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Picornavirus RNA polyadenylation by 3D^{pol}, the viral RNA-dependent RNA polymerase



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ABSTRACT

Poly(A) tails are functionally important features of all picornavirus RNA genomes. Some viruses have genomes with relatively short poly(A) tails (encephalomyocarditis virus) whereas others have genomes with longer poly(A) tails (polioviruses and rhinoviruses). Here we review the polyadenylation of picornavirus RNA as it relates to the structure and function of 3D^{pol}. Poliovirus 3D^{pol} uses template-dependent reiterative transcription mechanisms as it replicates the poly(A) tails of viral RNA (Steil et al., 2010). These mechanisms are analogous to those involved in the polyadenylation of vesicular stomatitis virus and influenza virus mRNAs. 3D^{pol} residues intimately associated with viral RNA templates and products regulate the size of poly(A) tails in viral RNA (Kempf et al., 2013). Consistent with their ancient evolutionary origins, picornavirus 3D^{pol} and telomerase reverse transcriptase (TERT) share structural and functional features. Structurally, both 3D^{pol} and TERT assume a “right-hand” conformation with thumb, palm and fingers domains encircling templates and products. Functionally, both 3D^{pol} and TERT use template-dependent reiterative transcription mechanisms to synthesize repetitive sequences: poly(A) tails in the case of picornavirus RNA genomes and DNA telomeres in the case of eukaryotic chromosomes. Thus, picornaviruses and their eukaryotic hosts (humans and animals) maintain the 3' ends of their respective genomes via evolutionarily related mechanisms.

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1. Introduction

Picornaviruses are ubiquitous and infect a diverse range of animals, insects and plants. The great variety of picornaviruses is consistent with their ancient origins (Koonin et al., 2008). Based on shared molecular features, picornaviruses are taxonomically organized by order, family, genus, species, and virus (Table 1) (Le Gall et al., 2008). The Picornavirales order includes five families: Picornaviridae, Dicistroviridae, Iflaviridae, Marnaviridae and Secoviridae (Le Gall et al., 2008). Hundreds of human and animal pathogens are distributed among 26 genera and 46 species groups in the Picornaviridae family (Knowles et al., 2012). Humans (CDC, 2010), apes and monkeys (Van Nguyen et al., 2014), pigs (Van Dung et al., 2014), cattle (Grubman and Baxt, 2004), mice (Denis et al., 2006), seals (Kapoor et al., 2008), shrimp (Aranguren et al., 2013), turtles (Farkas et al., 2014), birds (Boros et al., 2013) and bees (Chen et al., 2014) are but a few of the hosts frequently infected by these widespread viruses.

Poly(A) tails are a characteristic feature of viral RNA genomes in the Picornavirales order, with one potential exception (Sequiviruses) (Le Gall et al., 2008). There are inconsistent reports regarding the presence or absence of a poly(A) tail in some sequiviruses in the Secoviridae family: parsnip yellow fleck virus, lettuce mottle virus and dandelion yellow mosaic virus (Jadao et al., 2007; Menzel and Vetten, 2008; Turnbull-Ross et al., 1992). Two groups failed to detect poly(A) tails in these sequiviruses (Jadao et al., 2007; Turnbull-Ross et al., 1992) whereas another group reports the presence of a 3' terminal poly(A) tail in parsnip yellow fleck virus (Menzel and Vetten, 2008). Additional characterization of viruses in the Secoviridae family are warranted to confirm the presence or absence of a poly(A) tail in these viruses (Sanfacon et al., 2009). Here, we review the nature of picornavirus poly(A) tails and the manner in which they are maintained during viral replication.

2. Picornavirus RNA genomes and RNA replication

Picornavirus RNA genomes, like the enterovirus genome illustrated here (Fig. 1A), have a number of characteristic features, including a viral protein (VPg) at the 5' end and a poly(A) tail of variable length at the 3' end (Le Gall et al., 2008). One long open reading

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Table 1
Picornaviruses (Picornavirales order).

Family	Genus	Species groups	Type species
<i>Picornaviridae</i>	26 genera: <i>Aphtho-</i> , <i>Aquama-</i> , <i>Avihepato-</i> , <i>Avisi-</i> , <i>Cardio-</i> , <i>Cosa-</i> , <i>Dicipi-</i> , <i>Entero-</i> , <i>Erbo-</i> , <i>Galli-</i> , <i>Hepato-</i> , <i>Hunni-</i> , <i>Kobu-</i> , <i>Megri-</i> , <i>Mischi-</i> , <i>Mosa-</i> , <i>Osci-</i> , <i>Parecho-</i> , <i>Pasi-</i> , <i>Passeri</i> , <i>Rosa-</i> , <i>Sali-</i> , <i>Sapelo-</i> , <i>Seneca-</i> , <i>Tescho-</i> , & <i>Tremoviruses</i> .	Aphthoviruses Cardioviruses Enteroviruses A-H Rhinoviruses A-C others	Foot & mouth disease virus (FMDV) Encephalomyocarditis virus (EMCV) Polio, Coxsackie, Echo & Enteroviruses Rhinoviruses others
<i>Dicistroviridae</i>	<i>Cripavirus</i> <i>Apavirus</i>	<i>Cripivirus</i> <i>Apavirus</i>	Cricket paralysis virus (CrPV) Acute bee paralysis virus
<i>Iflaviridae</i>	<i>Iflavirus</i>	Deformed wing virus Slow bee paralysis virus 7 others	Deformed wing virus Slow bee paralysis virus
<i>Marnaviridae</i>	<i>Marnavirus</i>	Heterosigma akashiwo	Heterosigma akashiwo RNA virus
<i>Secoviridae</i>	<i>Como-</i> , <i>Faba-</i> and <i>Nepoviruses</i> <i>Chera-</i> , <i>Sadwa-</i> , <i>Sequi-</i> , <i>Torrado-</i> & <i>Waikaviruses</i>		Cowpea mosaic virus Parsnip yellow fleck virus

<http://www.picornavirales.org/>
<http://www.picornaviridae.com/>

Table 2
Reiterative transcription and polyadenylation of viral mRNAs.

Virus	RdRP	RNA template	Citations
VSV <i>Rhabdoviridae</i>	L protein	Intergenic U ₇	Schubert et al. (1980) Hunt et al. (1984) Barr and Wertz (2001) Hausmann et al. (1999)
Sendai <i>Paramyxoviridae</i>	L protein	Intergenic U ₇	
Influenza <i>Orthomyxoviridae</i>	PB1	U ₅ adjacent to panhandle	Robertson et al. (1981) Poon et al. (1999) Zheng et al. (1999)
Poliovirus <i>Picornaviridae</i>	3D ^{pol}	Poly(A) tail & VPg-linked poly(U)	Steil et al. (2010) Kempf et al. (2013)

frame encodes capsid proteins (VP1-VP4) and non-structural proteins associated with host cell interactions and RNA replication (2A^{pro}, 2B, 2C^{ATPase}, 3A, 3B^{VPg}, 3C^{pro} and 3D^{pol}). A comparison of *Picornaviridae* RNA genomes reveals some notable differences

between genera (Boros et al., 2014; Le Gall et al., 2008); however, common features suggest shared mechanisms of viral RNA replication. Shared genomic features important for RNA replication include phylogenetically related VPg proteins (Sun et al., 2014),

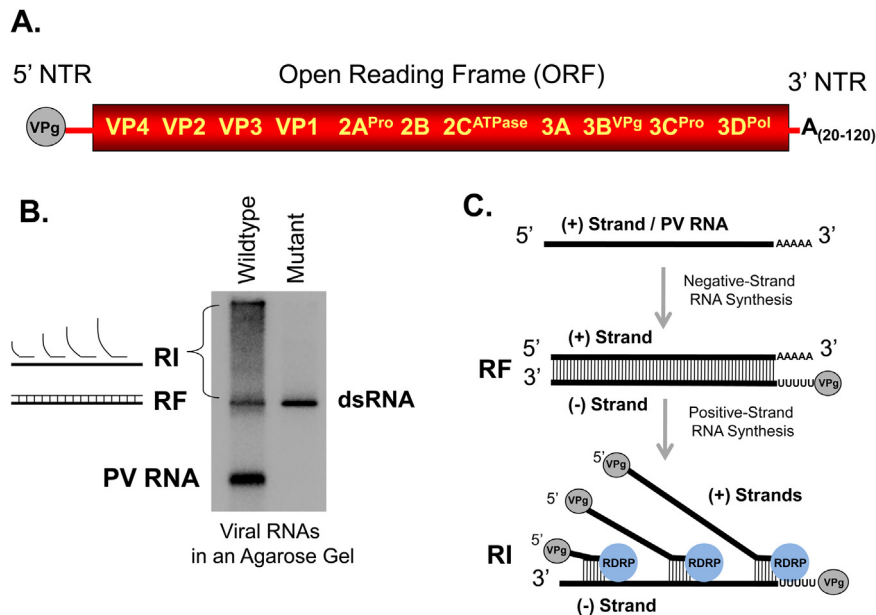


Fig. 1. Enterovirus RNA genomes and viral RNA replication. (A) Picornaviruses have a poly(A) tail of variable length at the 3' end of the viral RNA genome. (B) Cell-free replication of poliovirus RNA. Poliovirus RNA replicons [designated as DNVR27 and RNA2 in (Steil and Barton, 2008)] were incubated in HeLa cell-free translation reactions containing 2 mM guanidine HCl to form preinitiation RNA replication complexes (PIRCs). The PIRC were incubated for 1 h at 37 °C in reaction mixtures containing [α -³²P]CTP and nonradioactive ATP, GTP and UTP. Radiolabeled RNAs were fractionated by electrophoresis in a non-denaturing 1% agarose/Tris–Borate EDTA (TBE) gel and detected by phosphorimaging (Steil, 2008). The wildtype replicon (DNVR 27) has a wildtype 5' terminus whereas the mutant replicon (RNA2) has two non-viral Gs at the 5' end which inhibit positive-strand RNA synthesis (Steil, 2008; Steil and Barton, 2008). (C) Diagram of viral RNA replication. The viral RNA-dependent RNA polymerase (3D^{pol}) replicates the poly(A) tail, making VPg-linked poly(U) sequences at the 5' end of negative-strand RNA. Then, during positive-strand RNA synthesis, 3D^{pol} uses the poly(U) sequences at the 5' end of negative-strand RNA as the template for the polyadenylation of nascent positive-strands.

cis-acting replication elements (CREs) involved in VPg uridylylation (Cordey et al., 2008; Steil and Barton, 2009a), an ATPase required for RNA replication (Sweeney et al., 2010), the viral RNA-dependent RNA polymerase (Lescar and Canard, 2009) and 3' terminal poly(A) tails. While 3D^{pol} is consistently encoded at the 3' end of picornaviral ORFs (Le Gall et al., 2008; Son et al., 2014; Van Dung et al., 2014), CREs are located at variable locations in different picornavirus RNA genomes (Cordey et al., 2008; Steil and Barton, 2009a). CREs have been predicted and/or experimentally defined for various genera and species groups in the *Picornaviridae* family including group A, B and C rhinoviruses (Cordey et al., 2008; McKnight and Lemon, 1998; Yang et al., 2002), group A, B, C and D enteroviruses (Goodfellow et al., 2000, 2003; Paul et al., 2000; Shen et al., 2008; van Ooij et al., 2006b), aphthoviruses (Mason et al., 2002), hepatoviruses (Yang et al., 2008), cardioviruses (Lobert et al., 1999), parechoviruses (Al-Sunaidi et al., 2007) and sapeloviruses (Son et al., 2014). CREs have not been predicted or defined for viruses in the *Dicistroviridae*, *Iflaviridae*, *Marnaviridae* or *Secoviridae* families, so it remains to be determined whether all viruses in the *Picornavirales* order use template-dependent VPg uridylylation during viral RNA replication (Steil and Barton, 2009a). CRE-dependent VPg uridylylation and the initiation of picornavirus RNA synthesis are reviewed elsewhere in this issue by Paul and Wimmer (Paul and Wimmer, 2015). The diagram of RNA replication in Fig. 1C is simplified to emphasize common features of picornavirus replication which likely apply to other viruses throughout the *Picornavirales* order. In particular, we expect that the viral RNA-dependent RNA polymerases of viruses in the *Picornaviridae*, *Dicistroviridae*, *Iflaviridae*, *Marnaviridae* and *Secoviridae* families replicate the poly(A) tails of their RNA genomes (Fig. 1C). As discussed in some detail in this review, there is good evidence to indicate that 3D^{pol} replicates the poly(A) tail of poliovirus RNA (Kempf et al., 2013; Steil et al., 2010). Future experimental work will determine if other viruses in the *Picornavirales* order replicate the poly(A) tail of their respective genomes, as illustrated in Fig. 1C.

Picornavirus RNA genomes serve as both the viral mRNA required for viral protein synthesis and as a template for negative-strand synthesis during viral RNA replication. Following viral mRNA translation, non-structural proteins, in concert with cis-active RNA structures in the viral RNA templates, form membrane-anchored replication complexes in the cytoplasm of infected cells (reviewed in (Steil and Barton, 2009a)). Remarkably, all of the metabolic steps of viral replication (viral mRNA translation, polyprotein processing, RNA replication and virus assembly) are recapitulated in cell-free reactions containing cytoplasmic extracts from uninfected host cells (Molla et al., 1991). Cell-free virus replication, first achieved with poliovirus (Barton and Flanagan, 1993; Molla et al., 1991), was subsequently achieved with both encephalomyocarditis virus (EMCV) (Fata-Hartley and Palmenberg, 2005; Svitkin and Sonenberg, 2003) and rhinovirus RNAs (Todd et al., 1997). A cell-free replication system was also developed for positive-strand RNA plant viruses in the alpha-like and carmo-like virus supergroups (Komoda et al., 2004). The synchronous and sequential nature of viral mRNA translation (Kempf and Barton, 2008a,b), viral RNA replication (Barton and Flanagan, 1997) and virus assembly (Barton and Flanagan, 1993) within cell-free reactions has been exploited to better understand these individual steps of replication. Viral RNA replication is monitored in cell-free reactions by including radiolabelled NTPs in the reactions (Fig. 1B). Radiolabel from NTPs is incorporated into negative- and positive-strand viral RNAs as they are synthesized by the viral RNA-dependent RNA polymerase 3D^{pol}. The viral RNAs radiolabelled in cell-free reactions (Fig. 1B) are consistent with the expected intermediates of RNA replication (Fig. 1C).

Viral RNA replication occurs in sequential steps. First, positive-strand RNA templates are transcribed by 3D^{pol}, the picornavirus

RNA-dependent RNA polymerase, into complementary negative-strand products (Fig. 1C, negative-strand RNA synthesis). VPg and its uridylylated derivatives prime the initiation of negative-strand RNA synthesis (Steil and Barton, 2008, 2009b) using 3'-terminal poly(A) sequences on the viral RNA templates (Sharma et al., 2005), resulting in VPg-linked poly(U) products at the 5' end of negative-strand RNA intermediates (Steil et al., 2010). In turn, negative-strand RNA intermediates are used as templates for positive-strand RNA synthesis (Fig. 1C, positive-strand RNA synthesis). Uridylylated VPg (VPgUpU_{OH}) primes positive-strand RNA synthesis on complementary adenosine bases at the 3' end of negative-strand templates (Sharma et al., 2005; Steil and Barton, 2008, 2009b). Multiple copies of positive-strand RNA are made simultaneously on each negative-strand RNA template, leading to the formation of replicative-intermediate (RI) RNA. Mutations that specifically disable positive-strand RNA synthesis lead to the accumulation of replicative form (RF) RNA (Fig. 1B and C) (Morasco et al., 2003; Murray and Barton, 2003; Steil and Barton, 2008, 2009b).

Notably, 3D^{pol} replicates the poly(A) tail of viral RNA, synthesizing VPg-linked poly(U) at the 5' end of negative-strands (Fig. 1C) (Steil et al., 2010). Subsequently, VPg-linked poly(U) intermediates function as templates for the polyadenylation of nascent positive-strand RNA (Fig. 1C) (Steil et al., 2010).

3. Template-dependent reiterative transcription mechanisms

Viral RNA replication mechanisms need to ensure the faithful replication of viral RNA genomes. To maintain the integrity of picornavirus RNA genomes, it is imperative that poly(A) tails be regenerated on new viral RNAs during each round of viral replication. In theory, the poly(A) tails could be synthesized by host poly(A) polymerases (PAPs) (Laishram, 2014), by the viral RNA-dependent RNA polymerase, 3D^{pol} (as diagramed in Fig. 1C), or both. We found that 3D^{pol} is primarily responsible for the synthesis of poliovirus poly(A) tails (Kempf et al., 2013; Steil et al., 2010). Under normal conditions, 3D^{pol} replicates the poly(A) tail during viral RNA replication (Steil et al., 2010; Kempf et al., 2013). Cellular PAPs have been shown to restore viral poly(A) tails when they are deleted experimentally (Liu et al., 2008; Raju et al., 1999; Tacahashi and Uyeda, 1999; van Ooij et al., 2006); however, cellular PAPs do not appear to impact the overall size of poliovirus poly(A) tails, as 3D^{pol} alanine substitution mutations impact the overall size of poly(A) tails in viral RNA (Kempf et al., 2013). If cellular PAPs were primarily responsible for the size of poly(A) tails, then mutations in 3D^{pol} would not change the overall size of poly(A) tails to the extent that they do (Kempf et al., 2013). Based on these findings, poliovirus 3D^{pol} appears to use template-dependent reiterative transcription mechanisms analogous to those involved in the polyadenylation of *Mononegavirales* and *Orthomyxovirus* mRNAs (Table 2).

Template-dependent reiterative transcription by the viral polymerase involves the production of RNA products that are longer than the corresponding RNA template. VSV and Sendai L proteins use intergenic U₇ sequences to template the polyadenylation of viral mRNAs, where the poly(A) tail is substantially longer than the corresponding U₇ template sequence (Barr and Wertz, 2001; Hausmann et al., 1999; Hunt et al., 1984; Schubert et al., 1980) (Table 2). In a similar manner, influenza virus PB1 uses U₅ sequences for the polyadenylation of viral mRNAs (Poon et al., 1999; Pritlove et al., 1999; Robertson et al., 1981; Zheng et al., 1999) (Table 2). In the case of poliovirus poly(A) tails, the situation is more complicated due to the heterogeneous length of natural poly(A) tails, which range from 20 to 120 bases long (Fig. 2A, Polio). 3D^{pol} faithfully copies short poly(A) tails into short VPg-linked poly(U) intermediates (viral RNA with a poly A₃₂ → VPg-linked poly U₃₂

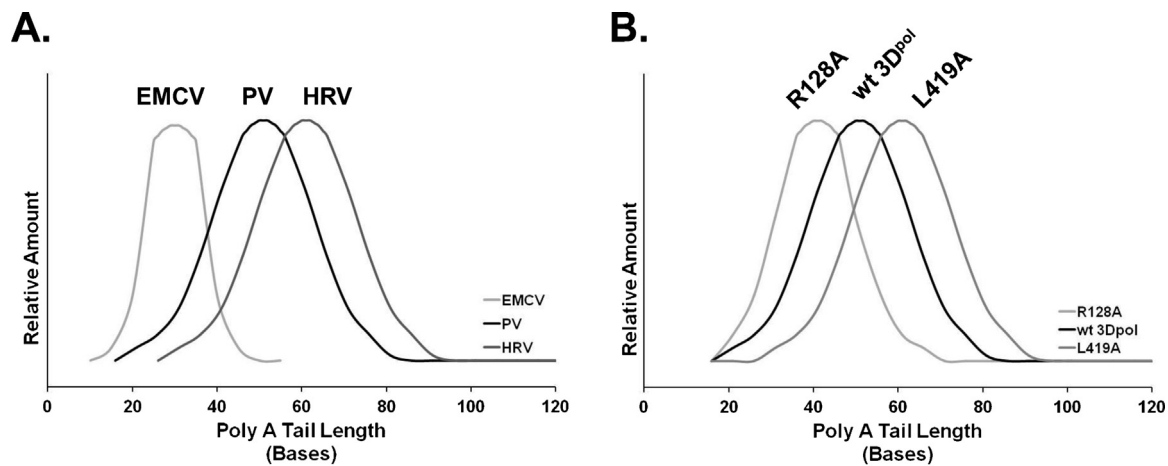


Fig. 2. Size distribution of poly(A) tails in picornavirus RNA genomes. (A) Picornavirus RNA genomes have poly(A) tails of variable length, with notable differences in the size distribution when comparing genus and species groups (Ahlquist and Kaesberg, 1979). Encephalomyocarditis virus (EMCV) RNA has relatively short poly(A) tails [range: 10–58 bases long; mean: 28 bases long] whereas poliovirus (PV) [range: 20–120; mean: 53 bases long] (Kempf et al., 2013) and rhinovirus (HRV) RNAs have longer poly(A) tails (Ahlquist and Kaesberg, 1979). (B) Alanine substitution mutations in poliovirus 3D^{pol} lead to changes in the size of poly(A) tails in RNA genomes (Kempf et al., 2013). 3D^{pol} R128A mutation results in shorter poly(A) tails (mean: 44 bases long) whereas L419A mutation results in longer poly(A) tails (mean: 66 bases long). The graphs display the size distribution of poly(A) tails for EMCV, PV and HRV RNAs based on published data (Ahlquist and Kaesberg, 1979; Kempf et al., 2013).

at the 5' end of negative-strand intermediates) (Steil et al., 2010). Then, in turn, the VPg-linked poly U₃₂ is used to make poly(A) tails from 20 to 120 bases long on new RNA genomes (Steil et al., 2010). In cases where the poly(A) tail is already long (greater than 52 bases in length), 3D^{pol} makes VPg-linked poly(U) products that are substantially longer (VPg-linked polyU_{>120}) (Steil et al., 2010). In turn, VPg-linked poly U₁₂₀ is used to make poly(A) tails 20–120 bases long (Steil et al., 2010). We do not yet appreciate the precise mechanisms used by 3D^{pol} to maintain poly(A) tails that range from 20 to 120 bases long; however, this is an active topic of investigation in our laboratory. Picornavirus poly(A) tails are highly diverse: EMCV maintains RNA genomes with relatively short poly(A) tails whereas poliovirus and rhinovirus maintain RNA genomes with longer poly(A) tails (Fig. 2A) (Ahlquist and Kaesberg, 1979). Features of poliovirus 3D^{pol} impact poly(A) tail lengths. Alanine substitution mutations in poliovirus 3D^{pol} change the size of poly(A) tails in virion RNA (Fig. 2B) (Kempf et al., 2013). Some mutations result in shorter poly(A) tails (R128A 3D^{pol}) whereas other mutations result in longer poly(A) tails (L419A 3D^{pol}). While there is comprehensive data regarding the size distribution of poly(A) tails in three picornavirus genomes (Fig. 2A, EMCV, PV and HRV), to the best of our knowledge there are no studies describing the size distribution of poly(A) tails in other picornaviruses. Nonetheless, as described below, we expect the data implicating poliovirus 3D^{pol} in the polyadenylation of viral RNA to be broadly applicable to other picornaviruses, and perhaps all polyadenylated positive-strand RNA viruses.

4. 3D^{pol} structures implicated in the polyadenylation of viral RNA

Picornavirus 3D^{pol} is well studied and its roles in viral RNA replication are well established. It shares common amino acid motifs with RNA-dependent RNA polymerases from retro, positive-strand, negative-strand and dsRNA virus families (Te Velthuis, 2014). Atomic resolution structures of 3D^{pol} are known for poliovirus (Gong and Peersen, 2010; Thompson and Peersen, 2004), coxsackievirus (Campagnola et al., 2008; Gong et al., 2013), rhinovirus (Appleby et al., 2005; Gong et al., 2013; Love et al., 2004), enterovirus 71 (Chen et al., 2013), foot-and-mouth disease virus (Ferrer-Orta et al., 2004, 2009), and EMCV (Vives-Adrian et al., 2014) (Fig. 3). In addition to solving several apo structures (3D^{pol}

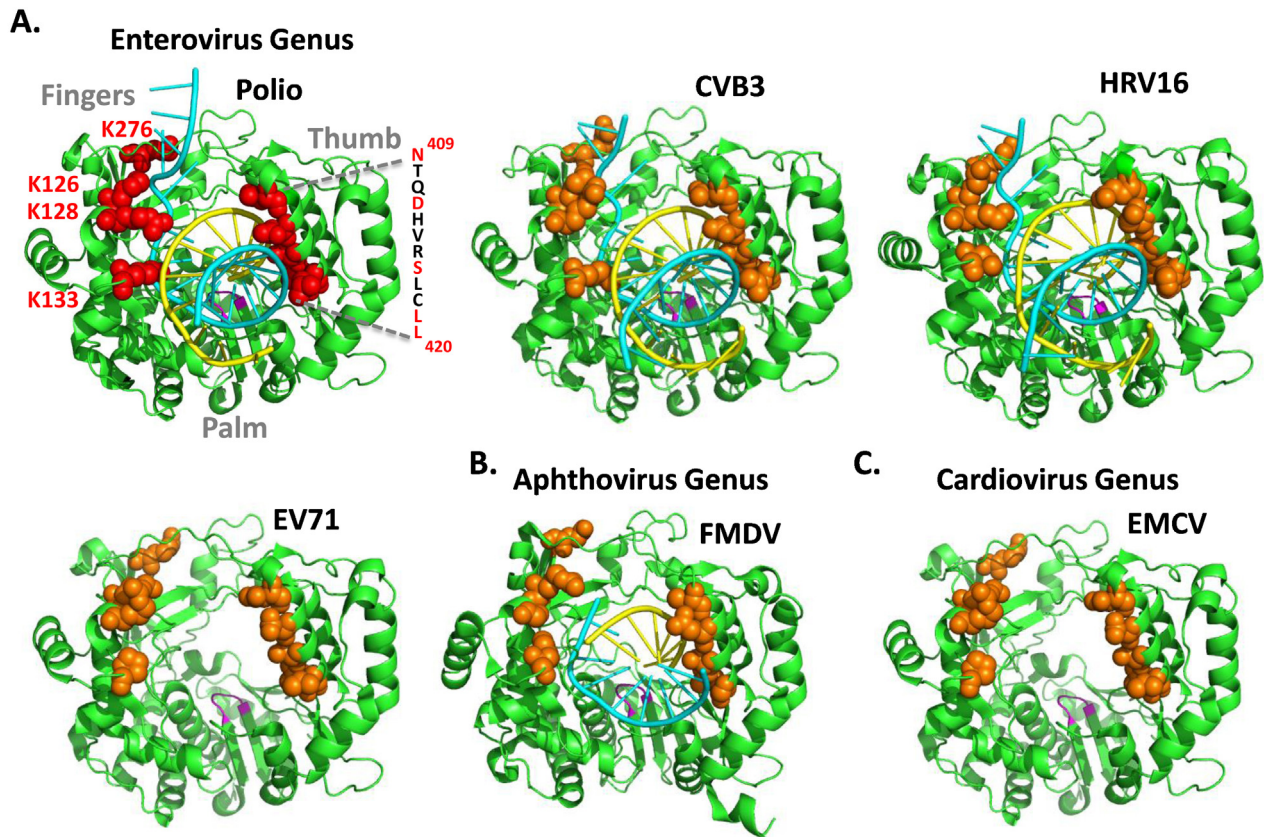
structures without RNA templates or products), the Peersen lab successfully isolated and crystallized 3D^{pol} elongation complexes (Gong et al., 2013; Gong and Peersen, 2010; Sholders and Peersen, 2014). The atomic structure of 3D^{pol} elongation complexes provides significant insight into the manner in which 3D^{pol} interacts with viral RNA templates and products (Fig. 3A). Ongoing work is focused on the manner in which viral RNA templates and products translocate as the polymerase synthesizes RNA (Sholders and Peersen, 2014). Template-dependent reiterative transcription mechanisms, like those envisioned in the polyadenylation of viral RNA, would require substantial structural rearrangements of viral RNA templates and products within 3D^{pol} elongation complexes.

Alanine substitution mutations implicate specific features of 3D^{pol} in the polyadenylation of viral RNA (Table 3 and Fig. 3A) (Kempf et al., 2013). Residues in the fingers and thumb domain of 3D^{pol} affect the size of poly(A) tails in poliovirion RNA (Table 3 and Fig. 3A). Charged amino acid residues in the fingers domain interact with viral RNA templates and products while a thumb domain α -helix fits within the minor groove of dsRNA products as they leave the active site (Fig. 4A). The structural orientation of these residues makes it clear that 3D^{pol} regulates the size of poly(A) tails, in part, through conserved elements that interact with viral RNA templates and products (Table 3 and Fig. 3). Therefore, based on the common mechanisms of picornaviral replication (Fig. 1) and the conserved features of poliovirus 3D^{pol} implicated in the polyadenylation of viral RNA (Table 3 and Fig. 3), we expect that 3D^{pol} is responsible for the polyadenylation of all picornavirus RNA genomes. Polymorphisms in 3D^{pol} among different picornaviruses likely affect the size of poly(A) tails in RNA genomes.

5. Unresolved questions

5.1. What is the biological impact of shorter or longer poly(A) tails?

At present, it is unclear why viruses like EMCV evolved to maintain relatively short poly(A) tails whereas others (polioviruses and rhinoviruses) evolved to maintain longer poly(A) tails (Fig. 2A). Considering circularized mRNPs, where eIF4G interacts with poly(A) binding protein (PABP) (Svitkin et al., 2001), it is possible that viral IRES elements and 2A^{pro} activity impact the optimal size of poly(A) tails. In addition, the size of poly(A) tails may impact



3D^{pol} (green). RNA template (cyan) and product (yellow). YGDD catalytic site (magenta). Residues implicated in polyadenylation of viral RNA (red / orange).

Fig. 3. 3D^{pol} structures implicated in the polyadenylation of viral RNA. (A) Enterovirus 3D^{pol} (Polio, CVB3, HRV16 and EV71) (B) Aphthovirus (FMDV) 3D^{pol}. (C) Cardiovirus (EMCV) 3D^{pol}. 3D^{pol}s (green). RNA template (cyan) and product (yellow). YGDD catalytic site (magenta). Residues implicated in the polyadenylation of viral RNA (red-poliovirus, orange-corresponding residues in other viruses as noted in Table 3) (Kempf et al., 2013). Protein Data Bank files for polio (4K4T), CVB3 (4K4X), HRV16 (4K50), EV71 (4IKA), FMDV (2E9T) and EMCV (4NZ0).

Table 3
Conserved structures of 3D^{pol} implicated in the polyadenylation of picornaviral RNA*.

Genus sp.	Virus	Fingers**	Thumb α -Helix**	PDB#	Citations
Enterovirus A	EV71	¹²⁶ KKRDILN ^{P133} ... ^{R277}	⁴¹⁰ NTQDHVRS ^{LCLL} ⁴²¹	4IKA	Chen et al., 2013
Enterovirus B	CVB3	¹²⁶ KKRDILSK ^{K133} ... ^{R277}	⁴¹⁰ NTQDHVRS ^{LCLL} ⁴²¹	3CDU, 3CDW 4K4X, 4K4Y, 4K4Z	Gruez et al., 2008 Gong et al., 2013
Enterovirus C	Polio	¹²⁶ KKRDILNK ^{K133} ... ^{K276}	⁴⁰⁹ NTQDHVRS ^{LCLL} ⁴²⁰	3OL6, 4K4S, 4K4T, 4K4U, 4K4V, 4K4W	Gong and Peersen 2010 Gong et al., 2013 Kempf et al., 2013
Rhinovirus A	HRV16	¹²⁶ KKKDLIN ^{N133} ... ^{K275}	⁴⁰⁸ QMQEHVLS ^{LCHL} ⁴¹⁹	1TP7, 4K50	Appleby et al., 2005 Gong et al., 2013
Rhinovirus B	HRV14	¹²⁶ KKRDILNK ^{K133} ... ^{R275}	⁴⁰⁸ NTQDHVRS ^{LCML} ⁴¹⁹	1XR5	Love et al., 2004
Aphthovirus	FMDV	¹²⁷ RRGALID ^{F134} ... ^{E286}	⁴¹⁹ TIQEK ^{LISVAGL} ⁴³⁰	2E9T, 2F8E	Ferrer-Orta et al., 2004 Ferrer-Orta et al., 2006
Cardiovirus	EMCV	¹²¹ RRTDVVD ^{W128} ... ^{E281}	⁴¹¹ TLSEK ^{LTSITML} ⁴²²	4NZ0	Vives-Adrian et al., 2014

* Red: Poliovirus 3D^{pol} residues implicated in the polyadenylation of viral RNA (Kempf et al., 2013).

Orange: Residues at corresponding locations in other 3D^{pol} sequences and structures.

** 3D^{pol} amino acid alignments (Chen et al., 2013; Gruez et al., 2008; Ferrer-Orta et al., 2004).

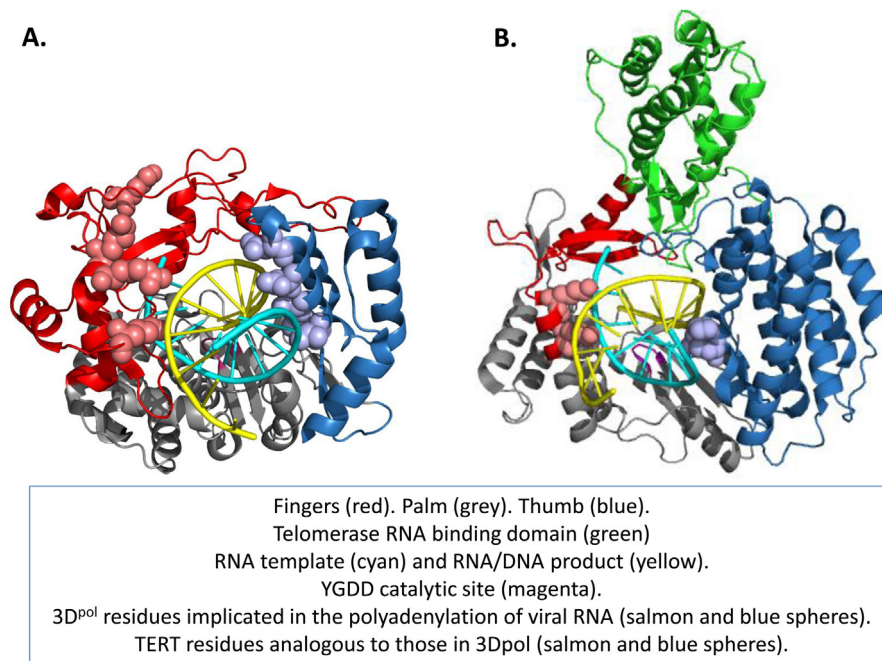


Fig. 4. Structural and functional parallels between 3D^{pol} and telomerase reverse transcriptase (TERT). (A) Poliovirus 3D^{pol} elongation complex (PDB: 4K4T) (Gong and Peersen, 2010). (B) TERT structure including RNA template and DNA product (PDB: 3KYL) (Mitchell et al., 2010).

the manner in which viral mRNAs interact with mRNA turnover machinery in cells, as deadenylase and Xrn1 are recruited specifically to polyadenylated mRNAs (Doidge et al., 2012). Poliovirus 2A^{pro} increases viral mRNA stability (Kempf and Barton, 2008a). Furthermore, 2A^{pro}-dependent increases in viral mRNA stability are coincident in time with the cleavage of eIF4G (Kempf and Barton, 2008a). The cleavage of eIF4G by 2A^{pro} liberates the NH-terminal portion of eIF4G from circularized viral mRNPs, and in so doing may also dissociate cellular mRNA turnover machinery from circularized viral mRNPs (Kempf and Barton, 2008a). Enteroviruses and rhinoviruses, which cleave eIF4G with 2A^{pro}, have longer poly(A) tails than EMCV, which does not cleave eIF4G. Thus, enterovirus and rhinovirus mRNAs are translated in 2A^{pro}-modified polysomes, perhaps to uncouple mRNA turnover machinery from viral mRNAs (Kempf and Barton, 2008a). Shorter poly(A) tails, like those in EMCV, may provide some relief from host mRNA turnover machinery, although this possibility has not been substantiated experimentally.

Stress granules are another consideration (White and Lloyd, 2012). Does the length of poly(A) tails impact the manner in which viral mRNAs interact with stress granules? Enterovirus and rhinovirus 3 C^{pro} cleave PABP and G3BP, stress granule proteins (White et al., 2007; White and Lloyd, 2011); however, the length of poly(A) tails as they relate to stress granule formation has not been examined experimentally.

5.2. Factors other than 3D^{pol} regulating the size of picornavirus poly(A) tails?

Our working model of reiterative transcription suggests that 3D^{pol} pauses during VPg-linked poly(U) and poly(A) synthesis, nascent dsRNA products melt, realign, reanneal and resume elongation, thereby making RNA products that are longer than the template (Steil et al., 2010). The 3' NTR of viral RNAs might impact the manner in which 3D^{pol} pauses during VPg-linked poly(U) synthesis. Likewise, VPg at the 5' end of negative-strand RNA templates could prevent 3D^{pol} from running off the end of RNA

templates during positive-strand RNA synthesis, provoking reiterative transcription during the polyadenylation of nascent (+) strands, especially on RNA templates with relatively short VPg-linked poly(U) sequences (Steil et al., 2010). 3D^{pol} oligomers (Bentham et al., 2012; Lyle et al., 2002) or protein complexes (Shen et al., 2008) might impact the manner in which nascent dsRNA products melt, realign and reanneal; however, there is no direct evidence implicating 3D^{pol} oligomers or protein complexes in these events. 3'NTR mutations are reported to impact the size of poly(A) tails (van Ooij et al., 2006a). Furthermore, PABP could influence the replication of poly(A) tails, although its contribution appears to be dispensable (Svitkin et al., 2007).

5.3. How are poly(A) tails maintained on other positive-strand RNA virus genomes?

Cellular PAPs synthesize poly(A) tails in a template-independent manner, downstream of characteristic polyadenylation signals (AAUAAA and AUUAAA) (Laishram, 2014). Poly(A) tails on herpesvirus mRNAs (Majerciak et al., 2013) and cellular mRNAs (Ni et al., 2013) are synthesized by cellular PAPs (Laishram, 2014). DNA viruses and retroviruses have 3' terminal polyadenylation signals that are used regularly by cellular PAPs (Schrom et al., 2013). Among polyadenylated positive-strand RNA viruses, only potexviruses have 3' terminal polyadenylation signals that could be used regularly by cellular PAPs (Osman et al., 2014). We expect that most polyadenylated positive-strand RNA viruses, like picornaviruses (Kempf et al., 2013; Steil et al., 2010), replicate their poly(A) tails with their viral RNA-dependent RNA polymerases. The presence of long poly(U) sequences in alphavirus (Sawicki and Gomatos, 1976) and coronavirus (Wu et al., 2013) negative-strand RNA intermediates are consistent with these predictions. Nonetheless, some polyadenylated positive-strand RNA viruses have been shown to use cellular PAPs to repair defective genomes lacking poly(A) tails (Liu et al., 2008; Raju et al., 1999; Tachahashi and Uyeda, 1999; van Ooij et al., 2006a).

6. Structural and functional parallels between 3D^{pol} and telomerase reverse transcriptase (TERT)

Consistent with their ancient evolutionary origins (Nakamura and Cech, 1998), picornavirus 3D^{pol} and TERT share structural and functional features. Structurally, both 3D^{pol} and TERT assume a “right-hand” conformation with thumb, palm and fingers domains encircling templates and products (Fig. 4) (Gillis et al., 2008; Gong and Peersen, 2010; Mason et al., 2011; Mitchell et al., 2010). Functionally, both 3D^{pol} and TERT use template-dependent reiterative transcription mechanisms to synthesize repetitive sequences at the ends of chromosomes: poly(A) tails in the case of picornavirus RNA genomes and DNA telomeres in the case of eukaryotic chromosomes (Blackburn, 1999; Kempf et al., 2013; Steil et al., 2010). These two enzymes have diverged to such a great degree that there is little amino acid homology evident beyond the catalytic residues in the palm domain. Nonetheless, there are strikingly similar fingers domain and thumb domain residues gripping the templates and products as they exit the respective molecules (Fig. 4). Based on these features of 3D^{pol} and TERT, it is reasonable to think of 3D^{pol} reiterative transcription mechanisms as telomerase-like aspects of viral RNA replication. Likewise, it is reasonable to consider poly(A) tails to be a telomere of picornavirus RNA genomes.

The concept of telomeres in positive-strand RNA virus genomes is not new (Rao et al., 1989). A tRNA-like element at the 3' end of brome mosaic virus RNA was ascribed telomere functions long ago (Rao et al., 1989). Telomeres have two characteristic features: (1) mechanisms to renew themselves, and (2) mechanisms to protect the remainder of the genome. Cellular CCA-adding enzyme can renew the integrity of the tRNA-like element at the 3' end of brome mosaic virus RNA genomes (Rao et al., 1989). Aminoacylation reinforces the integrity of the tRNA-like element (Rao et al., 1989). Furthermore, the tRNA-like element of brome mosaic virus RNA protects the viral RNA genome from host cell mRNA turnover machinery. In the case of picornaviruses, reiterative transcription mechanisms of 3D^{pol} renew 3' poly(A) tails on viral RNA genomes during viral RNA replication (Kempf et al., 2013; Steil et al., 2010). In turn, the poly(A) tail, via interactions with PABP and other factors, protects the viral RNA genome from mRNA turnover machinery (Kempf and Barton, 2008a,b). Thus, we think it is reasonable to consider both poly(A) tails and tRNA-like elements to be telomeres of positive-strand RNA virus genomes.

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