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Structure-Function Relationships Among RNA-Dependent RNA Polymerases

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Abstract

RNA-dependent RNA polymerases (RdRPs) play key roles in viral transcription and genome replication, as well as epigenetic and post-transcriptional control of cellular gene expression. In this article, we review the crystallographic, biochemical, and molecular genetic data available for viral RdRPs that have led to a detailed description of substrate and cofactor binding, fidelity of nucleotide selection and incorporation, and catalysis. It is likely that the cellular RdRPs will share some of the basic structural and mechanistic principles gleaned from studies of viral RdRPs. Therefore, studies of the viral RdRP establish a framework for the study of cellular RdRPs, an important yet understudied class of nucleic acid polymerases.

1 Introduction

Under physiological conditions, RNA-dependent RNA polymerases (RdRPs) catalyze the formation of phosphodiester bonds between ribonucleotides in an RNA template-dependent fashion. RdRPs have been found primarily in RNA viruses. In some cases, these enzymes are virion associated; in others, these enzymes are nonstructural proteins located in the cytoplasm but, on occasion, are located in the nucleus. In viral systems, the RdRP is responsible for transcription and replication of RNA virus genomes. Given the essential role of the RdRP for virus multiplication, the viral RdRP has been the subject of intensive study for many decades.

More than 30 years ago, RdRP activity was detected in the tissue of numerous plants that were thought to be uninfected (Astier-Manifacier and Cornuet 1971; Astier-Manifacier and Cornuet 1978; Boege and Sänger 1980; Duda et al. 1973). This observation eventually led to the cloning of a gene from tomato thought to be at least a component of this cellular activity of plants, although direct demonstration of RdRP activity associated with the cloned gene product was not possible (Schiebel et al. 1998). Subsequently, it was shown that the plant RdRP gene had homologs in fungi [e.g., QDE-1 in *Neurospora crassa* (Cogoni and Macino 1999)] and nematodes [EGO-1 and RRF genes in *Caenorhabditis elegans* (Smardon et al. 2000)]. In all cases, these genes were shown to be essential for gene silencing events: co-suppression in plants (Mourrain et al. 2000); quelling in *N. crassa* (Cogoni and Macino 1999); and RNA interference (RNAi) in *C. elegans* (Smardon et al. 2000). In particular, the RdRP is implicated in the genesis and/or maintenance of the gene silencing trigger, double-stranded RNA (dsRNA) (Nishikura 2001). Recently, RdRP activity was shown for the QDE-1 gene product, QDE-1p

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(Makeyev and Bamford 2002), and a structure of this enzyme is imminent (Laurila et al. 2005a). Unfortunately, at this time, the structure-function relationships of this class of RdRPs remain to be defined.

In this article, we will review our current understanding of the structure, function, and mechanism of viral RdRPs. It is likely that the unifying principles and corresponding methods described for viral RdRPs will be useful in guiding studies of cellular RdRPs required for RNAi.

2 RdRP Structures

Three-dimensional structural information is currently available for RdRPs from five families of positive-strand [*Picornaviridae*: poliovirus, human rhinovirus, foot-and-mouth-disease virus (FMDV); *Caliciviridae*: rabbit hemorrhagic disease virus, Norwalk virus; and *Flaviviridae*: hepatitis C virus, bovine viral diarrhea virus] and double-strand (*Cystoviridae*: phage ϕ6 and *Reoviridae*: reovirus) RNA viruses (Table 1). All enzymes share an overall structure that resembles a cupped "right hand" and contains "fingers," "palm," and "thumb" domains (Fig. 1). This architecture is shared with distantly related DNA-dependent DNA polymerases (DdDPs), DNA-dependent RNA polymerases (DdRPs), and RNA-dependent DNA polymerases (RdDPs or reverse transcriptases). The low level of amino acid sequence identity seen in polymerases from different classes strongly suggests that the structural elements that are conserved in evolutionarily distant species serve important functional roles. In addition to these three central domains, an N-terminal domain that bridges the fingers and thumb domains is found in all RdRPs. In the RdRPs from the *Flaviviridae, Cystoviridae, and Reoviridae*, C-terminal domains that enter or encircle the central cleft of the enzyme are also found.

Six sequence and structural motifs (designated A to F) have been identified in RdRPs (Bruenn 2003; Hansen et al. 1997; Kamer and Argos 1984; O'Reilly and Kao 1998). Most of these motifs are also shared with RdDPs, DdDPs, and DdRPs, indicating the fundamental importance of these structural elements in the enzymatic function of polymerases. Indeed, residues from most of these motifs have been shown to play critical roles in the binding of metal ions, nucleoside triphosphates, and RNA, all of which are critical for the nucleotidyltransferase reaction catalyzed by RdRPs. In the three-dimensional structures of RdRPs, these motifs line the central cavity that is responsible for binding substrates and cofactors, as well as catalyzing the nucleotidyltransferase reaction.

3 Structures of RdRP Complexes

3.1 Divalent Metal Ions

The dependence of polymerase activity upon divalent metal ions was initially demonstrated in early studies of DdDPs, and structural work on a wide range of phosphotransfer enzymes indicates that a basic mechanism involving two metal ions at the active site is a common feature of most if not all DdDPs, DdRPs, RdDPs, and RdRPs (Doublie and Ellenberger 1998; Doublie et al. 1999; Rothwell and Waksman 2005; Steitz 1998). In RdRPs, divalent metal ion dependence was initially demonstrated in poliovirus and subsequently shown to involve several of the most highly conserved residues in all RdRPs (Arnold et al. 1999; Flanegan and Baltimore 1977; Jablonski and Morrow 1995). Mutagenesis studies and crystal structures indicate that metal binding may occur at multiple sites near the active site. Two metal ions [designated A and B, according to Steitz (1998)] appear to be the most important for enzymatic activity. Metal ion A coordinates to the α-phosphate group of the nucleoside triphosphate (NTP) and the 3′-OH of the nascent primer, as well as the side chain carboxylate groups of the two consecutive Asp residues in motif C and the first Asp at the beginning of motif A (Fig. 2). Metal ion B

coordinates to the β- and γ-phosphate groups of the NTP, as well as the first two aspartic acid residues of motif A and the first of the two consecutive Asp residues in motif C.

Mutating the Asp residues in motifs A and C that coordinate to the divalent metal ions inactivates or alters the activity of several RdRPs (Arnold et al. 1999; Jablonski and Morrow 1995; Vazquez et al. 2000). In addition, altering the nature of the metal ions by introducing different ions such as Mg^{2+}, Mn^{2+}, Ca^{2+}, and Fe^{2+} affects the polymerase activity of RdRPs in a number of different ways. Properties observed in Mg^{2+} are most consistent with properties observed biologically. The structure of bacteriophage ϕ6 RdRP in complex with Ca^{2+} reveals an inactive arrangement of active site residues distinct from that seen in the enzyme bound to Mg^{2+} and Mn^{2+} (Salgado et al. 2004).

3.2 Nucleoside Triphosphates

The binding of NTPs to RdRPs primarily involves contacts with the triphosphate and sugar moieties, with the base forming interactions primarily with the primer and template (Fig. 2). The triphosphate moiety forms interactions with both divalent metal ions, as well as the positively charged side chains of Arg and Lys residues in motif F. The carboxylate side chain of a highly conserved Asp near the middle of motif A appears to form a critical hydrogen bond for distinguishing the 2′-OH of NTPs from the 2′-H of dNTPs (Arnold and Cameron 2004;Gohara et al. 2004;Gohara et al. 2000).

NTP-binding has also been observed in a number of RdRPs at sites other than the active site. In hepatitis C virus (HCV) and bovine viral diarrhea virus (BVDV) RdRPs, a regulatory guanosine triphosphate (GTP)-binding site has been localized (Bressanelli et al. 2002; Cai et al. 2005; Choi et al. 2006; Choi et al. 2004). In addition, a number of RdRP complexes have been obtained with NTPs in the absence of RNA (Ago et al. 1999; Bressanelli et al. 2002; O'Farrell et al. 2003; Thompson and Peersen 2004). Although the binding modes for NTPs that are observed in these complexes are sometimes similar to the productive mode expected for the phosphotransfer reaction, the absence of base-pairing with the template RNA strand usually leaves the base in a conformation that differs substantially from that expected in the productive mode.

3.3 RNA

At least two distinct modes of RNA binding have been seen in the two major divisions in the RdRP family. In the RdRPs from *Picornaviridae* and *Caliciviridae*, the RNA-binding cleft is approximately 15 Å wide and can fit an A-form RNA duplex, as seen in the FMDV RdRP-RNA complex (Fig. 1A) (Ferrer-Orta et al. 2004) and resembling the mode of DNA binding seen in numerous DdDPs and DdRPs. In contrast, the RdRPs from *Flaviviridae* and*Cystoviridae* contain protein structures that obstruct the cleft, preventing the binding of duplex RNA and providing a platform for the assembly of an initiation complex in the absence of an RNA primer (Fig. 1B,2A;Tao et al. 2002). RNA complexes from these RdRPs reveal a binding cleft that is suited more for binding a single strand of RNA template forming Watson-Crick base pairs with only a short segment of primer RNA (Butcher et al. 2001;O'Farrell et al. 2003). In the reovirus RdRP, a large C-terminal domain is situated in front of the active site cleft without blocking the entry of short RNA primers, thus forming a "cage" around the polymerase active site (Fig. 1C,2B;Tao et al. 2002). The initiation complex seen in this enzyme is similar to that seen in bacteriophage ϕ6 RdRP, with a priming loop extension of the palm domain forming a platform for dinucleotide synthesis. This loop moves away from the active site to allow for the formation of longer double-strand products, probably in a manner more similar to that expected for the primer-dependent RdRPs.

3.4 Proteins and Higher-Order Complexes

RdRPs have been shown to interact with a number of proteins produced by either the virus or the host, particularly during the initiation of RNA replication. In the *Picornaviridae*, a 22-amino-acid virally encoded initiator protein called VPg (virion protein genome linked) is uridylylated by the RdRP as an initial step in replication (Lee et al. 1977; Nomoto et al. 1977; Paul et al. 1998). The structure of the FMDV RdRP-VPg complex reveals interactions between VPg and the RdRP active-site cleft that position the side-chain hydroxyl group of Tyr 3 in VPg near the α-phosphate moiety of the uridine triphosphate (UTP) cosubstrate (Ferrer-Orta et al. 2006). In combination with mutational studies (Boerner et al. 2005; Lyle et al. 2002; Pathak et al. 2002), this structure reveals a number of residues in the active site cleft involved with the binding of VPg and with the uridylylation reaction involved with the initiation of RNA synthesis.

Higher-order complexes involving proteins and RNA structures are also formed by RdRPs and alternate forms of RdRPs, such as the proteinase-polymerase fusions seen in picornaviruses (Cornell and Semler 2002; Parsley et al. 1999; Ypma-Wong et al. 1988) and caliciviruses (Belliot et al. 2005; Belliot et al. 2003; Kaiser et al. 2006; Sosnovtseva et al. 1999; Wei et al. 2001). Although the formation of such complexes is best understood in the picornaviruses, especially poliovirus, no structural information on these complexes is available at present (Andino et al. 1999; Andino et al. 1993; Andino et al. 1990; Paul et al. 2003). It is likely that higher-order complexes involving RdRPs, other proteins, and RNA play critical roles in the initiation of RNA synthesis, translation, and RNA packaging for most, if not all, RNA viruses (Ortin and Parra 2006).

3.5 Inhibitors

Due to the severe threat to public health posed by HCV, an intensive search for novel antiviral therapies to treat HCV infection has been conducted in the past decade. A wide variety of inhibitors have been identified that target the RdRP from HCV. Most interesting among these have been a series of nonnucleoside inhibitors that appear to bind near the base of the thumb domain to allosterically inhibit polymerase activity, possibly by interfering with a conformational change required for normal catalytic activity (Biswal et al. 2005; Biswal et al. 2006; Dhanak et al. 2002; Di Marco et al. 2005; Gopalsamy et al. 2006; Harper et al. 2005; Love et al. 2003; Tomei et al. 2003; Wang et al. 2003). It is interesting to note that alternate conformational states have been observed in several RdRP structures (Biswal et al. 2005; Choi et al. 2004; Ng et al. 2002), suggesting that important conformational changes may accompany enzymatic catalysis as seen in other classes of polymerases (Doublie et al. 1999; Rothwell and Waksman 2005).

4 Phosphodiester Bond Formation

4.1 Two Metal Ion Mechanism

The chemistry at the active site of all nucleic acid polymerases studied to date is facilitated by a two metal ion mechanism that was proposed by Steitz based on his structural work on the magnesium-dependent exonuclease activity of DNA polymerase I from *Escherichia coli* (Steitz 1993). The model shown in Fig. 3 has been adapted for enzymes with a palm-based active site, which includes the viral RdRP. A magnesium (metal B)-nucleotide complex binds to the active site followed by binding of a second magnesium ion (metal A). The metal designations reflect the occurrence of metal A in some structures in the absence of nucleotide substrate. Metal A is thought to be involved in activation of the 3′-OH for nucleophilic attack by lowering its pK_a value. Metal B orients the β- and γ-phosphates of the nucleotide substrate and stabilizes the negatively charged pentavalent phosphorane transition state (Fig. 4).

The two metal ion mechanism implies that side chains of active site residues do not participate directly in catalysis, only indirectly as ligands for one or more of the magnesium ions (Steitz 1993). However, two proton transfer reactions must occur during the reaction. The 3′-OH nucleophile must be deprotonated and the pyrophosphate (PPi) leaving group must be protonated (Fig. 4). The acceptor and donor for these key proton transfer reactions is not known and is not likely to be solvent given the dearth of ordered solvent in structures of complexes thought to mimic the catalytically active polymerase-nucleic acid-nucleotide complex (Doublie et al. 1998; Franklin et al. 2001; Johnson et al. 2003; Sawaya et al. 1997; Yin and Steitz 2004).

4.2 Initiation Vs Elongation

Formally there are two mechanisms for initiation of RNA synthesis: primer independent (de novo) and primer dependent. De novo initiation requires formation of a phosphodiester bond between two ribonucleotides (Fig. 5A). For replication, initiation is templated by the extreme 3′-end of template; however, for transcription, initiation may be templated by internal positions. In Fig. 5A, the 3′ nucleotide defining the site of initiation has been designated “n.” Residues at the n and n+1 positions of template define the primer (P) and nucleotide (N) binding sites. The 3′-OH of the P-site NTP attacks the α-phosphorous of the N-site NTP to form a dinucleotide. Iterative rounds of incorporation and translocation will ultimately yield a stable elongation complex (Fig. 5B).

De novo initiation generally employs purine nucleotides, often with a preference for GTP at the P-site. With some enzymes, guanosine, guanosine monophosphate (GMP), and guanosine diphosphate (GDP) can substitute for a P-site GTP (Martin and Coleman 1989). Because base-pairing alone is insufficient to stabilize the P-site NTP and the triphosphate is not essential for P-site occupancy, specialized structural elements are employed. For example, in bacteriophage ϕ6, a specialized carboxyterminal domain presents at least one tyrosine for stacking with the P-site NTP (Butcher et al. 2001). The reovirus polymerase has a specialized loop that serves a similar function (Tao et al. 2002). The dinucleotide product is unstable. As a result, abortive cycling is often observed for polymerases that initiate de novo. Formation of a stable elongation complex generally coincides with formation of an RNA product long enough to form a stable duplex with the template and may require substantial conformational rearrangements of the polymerase (Yin and Steitz 2004).

Enzymes that employ a primer-dependent mechanism for initiation will use either a protein primer or an oligonucleotide of defined origin but random sequence (van Dijk et al. 2004). As discussed in Sect. 3.4, picornaviruses use the tyrosine hydroxyl group of VPg as the nucleophile. VPg binds to the RNA binding pocket of the polymerase independent of the template (Ferrer-Orta et al. 2006). This mechanism has all of the features of a stable elongation complex: limited, if any, abortive cycling and no requirement for large conformational rearrangements.

Transcription by the influenza virus RNA polymerase employs a “cap-snatching” mechanism. Capped mRNAs are cleaved by a subunit of the heterotrimeric polymerase complex to produce capped RNA oligonucleotides (10-15 nt) that are used to prime transcription (van Dijk et al. 2004). The cap is the major determinant for recognition by the endoribonuclease activity of the polymerase complex. The capped RNA product binds stably to the polymerase complex ($t_{1/2}$ ~1 h) (Olsen et al. 1996). The 3′-OH of the terminal nucleotide serves as the nucleophile, with the template being held in the complex independently. Again, this approach provides the advantages of the elongation complex described above. However, this approach can only be used for genome replication if the sequences at the ends of the genome lack information: coding sequence, *cis*-acting elements, etc.

5 Fidelity

RdRPs have often been described as error-prone polymerases. However, it has become increasingly clear that these polymerases are as faithful as replicative DNA polymerases in the absence of their proofreading exonuclease (Castro et al. 2005). Indeed, biochemical, phenotypic, and direct sequencing experiments have shown that RNA virus polymerases incorporate transition mutations at a frequency of 10^{-5} and transversions mutations at a frequency of 10^{-6}-10^{-7} (Castro et al. 2005). The kinetic and structural bases for fidelity of nucleotide selection is understood best for the RdRP from poliovirus (3Dpol).

5.1 Kinetic Basis

A complete kinetic mechanism for the single nucleotide addition cycle catalyzed by 3Dpol (E) is known. 3Dpol binds to a primer-template substrate (R_n) with a equilibrium dissociation constant in the micromolar range (Arnold and Cameron 2000). This complex isomerizes to form ER_n, a complex that has a half-life on the order of 2-4 h and is competent for binding nucleotide (Arnold and Cameron 2000). As shown in Fig. 6, binding of nucleotide to ER_n yields a complex, ER_nNTP, that undergoes a conformational change to produce a catalytically competent complex ($*ER_nNTP$) (Arnold and Cameron 2004). This conformational change has been suggested to be reorientation of the triphosphate moiety of the incoming NTP into a position suitable for catalysis and coordination of metal A (Arnold et al. 2004). Chemistry occurs ($ER_{n+1}PPi$) followed by translocation with concomitant release of PPi, placing the enzyme in the appropriate register for another round of nucleotide incorporation.

Binding of nucleotides to the ER_n complex is driven by the interaction of the triphosphate with motif F of the enzyme (Arnold and Cameron 2004). As a result, nucleotides with an incorrect base or sugar configuration (e.g., 2′-dNTPs) bind as well as the correct nucleotide. However, incorrect nucleotides are incapable of forming a $*ER_nNTP$ complex that is stable enough to undergo catalysis (Arnold and Cameron 2004; Arnold et al. 2004). In addition, the rate constant for chemistry is reduced significantly when an incorrect nucleotide is bound (Arnold and Cameron 2004; Arnold et al. 2004).

5.2 Structural Basis

Only one crystal structure is available for an RdRP complex that may represent ER_nNTP or $*ER_nNTP$ (Tao et al. 2002). However, the conserved nature of palmbased active sites combined with kinetic and thermodynamic analyses of site-directed mutants with nucleotide analogs has led to a structural model for nucleotide selection by 3Dpol that extrapolates well to other classes of nucleic acid polymerases (Gohara et al. 2004). Shown in Fig. 7 is a model for $*ER_nNTP$ (Gohara et al. 2004). The orientation of the triphosphate dictates both the stability of this complex and catalytic efficiency. The orientation of the triphosphate requires interaction with conserved structural motif A. Note that one residue of motif A, Asp-238, is located in the nucleoside binding pocket. Binding of a nucleotide with an incorrect base or ribose configuration will alter the dynamics or equilibrium position of Asp-238. This perturbation will be communicated to the active site by changes in the positions of the other motif A residues, placing the triphosphate in a suboptimal orientation and leading to a destabilized $*ER_nNTP$ complex with reduced catalytic efficiency.

6 RdRPs of RNAi

Very little is known about the structure, function, and mechanism of the RdRPs of RNAi. The most conserved region, based on sequence alignments, is shown in Fig. 8 and represents, at best, 20% of the protein (Huang et al. 2003). The DxDGD motif has been shown to be essential for RdRP activity (Makeyev and Bamford 2002) and is reminiscent of the metal-binding GDD

motif (motif C) of the viral RdRPs. The enzyme clearly has a requirement for divalent cation (Makeyev and Bamford 2002). Studies of the enzyme from *N. crassa* have suggested that the enzyme lacks template specificity and uses a de novo initiation mechanism, initiating both from the end and perhaps from internal positions (Makeyev and Bamford 2002). When initiating from an end, long products can be produced (Makeyev and Bamford 2002), consistent with the observation of transitive silencing in related systems (Sijen et al. 2001). The primary product of the reaction is single-stranded RNA on the order of 20 nt in length, a size appropriate for direct incorporation into the RISC complex (Sijen et al. 2001).

7 Concluding Remarks

Our current understanding of viral RdRPs has required the capacity to apply molecular genetic, biochemical, and structural approaches. Analysis of cellular RdRPs has not reached this stage but is well on its way. *N. crassa* is clearly an organism amenable to molecular genetics and a biochemical system is available for the RdRP from this organism. Importantly, a structure is imminent. Note added in proof: The crystal structure of the QDE-1, a cell-encoded RdRP from *N. crassa* was recently reported (Salgado et al. 2006). The publication reporting this structure is, These major advances in the *N. crassa* system will undoubtedly have a major impact on progress in other systems.

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References

Ago H, Adachi T, Yoshida A, Yamamoto M, Habuka N, Yatsunami K, Miyano M. Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus. Structure Fold Des 1999;7:1417–1426. [PubMed: 10574802]

Andino R, Rieckhof GE, Baltimore D. A functional ribonucleoprotein complex forms around the 5′ end of poliovirus RNA. Cell 1990;63:369–380. [PubMed: 2170027]

Andino R, Rieckhof GE, Achacoso PL, Baltimore D. Poliovirus RNA synthesis utilizes an RNP complex formed around the 5′-end of viral RNA. EMBO J 1993;12:3587–3598. [PubMed: 8253083]

Andino R, Boddeker N, Silvera D, Gamarnik AV. Intracellular determinants of picornavirus replication. Trends Microbiol 1999;7:76–82. [PubMed: 10081085]

Appleby TC, Luecke H, Shim JH, Wu JZ, Cheney IW, Zhong W, Vogeley L, Hong Z, Yao N. Crystal structure of complete rhinovirus RNA polymerase suggests front loading of protein primer. J Virol 2005;79:277–288. [PubMed: 15596823]

Arnold JJ, Cameron CE. Poliovirus RNA-dependent RNA polymerase [3D(pol)]. Assembly of stable, elongation-competent complexes by using a symmetrical primer-template substrate (sym/sub). J Biol Chem 2000;275:5329–5336. [PubMed: 10681506]

Arnold JJ, Cameron CE. Poliovirus RNA-dependent RNA polymerase (3Dpol): pre-steady-state kinetic analysis of ribonucleotide incorporation in the presence of Mg^{2+} Biochemistry 2004;43:5126–5137. [PubMed: 15122878]

Arnold JJ, Ghosh SK, Cameron CE. Poliovirus RNA-dependent RNA polymerase [3D(pol)]. Divalent cation modulation of primer, template, and nucleotide selection. J Biol Chem 1999;274:37060–37069. [PubMed: 10601264]

Arnold JJ, Gohara DW, Cameron CE. Poliovirus RNA-dependent RNA polymerase (3Dpol): pre-steady-state kinetic analysis of ribonucleotide incorporation in the presence of Mn^{2+} Biochemistry 2004;43:5138–5148. [PubMed: 15122879]

Astier-Manifacier S, Cornuet P. RNA-dependent RNA polymerase in Chinese cabbage. Biochim Biophys Acta 1971;232:484–493. [PubMed: 5572618]

Astier-Manifacier S, Cornuet P. Purification and molecular weight of an RNA-dependant RNA polymerase from Brassicae oleracea var. Botrytis (in French). C R Acad Sci Hebd Seances Acad Sci D 1978;287:1043–1046. [PubMed: 105817]

Belliot G, Sosnovtsev SV, Mitra T, Hammer C, Garfield M, Green KY. In vitro proteolytic processing of the MD145 norovirus ORF1 nonstructural polyprotein yields stable precursors and products similar to those detected in calicivirus-infected cells. J Virol 2003;77:10957–10974. [PubMed: 14512545]

Belliot G, Sosnovtsev SV, Chang KO, Babu V, Uche U, Arnold JJ, Cameron CE, Green KY. Norovirus proteinase-polymerase and polymerase are both active forms of RNA-dependent RNA polymerase. J Virol 2005;79:2393–2403. [PubMed: 15681440]

Biswal BK, Cherney MM, Wang M, Chan L, Yannopoulos CG, Bilimoria D, Nicolas O, Bedard J, James MN. Crystal structures of the RNA-dependent RNA polymerase genotype 2a of hepatitis C virus reveal two conformations and suggest mechanisms of inhibition by non-nucleoside inhibitors. J Biol Chem 2005;280:18202–18210. [PubMed: 15746101]

Biswal BK, Wang M, Cherney MM, Chan L, Yannopoulos CG, Bilimoria D, Bedard J, James MN. Non-nucleoside inhibitors binding to hepatitis C virus NS5B polymerase reveal a novel mechanism of inhibition. J Mol Biol 2006;361:33–45. [PubMed: 16828488]

Boege F, Sänger HL. RNA-dependent RNA polymerase from healthy tomato leaf tissue. FEBS Lett 1980;121:91–96.

Boerner JE, Lyle JM, Daijogo S, Semler BL, Schultz SC, Kirkegaard K, Richards OC. Allosteric effects of ligands and mutations on poliovirus RNA-dependent RNA polymerase. J Virol 2005;79:7803–7811. [PubMed: 15919933]

Bressanelli S, Tomei L, Roussel A, Incitti I, Vitale RL, Mathieu M, De Francesco R, Rey FA. Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus. Proc Natl Acad Sci U S A 1999;96:13034–13039. [PubMed: 10557268]

Bressanelli S, Tomei L, Rey FA, De Francesco R. Structural analysis of the hepatitis C virus RNA polymerase in complex with ribonucleotides. J Virol 2002;76:3482–3492. [PubMed: 11884572]

Bruenn JA. A structural and primary sequence comparison of the viral RNA-dependent RNA polymerases. Nucleic Acids Res 2003;31:1821–1829. [PubMed: 12654997]

Butcher SJ, Grimes JM, Makeyev EV, Bamford DH, Stuart DI. A mechanism for initiating RNA-dependent RNA polymerization. Nature 2001;410:235–240. [PubMed: 11242087]

Cai Z, Yi M, Zhang C, Luo G. Mutagenesis analysis of the rGTP-specific binding site of hepatitis C virus RNA-dependent RNA polymerase. J Virol 2005;79:11607–11617. [PubMed: 16140738]

Castro C, Arnold JJ, Cameron CE. Incorporation fidelity of the viral RNA-dependent RNA polymerase: a kinetic, thermodynamic and structural perspective. Virus Res 2005;107:141–149. [PubMed: 15649560]

Choi KH, Groarke JM, Young DC, Kuhn RJ, Smith JL, Pevear DC, Rossmann MG. The structure of the RNA-dependent RNA polymerase from bovine viral diarrhea virus establishes the role of GTP in de novo initiation. Proc Natl Acad Sci U S A 2004;101:4425–4430. [PubMed: 15070734]

Choi KH, Gallei A, Becher P, Rossmann MG. The structure of bovine viral diarrhea virus RNA-dependent RNA polymerase and its amino-terminal domain. Structure 2006;14:1107–1113. [PubMed: 16843892]

Cogoni C, Macino G. Gene silencing in Neurospora crassa requires a protein homologous to RNA-dependent RNA polymerase. Nature 1999;399:166–169. [PubMed: 10335848]

Cornell CT, Semler BL. Subdomain specific functions of the RNA polymerase region of poliovirus 3CD polypeptide. Virology 2002;298:200–213. [PubMed: 12127783]

Dhanak D, Duffy KJ, Johnston VK, Lin-Goerke J, Darcy M, Shaw AN, Gu B, Silverman C, Gates AT, Nonnemacher MR, Earnshaw DL, Casper DJ, Kaura A, Baker A, Greenwood C, Gutshall LL, Maley D, DelVecchio A, Macarron R, Hofmann GA, Alnoah Z, Cheng HY, Chan G, Khandekar S, Keenan RM, Sarisky RT. Identification and biological characterization of heterocyclic inhibitors of the hepatitis C virus RNA-dependent RNA polymerase. J Biol Chem 2002;277:38322–38327. [PubMed: 12167642]

Di Marco S, Volpari C, Tomei L, Altamura S, Harper S, Narjes F, Koch U, Rowley M, De Francesco R, Migliaccio G, Carfi A. Interdomain communication in hepatitis C virus polymerase abolished by small molecule inhibitors bound to a novel allosteric site. J Biol Chem 2005;280:29765–29770. [PubMed: 15955819]

Doublie S, Ellenberger T. The mechanism of action of T7 DNA polymerase. Curr Opin Struct Biol 1998;8:704–712. [PubMed: 9914251]

Doublie S, Tabor S, Long AM, Richardson CC, Ellenberger T. Crystal structure of a bacteriophage T7 DNA replication complex at 2.2 Å resolution. Nature 1998;391:251–258. [PubMed: 9440688]

Doublie S, Sawaya MR, Ellenberger T. An open and closed case for all polymerases. Structure 1999;7:R31–R35. [PubMed: 10368292]

Duda CT, Zaitlin M, Siegel A. In vitro synthesis of double-stranded RNA by an enzyme system isolated from tobacco leaves. Biochim Biophys Acta 1973;319:62–71. [PubMed: 4733693]

Ferrer-Orta C, Arias A, Perez-Luque R, Escarmis C, Domingo E, Verdaguer N. Structure of foot-and-mouth disease virus RNA-dependent RNA polymerase and its complex with a template-primer RNA. J Biol Chem 2004;279:47212–47221. [PubMed: 15294895]

Ferrer-Orta C, Arias A, Agudo R, Perez-Luque R, Escarmis C, Domingo E, Verdaguer N. The structure of a protein primer-polymerase complex in the initiation of genome replication. EMBO J 2006;25:880–888. [PubMed: 16456546]

Flanegan JB, Baltimore D. Poliovirus-specific primer-dependent RNA polymerase able to copy poly(A). Proc Natl Acad Sci U S A 1977;74:3677–3680. [PubMed: 198796]

Franklin MC, Wang J, Steitz TA. Structure of the replicating complex of a pol alpha family DNA polymerase. Cell 2001;105:657–667. [PubMed: 11389835]

Gohara DW, Crotty S, Arnold JJ, Yoder JD, Andino R, Cameron CE. Poliovirus RNA-dependent RNA polymerase (3Dpol): structural, biochemical, and biological analysis of conserved structural motifs A and B. J Biol Chem 2000;275:25523–25532. [PubMed: 10827187]

Gohara DW, Arnold JJ, Cameron CE. Poliovirus RNA-dependent RNA polymerase (3Dpol): kinetic, thermodynamic, and structural analysis of ribonucleotide selection. Biochemistry 2004;43:5149–5158. [PubMed: 15122880]

Gopalsamy A, Chopra R, Lim K, Ciszewski G, Shi M, Curran KJ, Sukits SF, Svenson K, Bard J, Ellingboe JW, Agarwal A, Krishnamurthy G, Howe AY, Orlowski M, Feld B, O'Connell J, Mansour TS. Discovery of proline sulfonamides as potent and selective hepatitis C virus NS5b polymerase inhibitors. Evidence for a new NS5b polymerase binding site. J Med Chem 2006;49:3052–3055. [PubMed: 16722622]

Hansen JL, Long AM, Schultz SC. Structure of the RNA-dependent RNA polymerase of poliovirus. Structure 1997;5:1109–1122. [PubMed: 9309225]

Harper S, Avolio S, Pacini B, Di Filippo M, Altamura S, Tomei L, Paonessa G, Di Marco S, Carfi A, Giuliano C, Padron J, Bonelli F, Migliaccio G, De Francesco R, Laufer R, Rowley M, Narjes F. Potent inhibitors of subgenomic hepatitis C virus RNA replication through optimization of indole-N-acetamide allosteric inhibitors of the viral NS5B polymerase. J Med Chem 2005;48:4547–4557. [PubMed: 15999993]

Huang, L.; Gledhill, J.; Cameron, CE. RNA-dependent RNA polymerase in gene silencing. In: Hannon, GJ., editor. RNAi: a guide to gene silencing. Cold Spring Harbor Laboratory Press; Cold Spring Harbor: 2003. p. 175-203.

Jablonski SA, Morrow CD. Mutation of the aspartic acid residues of the GDD sequence motif of poliovirus RNA-dependent RNA polymerase results in enzymes with altered metal ion requirements for activity. J Virol 1995;69:1532–1539. [PubMed: 7853486]

Johnson SJ, Taylor JS, Beese LS. Processive DNA synthesis observed in a polymerase crystal suggests a mechanism for the prevention of frameshift mutations. Proc Natl Acad Sci U S A 2003;100:3895–3900. [PubMed: 12649320]

Kaiser WJ, Chaudhry Y, Sosnovtsev SV, Goodfellow IG. Analysis of protein-protein interactions in the feline calicivirus replication complex. J Gen Virol 2006;87:363–368. [PubMed: 16432023]

Kamer G, Argos P. Primary structural comparison of RNA-dependent polymerases from plant, animal and bacterial viruses. Nucleic Acids Res 1984;12:7269–7282. [PubMed: 6207485]

Laurila MR, Salgado PS, Makeyev EV, Nettelship J, Stuart DI, Grimes JM, Bamford DH. Gene silencing pathway RNA-dependent RNA polymerase of Neurospora crassa: yeast expression and crystallization of selenomethionated QDE-1 protein. J Struct Biol 2005a;149:111–115. [PubMed: 15629662]

Laurila MR, Salgado PS, Stuart DI, Grimes JM, Bamford DH. Back-priming mode of phi6 RNA-dependent RNA polymerase. J Gen Virol 2005b;86:521–526. [PubMed: 15659773]

Lee YF, Nomoto A, Detjen BM, Wimmer E. A protein covalently linked to poliovirus genome RNA. Proc Natl Acad Sci U S A 1977;74:59–63. [PubMed: 189316]

Lesburg CA, Cable MB, Ferrari E, Hong Z, Mannarino AF, Weber PC. Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site. Nat Struct Biol 1999;6:937–943. [PubMed: 10504728]

Liu J, Tsai MD. DNA polymerase beta: pre-steady-state kinetic analyses of dATP alpha S stereoselectivity and alteration of the stereoselectivity by various metal ions and by site-directed mutagenesis. Biochemistry 2001;40:9014–9022. [PubMed: 11467964]

Love RA, Parge HE, Yu X, Hickey MJ, Diehl W, Gao J, Wriggers H, Ekker A, Wang L, Thomson JA, Dragovich PS, Fuhrman SA. Crystallographic identification of a noncompetitive inhibitor binding site on the hepatitis C virus NS5B RNA polymerase enzyme. J Virol 2003;77:7575–7581. [PubMed: 12805457]

Love RA, Maegley KA, Yu X, Ferre RA, Lingardo LK, Diehl W, Parge HE, Dragovich PS, Fuhrman SA. The crystal structure of the RNA-dependent RNA polymerase from human rhinovirus: a dual function target for common cold antiviral therapy. Structure 2004;12:1533–1544. [PubMed: 15296746]

Lyle JM, Clewell A, Richmond K, Richards OC, Hope DA, Schultz SC, Kirkegaard K. Similar structural basis for membrane localization and protein priming by an RNA-dependent RNA polymerase. J Biol Chem 2002;277:16324–16331. [PubMed: 11877407]

Makeyev EV, Bamford DH. Cellular RNA-dependent RNA polymerase involved in posttranscriptional gene silencing has two distinct activity modes. Mol Cell 2002;10:1417–1427. [PubMed: 12504016]

Martin CT, Coleman JE. T7 RNA polymerase does not interact with the 5′-phosphate of the initiating nucleotide. Biochemistry 1989;28:2760–2762. [PubMed: 2663058]

Mourrain P, Beclin C, Elmayan T, Feuerbach F, Godon C, Morel JB, Jouette D, Lacombe AM, Nikic S, Picault N, Remoue K, Sanial M, Vo TA, Vaucheret H. Arabidopsis SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. Cell 2000;101:533–542. [PubMed: 10850495]

Ng KK, Cherney MM, Vazquez AL, Machin A, Alonso JM, Parra F, James MN. Crystal structures of active and inactive conformations of a caliciviral RNA-dependent RNA polymerase. J Biol Chem 2002;277:1381–1387. [PubMed: 11677245]

Ng KK, Pendas-Franco N, Rojo J, Boga JA, Machin A, Alonso JM, Parra F. Crystal structure of Norwalk virus polymerase reveals the carboxyl terminus in the active site cleft. J Biol Chem 2004;279:16638–16645. [PubMed: 14764591]

Nishikura K. A short primer on RNAi: RNA-directed RNA polymerase acts as a key catalyst. Cell 2001;107:415–418. [PubMed: 11719182]

Nomoto A, Detjen B, Pozzatti R, Wimmer E. The location of the polio genome protein in viral RNAs and its implication for RNA synthesis. Nature 1977;268:208–213. [PubMed: 196204]

O'Farrell D, Trowbridge R, Rowlands D, Jager J. Substrate complexes of hepatitis C virus RNA polymerase (HC-J4): structural evidence for nucleotide import and de-novo initiation. J Mol Biol 2003;326:1025–1035. [PubMed: 12589751]

O'Reilly EK, Kao CC. Analysis of RNA-dependent RNA polymerase structure and function as guided by known polymerase structures and computer predictions of secondary structure. Virology 1998;252:287–303. [PubMed: 9878607]

Olsen DB, Benseler F, Cole JL, Stahlhut MW, Dempski RE, Darke PL, Kuo LC. Elucidation of basic mechanistic and kinetic properties of influenza endonuclease using chemically synthesized RNAs. J Biol Chem 1996;271:7435–7439. [PubMed: 8631770]

Ortin J, Parra F. Structure and function of RNA replication. Annu Rev Microbiol 2006;60:305–326. [PubMed: 16719717]

Parsley TB, Cornell CT, Semler BL. Modulation of the RNA binding and protein processing activities of poliovirus polypeptide 3CD by the viral RNA polymerase domain. J Biol Chem 1999;274:12867–12876. [PubMed: 10212275]

Pathak HB, Ghosh SK, Roberts AW, Sharma SD, Yoder JD, Arnold JJ, Gohara DW, Barton DJ, Paul AV, Cameron CE. Structure-function relationships of the RNA-dependent RNA polymerase from poliovirus (3Dpol). A surface of the primary oligomerization domain functions in capsid precursor processing and VPg uridylylation. J Biol Chem 2002;277:31551–31562. [PubMed: 12077141]

Paul AV, van Boom JH, Filippov D, Wimmer E. Protein-primed RNA synthesis by purified poliovirus RNA polymerase. Nature 1998;393:280–284. [PubMed: 9607767]

Paul AV, Peters J, Mugavero J, Yin J, van Boom JH, Wimmer E. Biochemical and genetic studies of the VPg uridylylation reaction catalyzed by the RNA polymerase of poliovirus. J Virol 2003;77:891–904. [PubMed: 12502805]

Pfefferkorn JA, Greene ML, Nugent RA, Gross RJ, Mitchell MA, Finzel BC, Harris MS, Wells PA, Shelly JA, Anstadt RA, Kilkuskie RE, Kopta LA, Schwende FJ. Inhibitors of HCV NS5B polymerase. Part 1. Evaluation of the southern region of (2Z)-2-(benzoylamino)-3-(5-phenyl-2-furyl)acrylic acid. Bioorg Med Chem Lett 2005a;15:2481–2486. [PubMed: 15863301]

Pfefferkorn JA, Nugent R, Gross RJ, Greene M, Mitchell MA, Reding MT, Funk LA, Anderson R, Wells PA, Shelly JA, Anstadt R, Finzel BC, Harris MS, Kilkuskie RE, Kopta LA, Schwende FJ. Inhibitors of HCV NS5B polymerase. Part 2. Evaluation of the northern region of (2Z)-2-benzoylamino-3-(4-phenoxy-phenyl)-acrylic acid. Bioorg Med Chem Lett 2005b;15:2812–2818. [PubMed: 15911260]

Powers JP, Piper DE, Li Y, Mayorga V, Anzola J, Chen JM, Jaen JC, Lee G, Liu J, Peterson MG, Tonn GR, Ye Q, Walker NP, Wang Z. SAR and mode of action of novel non-nucleoside inhibitors of hepatitis C NS5b RNA polymerase. J Med Chem 2006;49:1034–1046. [PubMed: 16451069]

Rothwell PJ, Waksman G. Structure and mechanism of DNA polymerases. Adv Protein Chem 2005;71:401–440. [PubMed: 16230118]

Salgado PS, Makeyev EV, Butcher SJ, Bamford DH, Stuart DI, Grimes JM. The structural basis for RNA specificity and Ca^{2+} inhibition of an RNA-dependent RNA polymerase. Structure 2004;12:307–316. [PubMed: 14962391]

Salgado P, Koivunen MRL, Makeyev EV, Bamford DH, Stuart DI, Grimes JM. The structure of an RNAi polymerase links RNA silencing and transcription. PLoS Biology 2006;4(12):e434. [PubMed: 17147473]

Sawaya MR, Prasad R, Wilson SH, Kraut J, Pelletier H. Crystal structures of human DNA polymerase beta complexed with gapped and nicked DNA: evidence for an induced fit mechanism. Biochemistry 1997;36:11205–11215. [PubMed: 9287163]

Schiebel W, Pelissier T, Riedel L, Thalmeir S, Schiebel R, Kempe D, Lottspeich F, Sanger HL, Wassenegger M. Isolation of an RNA-directed RNA polymerase-specific cDNA clone from tomato. Plant Cell 1998;10:2087–2101. [PubMed: 9836747]

Sijen T, Fleenor J, Simmer F, Thijssen KL, Parrish S, Timmons L, Plasterk RH, Fire A. On the role of RNA amplification in dsRNA-triggered gene silencing. Cell 2001;107:465–476. [PubMed: 11719187]

Smardon A, Spoerke JM, Stacey SC, Klein ME, Mackin N, Maine EM. EGO-1 is related to RNA-directed RNA polymerase and functions in germ-line development and RNA interference in C. elegans. Curr Biol 2000;10:169–178. [PubMed: 10704412]

Sosnovtseva SA, Sosnovtsev SV, Green KY. Mapping of the feline calicivirus proteinase responsible for autocatalytic processing of the nonstructural polyprotein and identification of a stable proteinase-polymerase precursor protein. J Virol 1999;73:6626–6633. [PubMed: 10400760]

Steitz TA. DNA- and RNA-dependent DNA polymerases. Curr Opin Struct Biol 1993;3:31–38.

Steitz TA. A mechanism for all polymerases. Nature 1998;391:231–232. [PubMed: 9440683]

Tao Y, Farsetta DL, Nibert ML, Harrison SC. RNA synthesis in a cage-structural studies of reovirus polymerase lambda3. Cell 2002;111:733–745. [PubMed: 12464184]

Thompson AA, Peersen OB. Structural basis for proteolysis-dependent activation of the poliovirus RNA-dependent RNA polymerase. EMBO J 2004;23:3462–3471. [PubMed: 15306852]

Tomei L, Altamura S, Bartholomew L, Biroccio A, Ceccacci A, Pacini L, Narjes F, Gennari N, Bisbocci M, Incitti I, Orsatti L, Harper S, Stansfield I, Rowley M, De Francesco R, Migliaccio G. Mechanism

of action and antiviral activity of benzimidazole-based allosteric inhibitors of the hepatitis C virus RNA-dependent RNA polymerase. J Virol 2003;77:13225–13231. [PubMed: 14645579]

van Dijk AA, Makeyev EV, Bamford DH. Initiation of viral RNA-dependent RNA polymerization. J Gen Virol 2004;85:1077–1093. [PubMed: 15105525]

Vazquez AL, Alonso JM, Parra F. Mutation analysis of the GDD sequence motif of a calicivirus RNA-dependent RNA polymerase. J Virol 2000;74:3888–3891. [PubMed: 10729164]

Wang M, Ng KK, Cherney MM, Chan L, Yannopoulos CG, Bedard J, Morin N, Nguyen-Ba N, Bethell RC, James MN. Non-nucleoside analogue inhibitors bind to an allosteric site on HCV NS5B polymerase: crystal structures and mechanism of inhibition. J Biol Chem 2003;278:9489–9495. [PubMed: 12509436]

Wei L, Huhn JS, Mory A, Pathak HB, Sosnovtsev SV, Green KY, Cameron CE. Proteinasepolymerase precursor as the active form of feline calicivirus RNA-dependent RNA polymerase. J Virol 2001;75:1211–1219. [PubMed: 11152494]

Yin YW, Steitz TA. The structural mechanism of translocation and helicase activity in T7 RNA polymerase. Cell 2004;116:393–404. [PubMed: 15016374]

Ypma-Wong MF, Dewalt PG, Johnson VH, Lamb JG, Semler BL. Protein 3CD is the major poliovirus proteinase responsible for cleavage of the P1 capsid precursor. Virology 1988;166:265–270. [PubMed: 2842953]

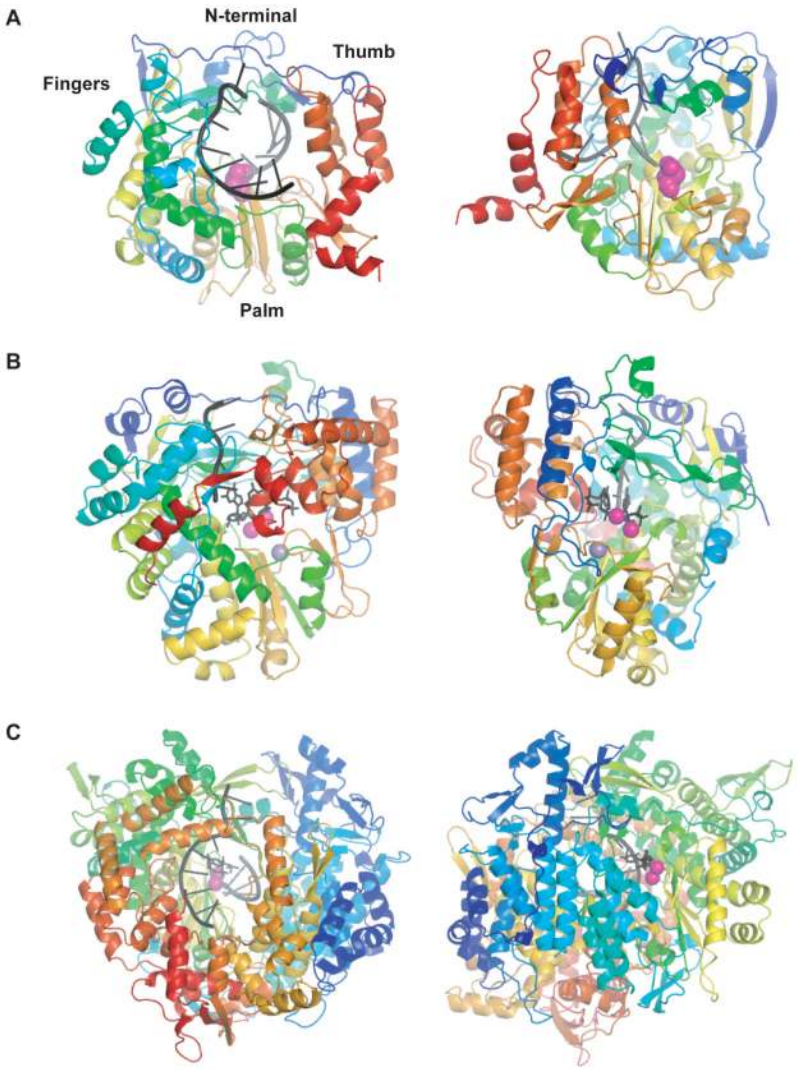

Fig. 1.

A-C Overall structures of RdRPs. Ribbon representations of RdRP structures (rainbow coloring with *blue* at the N-terminus and *red* at the C-terminus) bound to RNA template (*black*) and primer (*gray*) strands: **A** FMDV (1WNE) (Ferrer-Orta et al. 2004); **B** Bacteriophage ϕ6 (1HI0) (Butcher et al. 2001); **C** Reovirus (1N35) (Tao et al. 2002). Two views are presented for each structure, a "front" view down the axis of the RNA-binding, active site cleft (*left panel*) and a "side" or "back" view into the active site. Divalent metal ions at the active site in **B** and **C** are drawn as *magenta spheres*. Asp-338 in motif C of FMDV is drawn in space-filling representation as *magenta spheres* to mark the position of the active site in the absence of bound divalent metal ions

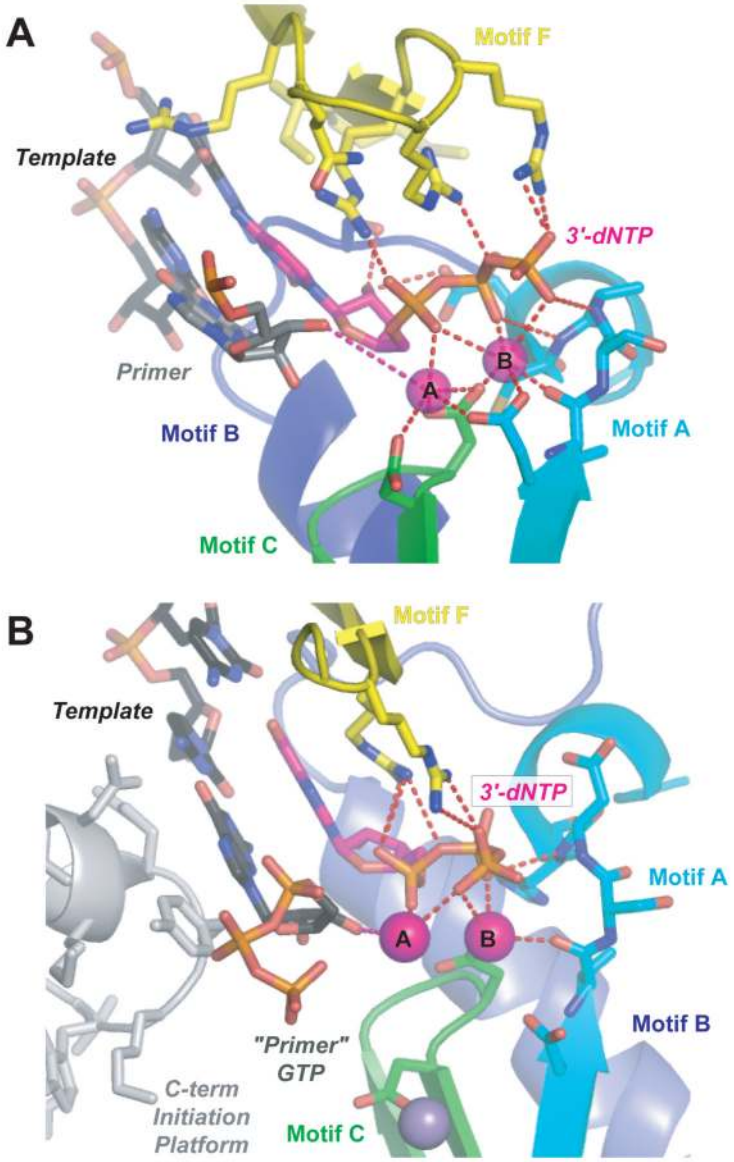

Fig. 2.

A, B Structures of E·RNA·NTP complexes. **A** RNA primer-dependent elongation complex formed by reovirus RdRP (1N35) (Tao et al. 2002). **B** Primer-independent (de novo) initiation complex formed by bacteriophage ϕ6 RdRP (1HI0) (Butcher et al. 2001). Divalent metal ions are drawn as *magenta spheres*. Coordination and hydrogen bonds are drawn as *dashed, red lines*. The 3′-terminal residue of the RNA primer in reovirus RdRP is drawn in *gray* and the two residues of the RNA template that are complementary to the 3′-terminal residue of the RNA primer and the 3′-dNTP are drawn in *black*. The long, 4.5-Å distance between the 3′-OH of the primer and metal ion A is drawn in *magenta* as a *dashed line*

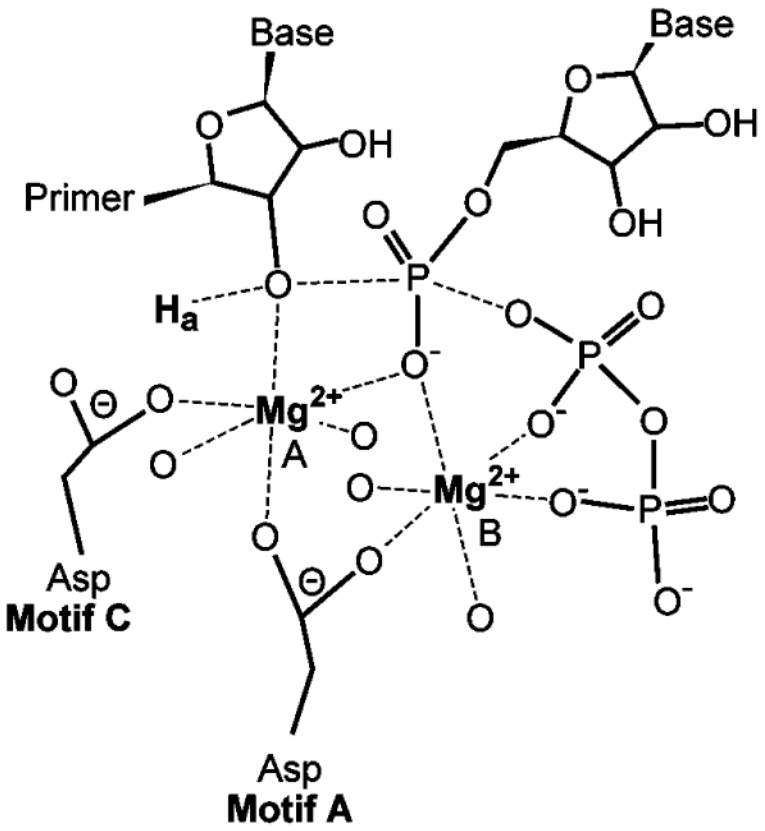

Fig. 3.

Two-metal-ion mechanism for nucleotidyl transfer. The nucleoside triphosphate enters the active site with a divalent cation (Mg^{2+}, metal B). This metal is coordinated by the β- and γ-phosphates of the nucleotide, by an Asp residue located in structural motif A of all polymerases, and likely water molecules (indicated as oxygen ligands to metal without specific designation). This metal orients the triphosphate in the active site and may contribute to charge neutralization during catalysis. Once the nucleotide is in place, the second divalent cation binds (Mg^{2+}, metal A). Metal A is coordinated by the 3′-OH, the α-phosphate, and Asp residues of structural motifs A and C. This metal lowers the pK_a of the 3′-OH facilitating catalysis at physiological pH. (Adapted from Liu and Tsai 2001)

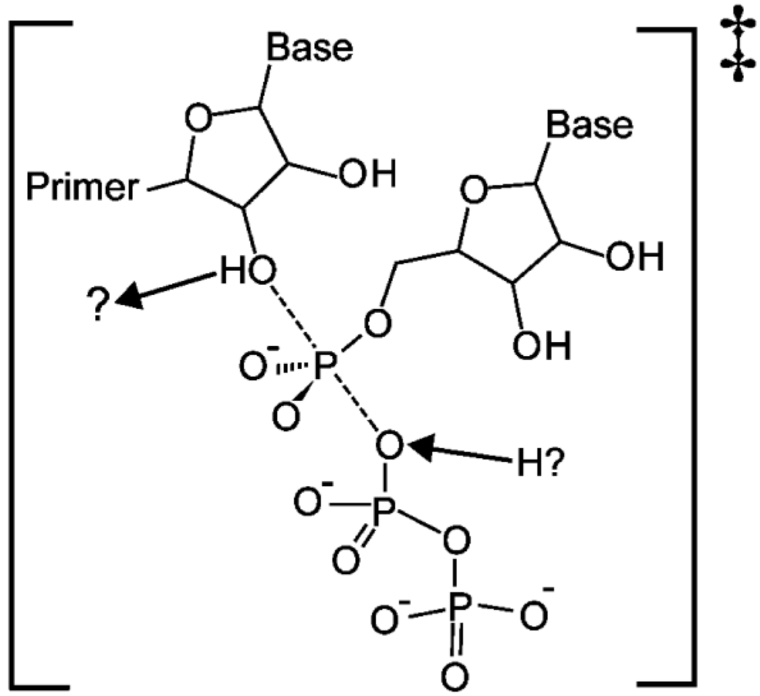

Fig. 4.
Pentavalent phosphorane transition state. During the nucleotidyl transfer reaction, two proton transfer reactions must occur. The proton from the 3′-OH nucleophile must be removed; a proton must be donated to the pyrophosphate leaving group. To date there is no information on these steps of the nucleotidyl transfer reaction

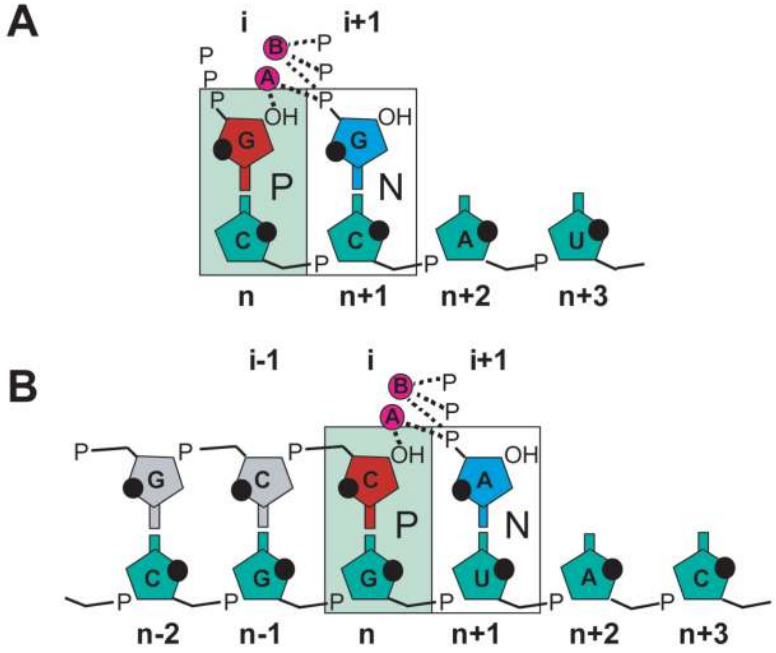

Fig. 5.

A, B De novo initiation and elongation complexes. **A** De novo initiation of RNA synthesis involves binding of the initiating nucleotide (GTPi; *red*) at the priming or initiation site (P-site;*green box*) and binding of the first NTP substrate (GTPi+1; *blue*) to the nucleotide binding site (N-site; *white box*). Specific binding sites for divalent cations (*pink circles A* and *B*) are shown in close proximity to the α-, β-, and γ-phosphates of the first nucleotide substrate. **B** Elongation complex. Nucleotide addition during elongation involves binding of the nascent RNA primer strand, positioning of the 3′-terminal nucleotide in the P-site, and binding of the first NTP substrate (i+1, *blue*) to the nucleotide binding site (N-site; *white box*)

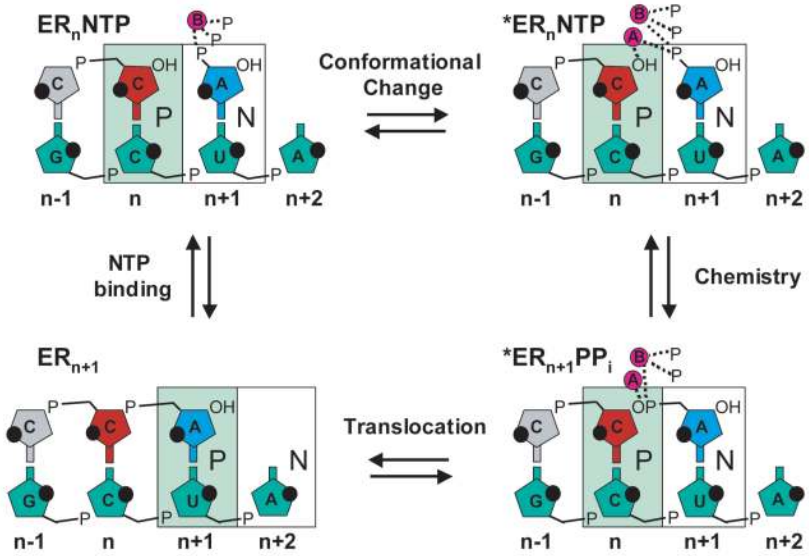

Fig. 6.

Elongation cycle. The stages of RNA synthesis can be divided into four steps: nucleotide binding (step 1), a conformational-change step, thought to be orientation of the triphosphate for catalysis (step 2), chemistry (step 3), and translocation (step 4)

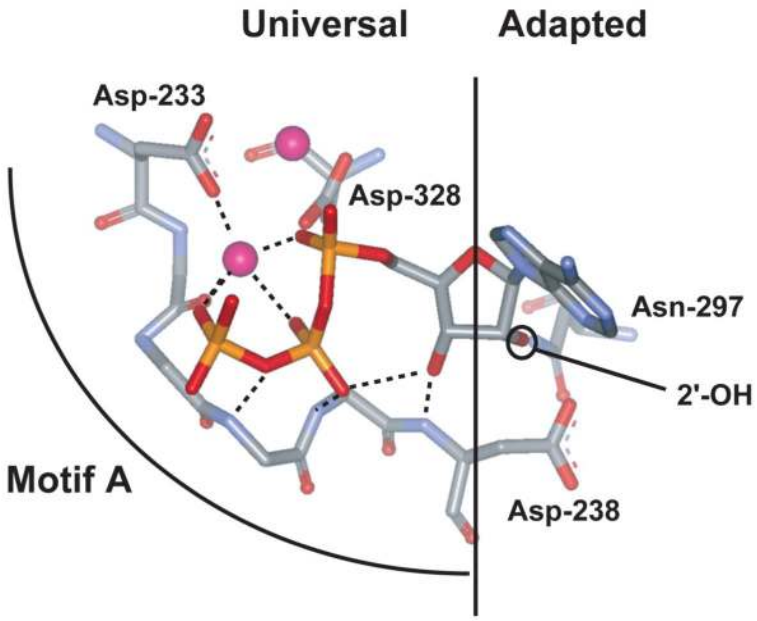

Fig. 7.

Structural basis for fidelity. The nucleotide-binding pocket of all nucleic acid polymerases with a canonical "palm"-based active site is highly conserved. The site can be divided into two parts: a region that has "universal" interactions mediated by conserved structural motif A that organize the metals and triphosphate for catalysis, and a region that has "adapted" interactions mediated by conserved structural motif B that dictate whether ribo- or 2′deoxyribonucleotides will be utilized. In the classical polymerase, there is a motif A residue located in the sugar-binding pocket capable of interacting with the motif B residue(s) involved in sugar selection. This motif A residue in other polymerases could represent the link between the nature of the bound nucleotide (correct vs incorrect) to the efficiency of nucleotidyl transfer as described herein for Asp-238 of $3D^{pol}$. (Gohara et al. 2004)

Fig. 8.

Alignment of conserved regions of RNAi RdRPs. Comparison of putative RdRP amino acid sequence from different organisms including tomato plant, *Neurospora* (*QDE-1*), *C. elegans* (*EGO-1, RRF-1*), *Arabidopsis* (*SDE1*), and *Dictyostelium discoideum* (*RrpA*). Amino acids in *red* indicate conserved residues in all sequences in the alignment. Those in *blue* and *green* indicate conservative substitutions and semi-conservative substitutions, respectively

Table 1

Crystal structures of RdRPs

Virus	PDB	Res. (Å)	Details	Reference(s)
A. Apo and metal-liganded polymerase structures				
PV type 1	1RDR	2.4	Partial structure, non-native N-terminus	Hansen et al. 1997
	1RA6	2.0	Full-length with native N-terminus	Thompson and Peersen 2004
	1RAJ	2.5	68-residue N-terminal truncation	
	1TQL	2.3	G1A N-terminal residue mutant	
HRV-1B	1XR6	2.5	Full-length complex with K^+	Love et al. 2004
HRV-14	1XR5	2.8	Full-length complex with Sm^{3+}	
HRV-16	1XR7	2.3	Full-length native	
	1TP7	2.4	Full-length native with C-terminal His-tag	Appleby et al. 2005
FMDV	1UO9	1.9	Full-length native	Ferrer-Orta et al. 2004
RHDV	1KHV	2.5	Full-length complex with Lu^{3+}	Ng et al. 2002
	1KHW	2.7	Full-length complex with Mn^{2+}	
NV	1SH0	2.2	Full-length native	Ng et al. 2004
	1SH2	2.3		
	1SH3	2.9		
	2B43	2.3	Full-length native	N/A
HCV	1C2P	1.9	21-residue C-terminal truncation	Lesburg et al. 1999
	1CSJ	2.8	55-residue C-terminal truncation	Bressanelli et al. 1999
	1NB4	2.0	21-residue C-terminal truncation+C-terminal His-tag	O'Farrell et al. 2003
	1QUV	2.5	21-residue C-terminal truncation	Ago et al. 1999
BVDV	2CJQ	2.6	Residues 92-672, not domain-swapped	Choi et al. 2006
	1S48	3.0	Residues 92-679, domain-swapped N-terminus	Choi et al. 2004
	1S4F	3.0	Residues 92-674, domain-swapped N-terminus	
Reovirus	1MUK	2.5	Full-length native	Tao et al. 2002
Phage ϕ6	1HHS	2.0	Full-length complex with Mn^{2+}	Butcher et al. 2001
	1HI8	2.5	Selenomethionine derivative with Mg^{2+}	
	1WAC	3.0	Initiation platform mutant	Laurila et al. 2005b
B. RdRP complexes with NTPs, RNA, and proteins				
PV type 1	1RA7	2.3	GTP complex	Thompson and Peersen 2004
FMDV	1WNE	3.0	Primer-template complex	Ferrer-Orta et al. 2004
	2D7S	3.0	VPg complex	Ferrer-Orta et al. 2006
	2F8E	2.9	VPg-UMP complex	
Reovirus	1MWH	2.5	Cap complex	Tao et al. 2002
	1N1H	2.8	Initiation complex with GTP+template RNA	
	1N38	2.8	Short elongation complex	
	1N35	2.5	Long elongation complex	
Phage ϕ6	1HHT	2.9	RNA template complex	Butcher et al. 2001
	1HI0	3.0	Initiation complex with GTP+template RNA	
	1HI1	3.0	ATP complex	
	1UVI	2.1	Complex with 6 nt RNA	Salgado et al. 2004
	1UVJ	1.9	Complex with 7 nt RNA	
	1UVK	2.4	Dead-end complex	
	1UVL	2.0	Complex with 5 nt RNA, conformation A	
	1UVM	2.0	Complex with 5 nt RNA, conformation B	
	1UVN	3.0	Ca^{2+} inhibition complex+RNA+NTPs	
C. RdRP complexes with inhibitors				
HCV	1GX5	1.7	GTP+Mn^{2+} complex	Bressanelli et al. 2002
	1GX6	1.8	UTP+Mn^{2+} complex	
	1NB6	2.6	UTP complex	O'Farrell et al. 2003
	1NB7	2.9	U_4 complex	
	1NHU	2.0	Non-nucleoside inhibitor complexes	Wang et al. 2003
	1NHV	2.9		
	1OS5	2.2	Non-nucleoside inhibitor complex	Love et al. 2003
	1YVF	2.5	Non-nucleoside inhibitor complexes	Pfefferkorn et al. 2005a,b
	1Z4U	2.8		
	1YVX	2.0	Non-nucleoside inhibitor complexes	Biswal et al. 2005
	1YVZ	2.2		
	2AWZ	2.1	Covalent inhibitor complexes	Powers et al. 2006
	2AX0	2.0		
	2AX1	2.1		
	2BRK	2.3	Non-nucleoside inhibitor complexes	Di Marco et al. 2005
	2BRL	2.4		
	2D3U	2.0	Non-nucleoside inhibitor complexes	Biswal et al. 2006
	2D3Z	1.8		
	2D41	2.1		
	2GC8	2.2	Non-nucleoside inhibitor complex	Gopalsamy et al. 2006

BVDV, bovine viral diarrhea virus; FMDV, foot-and-mouth disease virus; HCV, hepatitis C virus; HRV, human rhinovirus; N/A, Reference not currently available; NV, norovirus; PDB, Protein Data Bank; PV, poliovirus; Res., maximum resolution limit of diffraction; RHDV, rabbit hemorrhagic disease virus