Structural Insights into Mechanisms of Catalysis and Inhibition in Norwalk Virus Polymerase*

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Crystal structures of Norwalk virus polymerase bound to an RNA primer-template duplex and either the natural substrate CTP or the inhibitor 5-nitrocytidine triphosphate have been determined to 1.8 Å resolution. These structures reveal a closed conformation of the polymerase that differs significantly from previously determined open structures of calicivirus and picornavirus polymerases. These closed complexes are trapped immediately prior to the nucleotidyl transfer reaction, with the triphosphates of the nucleotides poised for reaction to the 3'-hydroxyl group of the RNA primer. The positioning of the 5-nitrocytidine triphosphate nitro group between the α-phosphate and the 3'-hydroxyl group of the primer suggests a novel, general approach for the design of antiviral compounds mimicking natural nucleosides and nucleotides.

Norwalk virus (NV)4 is the prototype species of the Norovirus genus within the Caliciviridae and is a major cause of gastroenteritis outbreaks in developed countries (1). Unfortunately, effective treatments are not currently available for many important diseases caused by NV and related RNA viruses. The virally encoded RNA-dependent RNA polymerase (RdRP) is the central enzyme required for replication (2) and is one of the key targets for the development of novel antiviral agents. Recently, 5-nitrocytidine triphosphate (NCT) was identified as a potent inhibitor of picornaviral polymerases, and the nucleoside 5'-nitrocytidine was found to have low toxicity and significant antiviral activity in a cultured cell viral infection model (3). A structural and mechanistic basis for rationalizing the inhibitory activity of NCT and related inhibitors is currently lacking because of a shortage of high resolution structural information on RdRP replication complexes.

Details on the structure and mechanism of viral RdRPs are clearly required to understand the replication of RNA viruses and to develop more effective antiviral agents. Previous structural studies of viral RdRPs from positive strand RNA viruses and double-strand RNA viruses indicate that the general features of RdRP architecture are highly conserved throughout a diverse range of viruses (reviewed in Refs. 2 and 4). The three-dimensional arrangement of N-terminal, fingers, palm, and thumb domains, as well as the active site residues in motifs A–F are nearly universally shared (5).

The structural conservation seen in RdRPs suggests that the enzymatic mechanism of nucleotidyl transfer is also highly conserved. Studies primarily on poliovirus RdRP have revealed many of the basic features underlying the nucleotidyl transfer reaction in RdRPs (6, 7). These studies and others indicate that RdRPs, like other polynucleotide polymerases, follow a five-step reaction cycle involving (i) the binding of an NTP complex to the active site, (ii) conformational changes of the thumb domain, which are necessary for the formation of a new thumb domain active site, (iii) nucleotidyl transfer and translocation, (iv) a second conformational change, and finally (v) the release of pyrophosphate (see Fig. 1). Experimentally determined structural information is clearly needed to corroborate and further elaborate the different states identified from kinetic studies, but trapping these different states has proven to be very challenging in RdRPs and other polymerases. Crystal structures of RdRP-RNA-NTP complexes from reovirus (8) and foot-and-mouth disease virus (FMDV) (9) have revealed important aspects of RNA and NTP binding, but key details of the closed

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4 The abbreviations used are: NV, Norwalk virus; FMDV, foot-and-mouth disease virus; HIV, human immunodeficiency virus; NCT, 5-nitrocytidine triphosphate; RdRP, RNA-dependent RNA polymerase; DTT, dithiothreitol; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonic acid.

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Norwalk Virus RdRP-RNA-NTP Complexes

catalytic complex formed in step iii remain poorly determined. The reovirus RdRP-RNA-GTP initiation complex (Protein Data Bank code 1N1H) contains two divalent metal ions at the active site and the NTP bound near the RNA primer terminus. Unfortunately, the 2.8-Å diffraction limit prevents a detailed analysis of molecular conformation, hydrogen bonding, and water structure at the active site. The FMDV structures were determined at a similar resolution (2.5–3.0 Å) and appear to be open complexes trapped at steps ii or v. The NTP is bound too far from the primer terminus for the nucleotidyl transfer reaction to occur in the open complexes, and one of the two divalent metal ions needed for the two-metalion mechanism of catalysis (10) is missing from the active site.

To provide a more detailed structural basis for understanding the nucleotidyl transfer reaction in viral RdRPs, we have determined high resolution structures of NV RdRP bound to a self-complementary oligonucleotide (5′-UGCCCGGG-3′), two Mn$^{2+}$ ions, and either the natural substrate CTP or the nucleotide inhibitor NCT. These structures reveal for the first time details of ternary RdRP-RNA-NTP complexes trapped immediately prior to the nucleotidyl transfer reaction (the closed complex iii in Fig. 1A). Comparisons of the structures of the CTP and NCT complexes also suggest a novel mechanism for inhibition that may be exploited for the design of more effective antiviral agents.

EXPERIMENTAL PROCEDURES

Synthesis of 5-Nitrocytidine Triphosphate (NCT) and RNA—NCT was synthesized as previously described (3). The 5′-UGCCCGGG-3′ sequence was synthesized by the University of California Core DNA Services facility (University of California). The desalted oligonucleotide was dissolved at a concentration of 3.5 mM RNA duplex (7 mM single-stranded RNA) in 1 mM sodium citrate, pH 6.2, 50 mM potassium chloride. To anneal the self-complementary oligonucleotide into a duplex form, the oligonucleotide was heated to 80 °C and cooled at the rate of 5 °C/min using a thermocycler.

Expression and Purification of NV Polymerase—NV polymerase was expressed and purified as previously described (11), except for the following modifications. 1-Liter cultures of Escherichia coli XL1-Blue transformed with pGEX-NV-3D were induced with 0.2 mM isopropyl-β-D-thiogalactoside and then grown at 25 °C for 20 h. The cells were harvested by centrifugation and resuspended in 30 ml of buffer A (50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 0.25 mM EDTA). The cells were lysed by sonication in the presence of lysozyme and DNase I. The concentration of sodium chloride was then raised to 0.3 M, and polyethyleneimine (Sigma) was added dropwise to a final concentration of 0.1% (w/v) to help remove nucleic acids. The extract was clarified by centrifugation and loaded sequentially onto two 2-ml columns of glutathione-Sepharose 4B (GE Healthcare) equilibrated in buffer B (20 mM Tris-Cl, 450 mM NaCl, 1 mM EDTA, 4 mM DTT). The columns were washed with 50 ml buffer of B and 20 ml of buffer C (25 mM Na-HEPES, pH 7.5, 100 mM NaCl, 0.5 mM EDTA, 4 mM DTT, and 10% glycerol). Thrombin (20 units, GE Healthcare) was diluted into 1 ml of buffer C and incubated for 90 min with each of the two 2-ml columns of glutathione-Sepharose. Each column was eluted with 10 ml of buffer C, and the thrombin was inactivated with 0.5 mM 4-(2-aminoethyl) benzenesulfonyl fluoride. The digestion was repeated for each column, and all of the eluates were pooled. The pooled eluates were dialyzed overnight against 1 liter of buffer D (25 mM Na-HEPES, pH 7.0, 25 mM of NaCl, 0.5 mM EDTA, 4 mM DTT, and 15% glycerol) and then loaded onto a 5-ml Hitrap SP-Sepharose column. The column was washed with 30 ml of buffer D containing 30 mM NaCl, and NV polymerase was eluted with an 80-ml linear gradient of buffer D containing 30–150 mM NaCl. Peak fractions were diluted with RNase-free buffer E (25 mM Tris-HCl, pH 8.0, and 15% glycerol (Fluka)) to reduce the NaCl concentration to 20 mM and loaded onto a Sartobind Q15F filter (Sartorius). The filter was washed with buffer E and eluted with RNase-free buffer F (10 mM Tris, pH 8.0, 300 mM NaCl, 6 mM DTT, and 15% glycerol). NV polymerase was diluted into buffer G (10 mM Tris, pH 8.0, 150 mM NaCl, 6 mM DTT, and 15% glycerol) and concentrated with an Amicon Ultrafree-4 (10,000 molecular weight cut-off) centrifugal filter to 7.5 mg/ml (0.13 mM, concentration estimated by UV absorption using a calculated extinction coefficient of 1.43 OD/(mg/ml)). NV polymerase was filtered with a 0.22-μm Spin-X cellulose acetate centrifugal filter (Costar) and stored on ice.

Crystallization—CHAPS (0.2% w/v) and Superase-In ribonuclease inhibitor (1 unit/μl; Ambion) were added to NV polymerase prior to crystallization. The annealed RNA duplex (18 μl) was mixed with 4.5 μl of NCT or CTP (25 mM) and 31.5 μl water to prepare a mixture containing 1.16 mM RNA duplex and 2.1 mM NTP. A reservoir solution containing 16% polyethylene glycol 8000, 25% glycerol, 100 mM Tris-HCl, pH 7.0, 50 mM KCl, 4 mM MgCl$_2$, 10 mM MnCl$_2$, and 0.1% 2-mercaptoethanol was prepared.

Reservoir solution (3 μl) was mixed with the RNA-NTP mixture (6 μl) and NV polymerase (4 μl). This mixture was suspended as a hanging drop and equilibrated against 1 ml of reservoir solution at 22 °C. Crystals appeared within a week and grew to full size (~0.2 × 0.1 × 0.05 mm) after 2–3 weeks.

Crystal Structure Determination—A single crystal (0.2 × 0.1 × 0.05 mm) grown in the presence of NCT was quickly mounted in a polymer fiber loop (Hampton Research) and flash-cooled in a nitrogen gas stream at ~100 K. Diffraction data were initially measured using a MAR 345 image plate and X-rays produced with a rotating copper anode (Rigaku RUH3R). The crystal was stored in liquid nitrogen and transferred to the Canadian Light Source beamline 08ID-1 for data collection using a Marmosaic CCD225 detector. The data were processed and scaled using XDS (12) (data quality and refinement statistics are given in Table 1). The structure of unliganded NV polymerase (Protein Data Bank code 1SH0) was used as the search model for molecular replacement calculations using PHASER (13). Very clear solutions were obtained for the rotational (z = 20.8) and translational (z = 26.3) placement of a single polymerase molecule, yielding a solvent content of 59% and V$_m$ = 3.0. Initial electron density maps clearly indicated that an RNA duplex, metal ions, and NTP were present. Initial maps also showed that the C-terminal segment was missing and that residues 437–452 in the thumb domain needed to be rebuilt. A model for the RNA segment was built with reference
to the FMDV-RNA complexes and high resolution RNA helices deposited in the NDB. Coot (14) and MI-Fit (15) were used for model building, and Refmac (16) was used for refinement. The electron density maps clearly showed the incorporation of a single NCT residue in the distal end of the primer-template duplex bound to NV RdRP. This residue must have been added by the polymerase onto this end of the primer, and the extended duplex and NTP bound in the active site cleft (Figs. 1B and 2). As predicted previously, RNA binding displaces the C-terminal tail from the active site cleft of NV RdRP (11). Residues 489–516 are not defined by electron density, suggesting that the C-terminal tail is unstructured. The binding of RNA also causes the central helix of the thumb domain (residues 435–449) to rotate by 22°, thus inducing the formation of a binding groove for the primer strand (Fig. 2). The rotation of this helix appears to be coupled to the movement of the C-terminal tail away from the active site cleft in the unbound conformation of NV and Sapovirus RdRPs, the proximal portion of the C-terminal tail (residues 489–502 in NV) packs against this central helix, acting as a brace to resist the outward rotation of the helix seen in the RNA-bound conformation (11, 19). The movement of the C-terminal tail away from the active site cleft allows the central helix to rotate and interact with the primer strand and minor groove of the primer-template duplex.

These conformational changes are reminiscent of changes seen in DNA polymerases following the binding of DNA and dNTPs (20) and are larger than the conformational changes previously seen in viral RdRPs crystallized in different packing environments (21, 22) or bound to allosteric inhibitors (23).

### RESULTS

**Overall Structure of NV RdRP-RNA-NTP Complexes**—Ternary complexes of NV RdRP reveal an RNA primer-template duplex and NTP bound in the active site cleft (Figs. 1B and 2). As predicted previously, RNA binding displaces the C-terminal tail from the active site cleft of NV RdRP (11). Residues 489–516 are not defined by electron density, suggesting that the C-terminal tail is unstructured. The binding of RNA also causes the central helix of the thumb domain (residues 435–449) to rotate by 22°, thus inducing the formation of a binding groove for the primer strand (Fig. 2). The rotation of this helix appears to be coupled to the movement of the C-terminal tail away from the active site cleft in the unbound conformation of NV and Sapovirus RdRPs, the proximal portion of the C-terminal tail (residues 489–502 in NV) packs against this central helix, acting as a brace to resist the outward rotation of the helix seen in the RNA-bound conformation (11, 19). The movement of the C-terminal tail away from the active site cleft allows the central helix to rotate and interact with the primer strand and minor groove of the primer-template duplex.

These conformational changes are reminiscent of changes seen in DNA polymerases following the binding of DNA and dNTPs (20) and are larger than the conformational changes previously seen in viral RdRPs crystallized in different packing environments (21, 22) or bound to allosteric inhibitors (23). The conformational changes seen in the NV RdRP-RNA-NTP complex are also much larger than the changes seen in the FMDV RdRP-RNA-NTP complexes, in which the conformation...
Norwalk Virus RdRP-RNA-NTP Complexes

![Diagram](image)

FIGURE 1. A, general kinetic scheme for nucleotide incorporation in RdRPs and other polymerases (36, 37). E, RdRP enzyme; n, RNA oligonucleotide containing n nucleotides; n + 1, RNA oligonucleotide containing n + 1 nucleotides; NTP, nucleoside triphosphate; PP, pyrophosphate. B, stereoscopic view of the NV RdRP-ErNTP complex with the primer RNA strand colored yellow, template RNA strand in magenta, fingers domain in blue, palm domain in green, and thumb domain in red.

The intrinsic network of interactions surrounding the bound nucleotide reveals at high resolution several key elements underlying substrate binding and catalysis in RdRPs (Fig. 3). The NTP bases form hydrogen bonds with the complementary guanosine base of the template and stack against the 3’-terminal base of the primer, as well as the Arg-182 guanidino group (Fig. 3). Arg-182 is highly conserved in RdRPs (Motif F3) (5) and is near the NTP α-phosphate (3.5 Å). Arg residues occupy a similar position in all viral RdRPs with known structures, as well as distantly related enzymes like HIV reverse transcriptase (Table 2). The cytosine and nitrocytosine bases stack tightly against Arg-182, unlike the looser interactions seen in the more open FMDV complexes (9).

The interactions made by the ribose and triphosphate moieties of the bound nucleotide indicate that the NV RdRP complexes are perfectly positioned for nucleotidyl transfer (Fig. 3). The 2’-OH accepts a hydrogen bond from Asn-309 and donates a hydrogen bond to Ser-300, which in turn donates a hydrogen bond to Asp-247. This pattern of hydrogen bonding reveals how these highly conserved residues (equivalent to poliovirus RdR residues 297, 288, and 238, respectively) distinguish ribonucleotides from 2’-deoxyribonucleotides (24–26). A similar network of hydrogen bonds is seen in one of the reovirus-NTP-RNA complexes (8), but the more open FMDV complexes or binary poliovirus RdRP-NTP complexes do not form the same hydrogen-bonding pattern, suggesting that ribonucleotide selection occurs in the closed complex formed immediately before catalysis (9, 27).

Two Mn2+ ions also coordinate to three highly conserved Asp residues and the NTP triphosphate moiety to mediate catalysis through the two-metal-ion mechanism (10, 28) (Fig. 3). Metal ion A octahedrally coordinates to the 3’-OH nucleophile of the primer, the NTP α-phosphate, the carboxylate groups of Asp-242, Asp-343, and Asp-344, as well as a water molecule. By donating hydrogen bonds to the negatively charged primer phosphodiester and NTP α-phosphate groups, this water molecule may be activated to become a better general base catalyst (7), thus allowing it to abstract a proton from the primer 3’-OH (2.8 Å away) following a mechanism similar to that proposed for DNA polymerase β (29, 30). Metal ion B also octahedrally coordinates to the side chain carboxylate groups of Asp-242 and Asp-343, the main chain carbonyl group of Tyr-243, as well as a single oxygen atom from each of the α, β, and γ phosphate groups of the NTP.

DISCUSSION

Trapping of a Preinsertion Conformation of NV RdRP via Constraints from Crystal Packing—The recombinant form of NV RdRP used in these studies is enzymatically active (11), and there is surprisingly clear evidence of this activity in the crystal structure itself. Because the primer-template oligonucleotide is self-complementary and symmetrical, each oligonucleotide duplex contains two 5’-overhangs where primer extension can occur. In the NV RdRP-RNA-NTP complexes, the end of the duplex found in the enzyme active site reveals the presence of a 3’-terminal guanosine residue in the primer and a 3’-GU-5’ sequence for the single-stranded 5’-overhang of the template strand. CTP or NCT forms a Watson-Crick base pair with the guanosine residue of the template, but crystal packing interactions at the distal end of the primer-template duplex appear to impede the translocation event following nucleotidyl transfer, thus trapping this complex. Surprisingly, the end of the primer-template duplex that is located distal from the active site reveals that either a cytidine or 5-nitrocytidine residue has been added to the primer strand, presumably prior to crystallization. This residue must have been added when this end of the duplex was bound at the active site. Following the addition of this single residue, the duplex must be dissociated from the enzyme and bound in the opposite orientation prior to crystallization.
The packing arrangement of molecules in this crystal form appears to be critical for arresting the normal reaction cycle of NV RdRP at the point immediately prior to nucleotidyl transfer. Previous studies on a wide range of polynucleotide polymerases indicate the necessity of a translocation step immediately following nucleotidyl transfer (20). All of the previously determined crystal structures of polymerases trapped at this pre-insertion step of the reaction cycle have been chemically trapped by the use of substrate analogues like primer strands missing the 3′-hydroxyl group or a nucleoside 5′-(α,β)-imido-triphosphate analogue (29, 31–35). Such complexes provide a valuable but slightly distorted view of the natural complex. The fortuitous molecular packing arrangement in the NV RdRP-RNA-NTP crystal form has allowed us to trap a critical intermediate in the reaction cycle without introducing distortions through chemical modifications. The most similar, active polymerase complex for which a high resolution structure has been previously determined is the DNA polymerase β-DNA-2′:deoxyuridine-5′-[(α,β)-imido]triphosphate complex determined to 1.65 Å resolution (29). The trapped catalytic complex seen in DNA polymerase β closely resembles the NV RdRP ternary complex, including the presence of water molecules that may participate in catalysis.

A Novel and General Mechanism of RdRP Inhibition by NCT—The structure of the NCT complex suggests a novel mechanism of inhibition. The nitro group is 2.7 Å from the NCT α-phosphate and 4.3 Å from the 3′-OH nucleophile of the primer. If the pentavalent transition state forms halfway between the ground state positions of the 3′-OH nucleophile and the α-phosphate electrophile, the proximity of the negatively charged nitro group (~3 Å) to the negatively charged oxygen atoms of the transition state could destabilize the transition state of the nucleotidyl transfer reaction. The stacking of Arg-182 and the 3′-end of the primer against the NTP base, as well as base-pairing interactions with the template strand all act to fix the position of the NTP base, thus keeping the nitro group close to the negatively charged transition state. Finally, the nitro group displaces a water molecule that forms hydrogen bonds with the α-phosphate.
FIGURE 3. Stereoscopic views of the active site of the NCT (A and C) and CTP (B and D) complexes. In panels A and B, coordination bonds (red dashes) with Mn^{2+} ions A and B (pink spheres), and hydrogen bonds (red dashes) between the bound nucleotide (magenta), key water molecules (red spheres), and the protein are drawn. In panels C and D, Arg-182, the bound nucleotide, Mn^{2+} ions, and the terminal nucleotide of the primer were removed prior to 20 rounds of refinement and (|F_o| - |F_c|) electron density map calculation (3σ contour). Figs. 2 and 3 were prepared using PyMOL (38).
with the $\alpha$-phosphate of CTP and the 3'-terminal phosphodiester of the primer in the ground state. Loss of this water molecule in the NCT complex could further destabilize the transition state by reducing hydration.

Apart from the positioning of the nitro group and displacement of a water molecule from the vicinity of the reaction pathway, there are a few additional differences between the CTP and NCT complexes that may also contribute to the mechanism of inhibition of NCT. One important difference is a longer than expected coordination distance of 2.6 Å between Asp-344 and metal ion A in the NCT complex, when compared with the expected distance of 2.1 Å seen in the CTP complex (Fig. 3). The structures of the two complexes do not indicate a direct connection between the presence of the nitro group of NCT and effects on metal ion coordination. If this altered coordination geometry is an indirect effect of the nitro group from NCT, however, then this longer coordination distance may be related to an impairment of the nucleotidyl transfer reaction.

A second difference between the NCT and CTP complexes is the positioning of the side chain of Arg-182 (Fig. 3). The $\chi_4$ torsion angle of Arg-182 differs by 180° between the conformations adopted in the NCT and CTP complexes. As a result, the conformation adopted in the NCT complex appears to form better $\pi$-stacking and electrostatic interactions with the nitro group (Fig. 3). The guanidino group of the side chain in both complexes forms good stacking interactions with the central portion of the base. Because the guanidino group is closer to the nitro group in the NCT complex, however, this group is farther from the $\beta$-phosphate than in the CTP complex. As a result, the Ne atom of Arg-182 forms a water-mediated hydrogen bond with the $\beta$-phosphate in the NCT complex, and one of the terminal nitrogen atoms forms a direct hydrogen bond with the oxygen atom bridging the $\alpha$- and $\beta$-phosphates. In the CTP complex, however, the two terminal nitrogen atoms of Arg-182 form direct hydrogen bonds with the oxygen atom bridging the $\alpha$- and $\beta$-phosphates, as well as a nonbridging oxygen atom on the $\beta$-phosphate. By altering the position of the guanidino group relative to the triphosphate moiety, the 5-nitro group may reduce the ability of Arg-182 to stabilize the pyrophosphate leaving group during the course of nucleotidyl transfer.

The mechanism of inhibition suggested by the structure of the NCT complex provides a general, new approach for inhibitor design. Large, negatively charged substituents at the 5- and 6-positions of pyrimidine bases may be effective at destabilizing the transition state without disturbing essential substrate binding interactions. Effective inhibitors should preserve favorable $\pi$-stacking and electrostatic interactions with the primer and Arg-182, as well as base pairing with the template. Substituents like the 5-nitro group that interact well with Arg-182 may bind viral RdRPs more effectively than host polymerases, because Arg is almost universally conserved at this position in viral RdRPs, but Arg is not conserved at this position in any of the family A, B, and Y DNA polymerases, eukaryotic RdrPs, and phage, prokaryotic, and eukaryotic RNA-dependent RNA polymerases. A neutral aromatic residue seems to occupy this position in most other polymerases.

The NV RdRP-RNA-NTP complexes reveal for the first time several structural features underlying the nucleotidyl transfer reaction that may be exploited for developing novel antiviral inhibitors. The synthesis and evaluation of novel inhibitors will provide powerful and practical tests for the mechanisms proposed in this paper.

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TABLE 2
Motif F3 in RdRPs and HIV reverse transcriptase with known three-dimensional structures

| Virus   | Genome | Family        | Protein Data Bank code | Start | End | F3 motif   |
|---------|--------|---------------|------------------------|-------|-----|------------|
| NV      | ssRNA(+)| Caliciviridae | 1SH4                   | 179   | 184 | IKKRLL     |
| RHDV    | ssRNA(+)| Caliciviridae | 1KHW                   | 185   | 190 | GKRRL     |
| SV      | ssRNA(+)| Caliciviridae | 2CKW                   | 179   | 184 | GKRRL     |
| PV      | ssRNA(+)| Picornaviridae| 2IM0                   | 171   | 176 | GKSRL     |
| HRV     | ssRNA(+)| Picornaviridae| 1TP7                   | 171   | 176 | GKSRL     |
| FMDV    | ssRNA(+)| Picornaviridae| 1U09                   | 176   | 181 | GKTIV     |
| HCV     | ssRNA(+)| Flaviviridae  | 1CSJ                   | 155   | 160 | KPARL     |
| DENV    | ssRNA(+)| Flaviviridae  | 2J7U                   | 203   | 208 | KGRAI     |
| WNV     | ssRNA(+)| Flaviviridae  | 2HCN                   | 161   | 166 | KGRAI     |
| BVDV    | ssRNA(+)| Flaviviridae  | 1S48                   | 217   | 217 | KPRVI     |
| IBDV    | dsRNA  | Birnaviridae  | 2PGG                   | 302   | 307 | TKTRNI    |
| Phi6    | dsRNA  | Cystoviridae  | 1HHS                   | 267   | 272 | ERRRTA    |
| Reovirus| dsRNA  | Reoviridae    | 1N35                   | 523   | 528 | RRPRSI    |
| HIV     | ssRNA-RT(+) | Betroviridae | 1RTD                   | 69    | 74  | TKWRL     |
Norwalk Virus RdRP-RNA·NTP Complexes

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