulting in a half-life of ≈1 h. Incorporation of 1–4 nucleotides further stabilize the complex, increasing its half life to ≈4 h or more and resulting in the highly processive elongation complex (EC) that is capable of replicating genome length RNAs without dissociating. Stalled ECs remain functional for hours on the bench (Arnold and Cameron, 2000), including in high salt concentrations that eliminate RNA rebinding (Hobdey et al., 2010), and they are stable for over a week at 16 °C as purified complexes for crystallization (Gong and Peersen, 2010). Notably, this high stability has allowed us to purify and crystallize multiple stalled elongation complexes, leading to structures that have provided tremendous structural insights into the $3D^{pol}$ catalytic cycle (Gong et al., 2013).

Replicative polymerases share a catalytic mechanism in which two magnesium ions are locked into the active site *via* interactions with aspartic acid residues on the protein, the priming nucleotide 3′ hydroxyl group, and the NTP triphosphate (Steitz, 1998). The magnesium ions stabilize the transition state and help deprotonate the primer 3′ hydroxyl group, setting it up for a nucleophilic attack on the NTP alpha phosphate. The picornaviral $3D^{pol}$ enzymes utilize this highly conserved mechanism and structural framework for catalysis, but notably they arrive at the final catalytic conformation by molecular motions that are distinct from those used by other sin-

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