The Picornaviral Polymerase Fingers Domain Controls RNA Binding and Translocation

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Abstract: The picornavirus family of viruses includes poliovirus, the causative agent of paralytic polio and coxsackievirus, which is responsible for viral-heart-disease. Picornaviruses contain a single-stranded positive-sense RNA genome replicated by 3D^{pol}, an RNA-dependent RNA polymerase (RdRP). Crystal structures of 3D^{pol} from multiple picornaviruses have shown a conserved polymerase fold analogous to a "right hand" composed of fingers, palm and thumb domains. These crystal structures also identified unique regions in the fingers domain whose function in 3D^{pol} were unknown. Through biochemical kinetic analysis we have now determined the purpose of these regions, and their effects on the catalytic cycle of 3D^{pol}.

Poliovirus Lifecycle

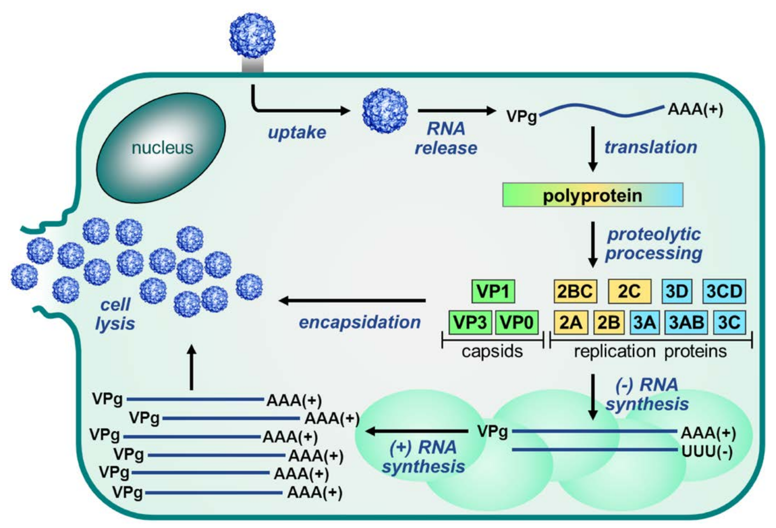

Figure 1: The poliovirus lifecycle during a viral infection. Virus uptake leads to RNA genome release and polyprotein translation by host cell ribosomes. Polyprotein proteolytic processing occurs via viral proteases. RNA genome is copied by the viral polymerase and packaged into new virus particles capable of infecting additional cells.

Picornavirus Polymerase Structure

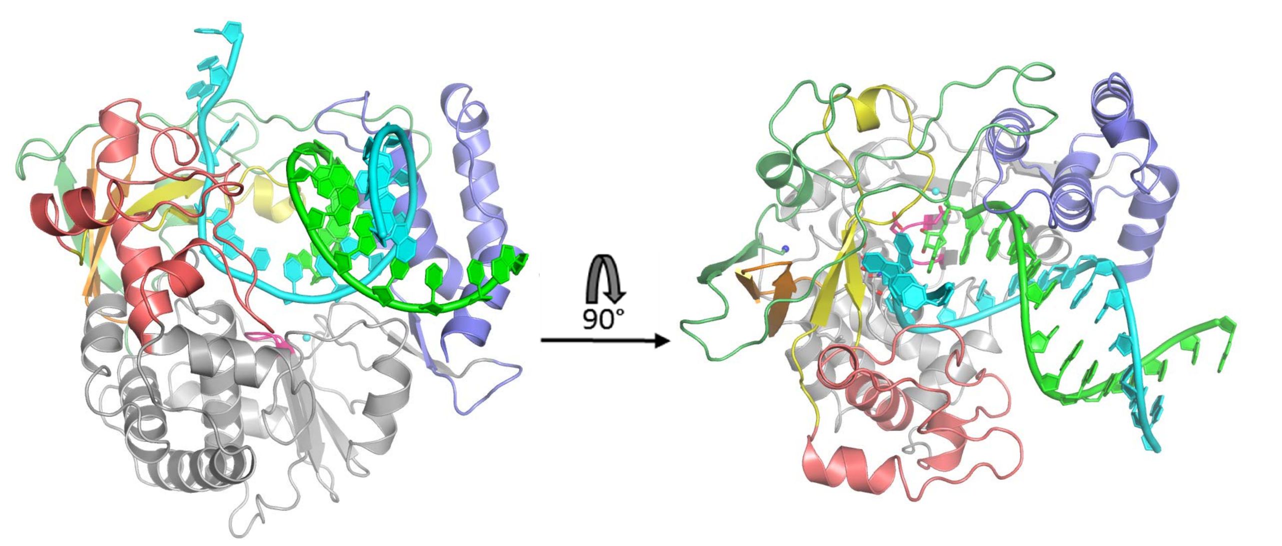

Figure 2: The poliovirus polymerase is an RNA-dependent RNA-polymerase, with a structure analogous to a "right hand" including a fingers, palm and thumb domain. Elongation complex shown with RNA passing through the polymerase. The template strand is shown in cyan, and the product strand is shown in green.

Catalytic Cycle

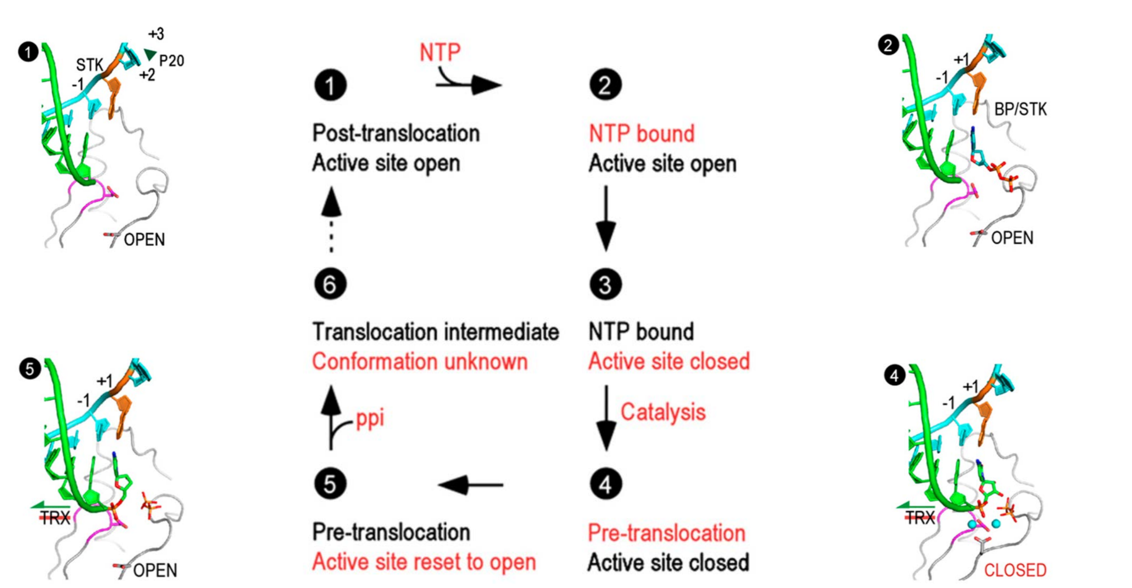

Figure 3: Six step catalytic cycle of 3D^{pol} (Gong & Peersen, 2010, PNAS, 107, 22505–10). Templating RNA base in the +1 position unpaired with an NTP, 1. Incoming NTP positioning by base pairing, 2. Active site closure, 3. Catalysis, 4. Active site opening, 5. Translocation, 6.

General Mechanism of a Polymerase

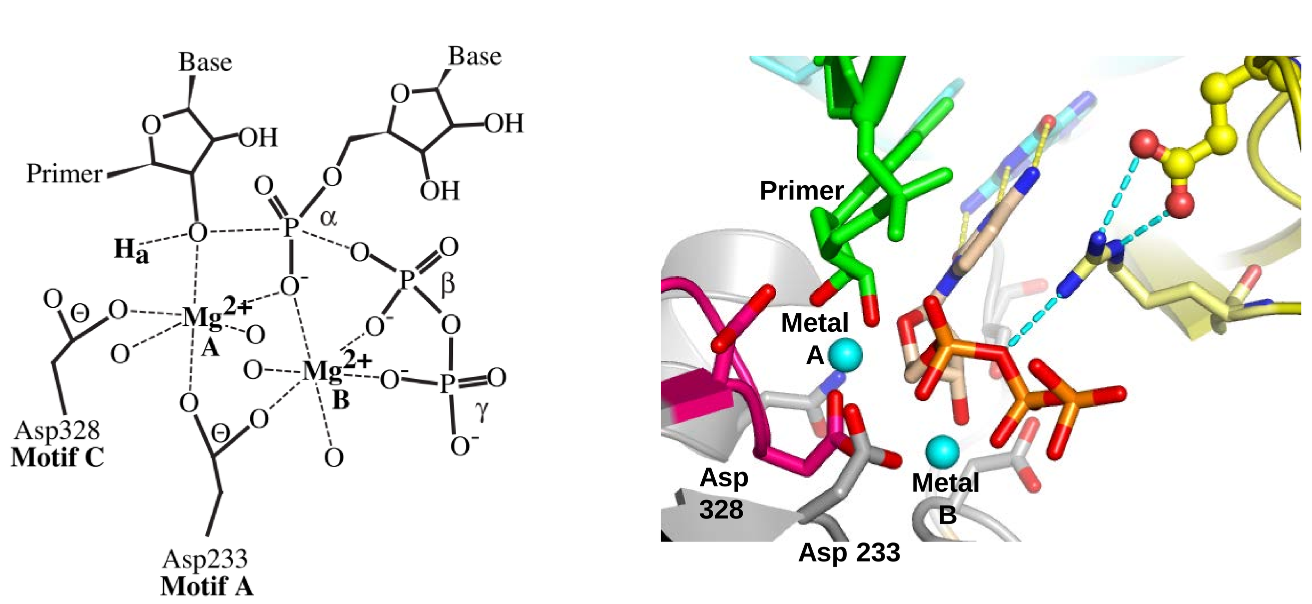

Figure 4: All polymerases have a conserved active site geometry and mechanism for nucleotide incorporation (Steitz, T. A., 1998 *Nature* 391, 231–2). A general acid, general base, and two metal ions are required for chemistry to occur.

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Targeted Mutations

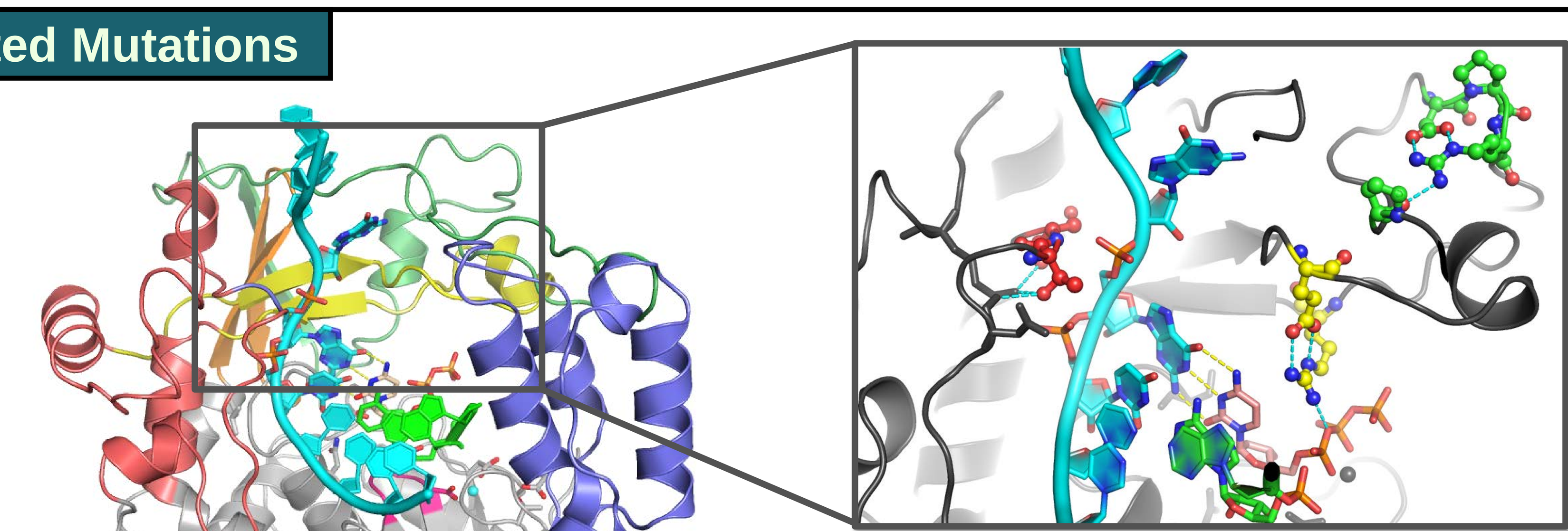
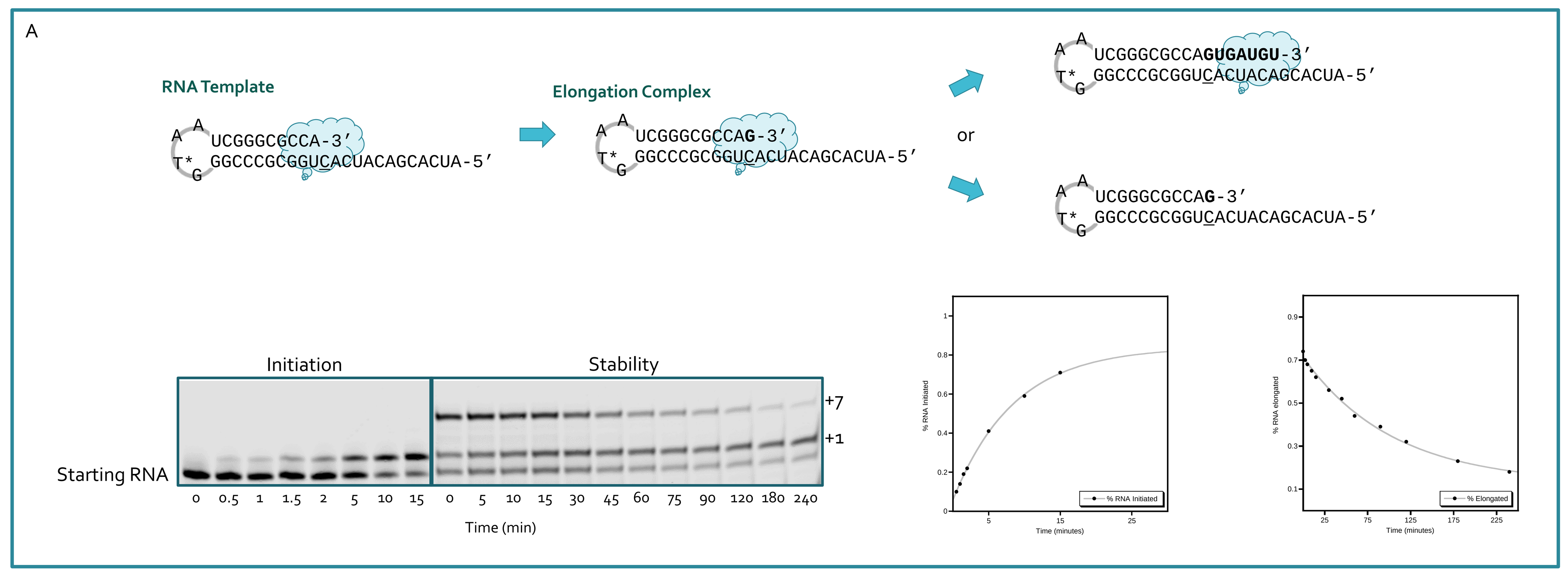
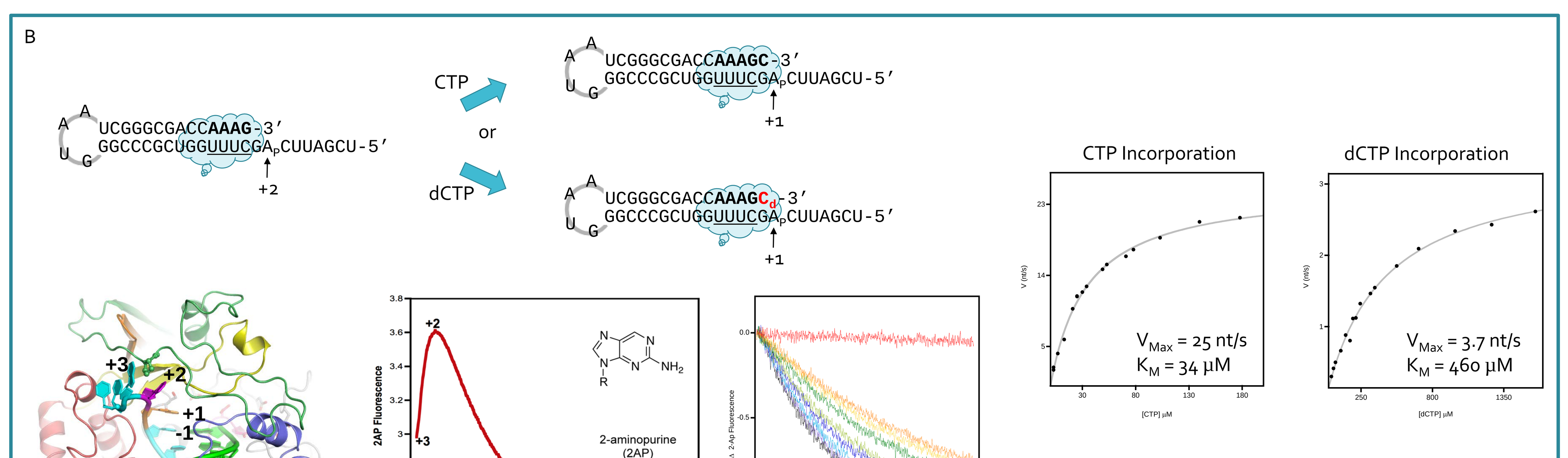


Figure 5: Targeted mutations to the fingers. Targeted mutations within poliovirus 3D^{pol} fingers domain. Side view of 3D^{pol} down the RNA exit channel showing the three regions targeted for mutation within the fingers domain. Individual residues targeted are shown as spheres; kink residues shown in green, gateway residues in red, and sensor residues in yellow.

Kinetic Assays





$$Discrimination\ Factor = \frac{\left(\frac{V_{Max}}{K_M}\right)_{CTP}}{\left(\frac{V_{Max}}{K_M}\right)_{dCTP}}$$

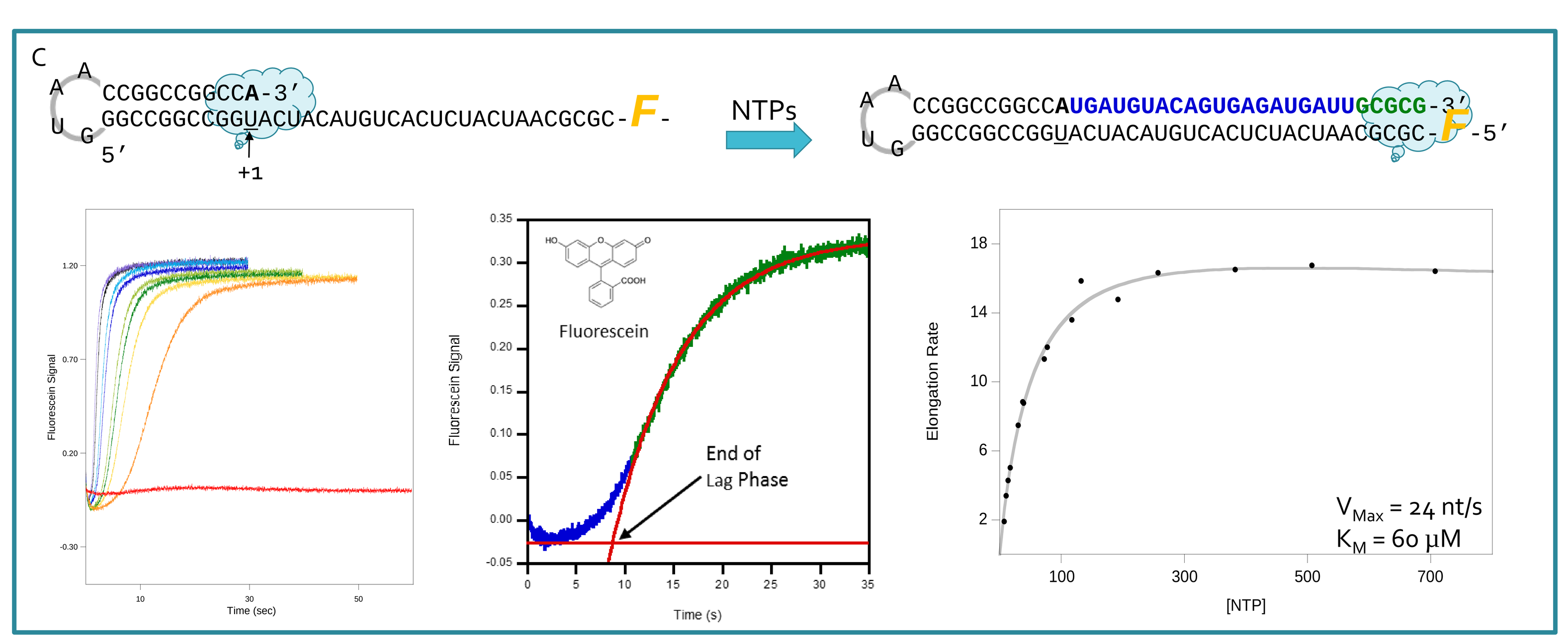
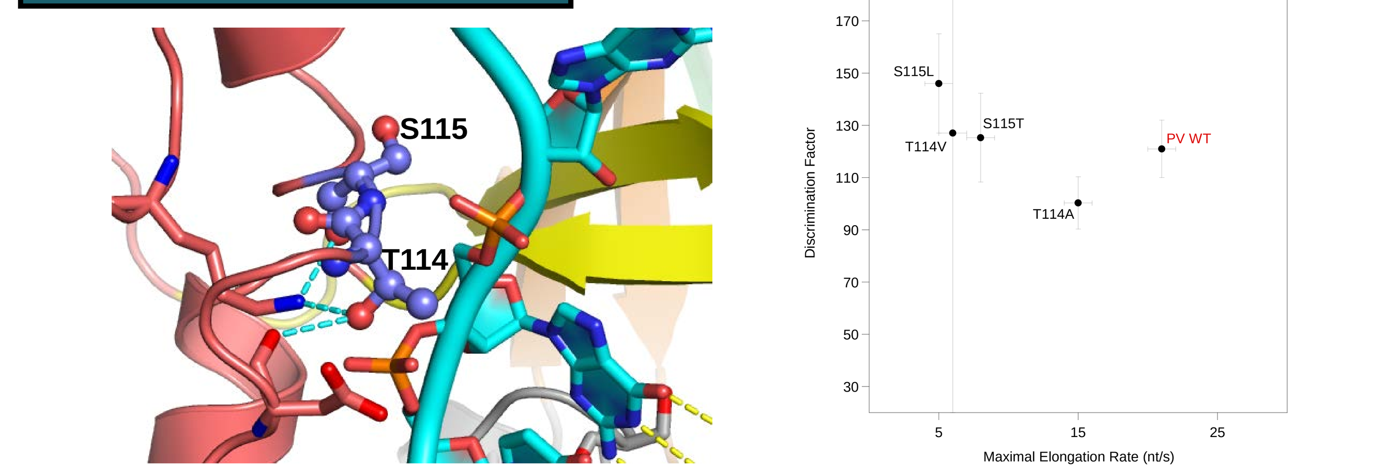


Figure 6: Kinetic Assays. (A) Initiation and Stability. Polymerases were mixed with RNA templates and NTPs, and incubated at room temperature before EDTA quench at multiple time points. Curve fitting was done to a single exponential equation for fraction elongated product at each time point, representative graph shown. **(B) Single NTP rate and fidelity determination.** 3D^{pol}-RNA complex was mixed with varying concentrations of CTP or dCTP and rate determined by 2-aminopurine fluorescence change with time to generate a Michaelis-Menten curve and calculate discrimination factor. **(C) Processive elongation rate determination.** 3D^{pol}-RNA complex was mixed with varying NTP concentrations and rate determined by fluorescence change. Lag phase length was used to determine processive elongation rate (first ~20 NTPs added) at each NTP concentration.

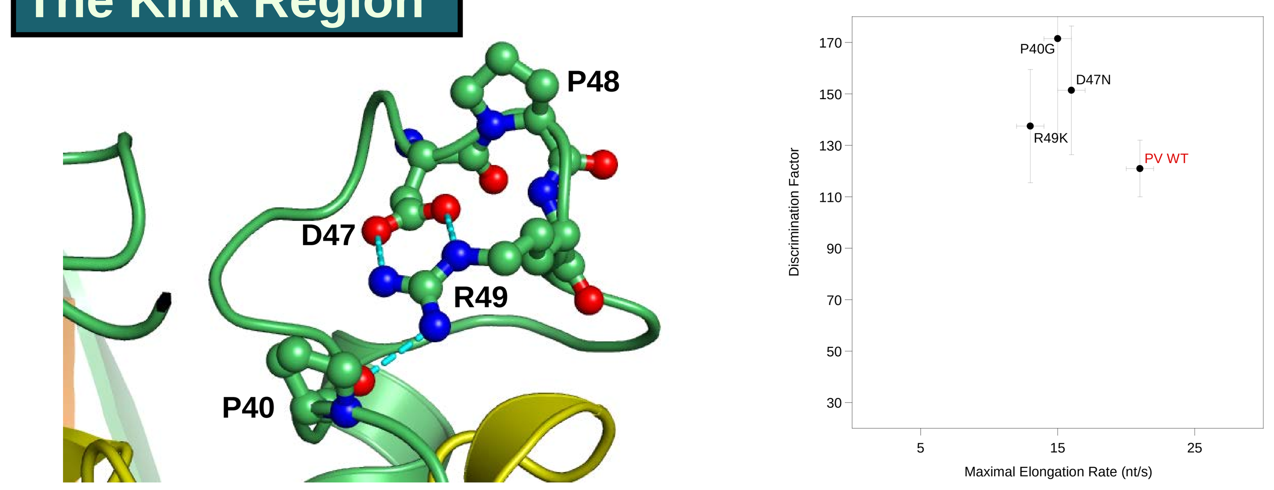
The Gateway Region



3D^{pol}	RNA Initiation (min)	EC Stability (min)	Single Nucleotide k_{pol} (nt/sec)	Single Nucleotide K_M (µM)	Processive Elongation k_{pol} (nt/sec)	Processive Elongation K_M (µM)	Discrimination Factor
WT	4 ± 2	130 ± 20	25 ± 1	20 ± 2	20 ± 1	49 ± 4	120 ± 10
Gateway Mutants							
T114A	10 ± 2	57 ± 10	25 ± 2	31 ± 5	**14 ± 1**	63 ± 6	100 ± 10
T114L	**33 ± 7**	**5 ± 1**	26 ± 3	90 ± 20	**10 ± 1**	100 ± 10	-
T114S	4 ± 1	82 ± 9	26 ± 1	23 ± 1	20 ± 1	52 ± 4	-
T114V	**18 ± 4**	**9 ± 2**	27 ± 6	150 ± 40	**5 ± 1**	80 ± 40	130 ± 100
S115A	5 ± 1	90 ± 20	28 ± 2	60 ± 7	**11 ± 1**	95 ± 6	-
S115L	**17 ± 3**	**15 ± 1**	19 ± 4	110 ± 30	**5 ± 1**	30 ± 10	150 ± 20
S115T	5 ± 1	**13 ± 1**	23 ± 2	61 ± 7	**8 ± 1**	120 ± 10	130 ± 20
S115V	**18 ± 3**	**25 ± 2**	29 ± 1	38 ± 3	16 ± 1	74 ± 4	-

Conclusion: Gateway residues act as an energy barrier to RNA template translocation and are involved in RNA positioning.

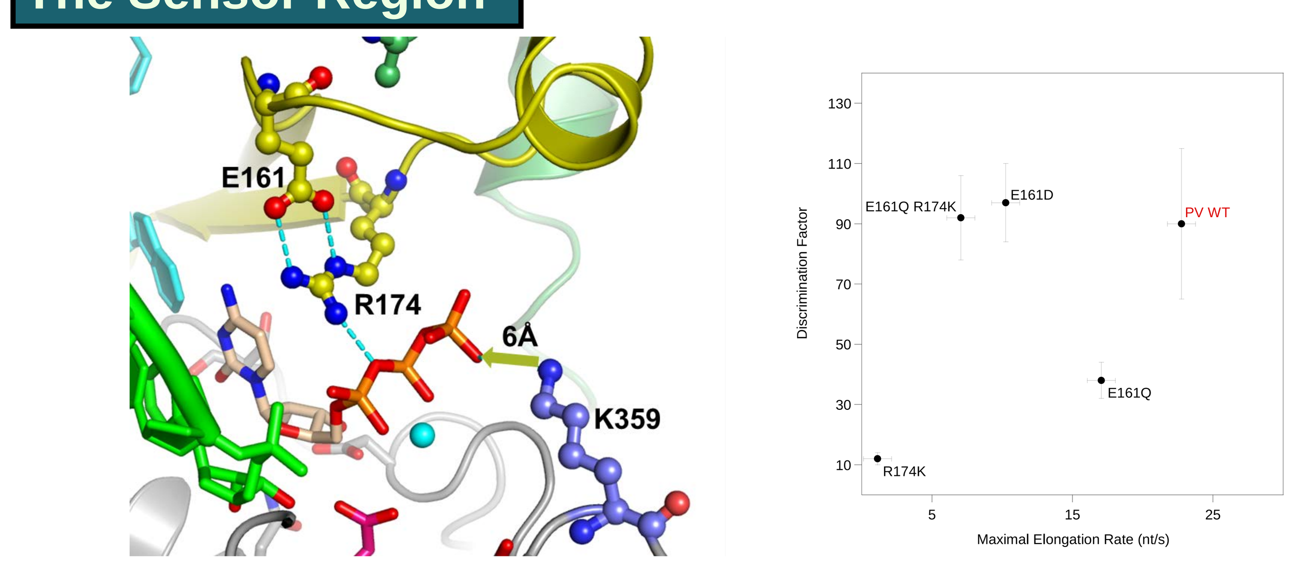
The Kink Region



3D^{pol}	RNA Initiation (min)	EC Stability (min)	Single Nucleotide k_{pol} (nt/sec)	Single Nucleotide K_M (µM)	Processive Elongation k_{pol} (nt/sec)	Processive Elongation K_M (µM)	Discrimination Factor
WT	4 ± 2	130 ± 20	25 ± 1	20 ± 2	20 ± 1	49 ± 4	120 ± 10
Kink Mutants							
P40G	**13 ± 1**	**29 ± 2**	**22 ± 1**	37 ± 3	**13 ± 1**	100 ± 10	170 ± 40
D47N	**15 ± 1**	**19 ± 2**	**20 ± 1**	46 ± 6	**15 ± 1**	55 ± 7	150 ± 30
P48G	6 ± 1	100 ± 20	27 ± 1	23 ± 3	20 ± 1	52 ± 4	-
R49K	**19 ± 2**	**29 ± 4**	**17 ± 2**	40 ± 10	**11 ± 1**	90 ± 20	140 ± 20

Conclusion: Kink residues play a role in RNA binding and resetting the polymerase active site after translocation.

The Sensor Region



3D^{pol}	RNA Initiation (min)	EC Stability (min)	Single Nucleotide k_{pol} (nt/sec)	Single Nucleotide K_M (µM)	Processive Elongation k_{pol} (nt/sec)	Processive Elongation K_M (µM)	Discrimination Factor
WT	2 ± 1	92 ± 16	25 ± 1	34 ± 2	23 ± 1	65 ± 4	90 ± 30
Sensor Mutants							
E161Q	10 ± 3	84 ± 15	22 ± 1	9 ± 1	17 ± 1	19 ± 2	**38 ± 6**
E161D	5 ± 1	65 ± 15	24 ± 1	19 ± 2	**10 ± 1**	32 ± 2	100 ± 10
R174K	3 ± 1	62 ± 12	**2 ± 1**	69 ± 7	**1 ± 1**	18 ± 1	**12 ± 2**
E161Q + R174K	8 ± 1	82 ± 18	**5 ± 1**	16 ± 2	**7 ± 1**	53 ± 9	90 ± 10

Conclusion: Sensor residues position the proton donor for catalysis and act as the signal that catalysis has occurred.