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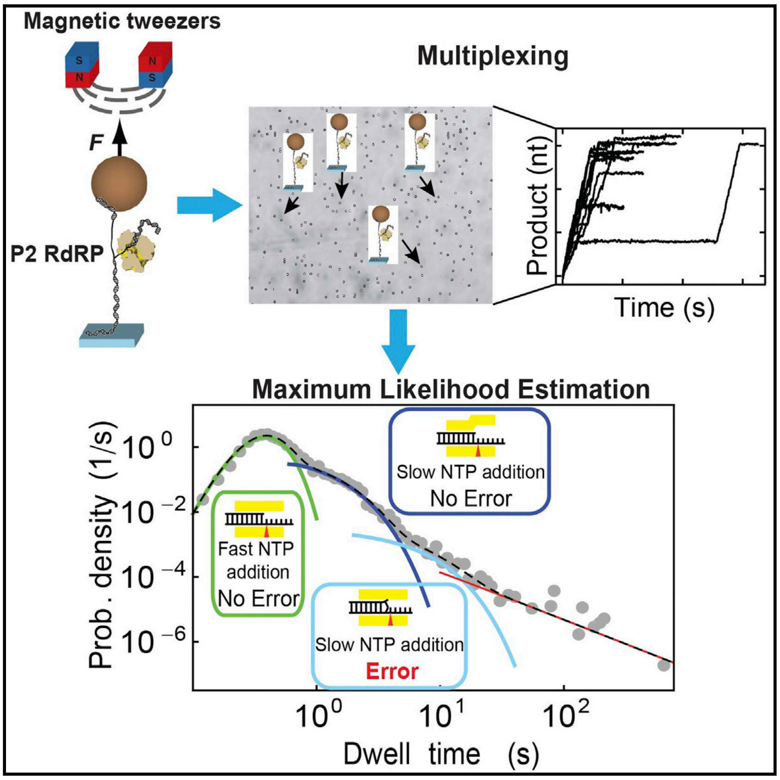
Article

Elongation-Competent Pauses Govern the Fidelity of a Viral RNA-Dependent RNA Polymerase

Graphical Abstract



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In Brief

Using high-throughput single-molecule magnetic tweezers, Dulin et al. show that viral RdRP elongation is highly dynamic. Applying an unbiased analysis based on maximum likelihood estimation, they demonstrate that short pauses are the signature of an unknown error-prone nucleotide incorporation pathway.

Highlights

- The RNAP elongation dynamic of dsRNA virus is captured by magnetic tweezers
- Large sets of high-resolution data allow quantification of polymerase fidelity
- Maximum likelihood estimation allows extraction of critical model parameters
- Errors are predominantly incorporated via new error-prone catalytic pathway

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Elongation-Competent Pauses Govern the Fidelity of a Viral RNA-Dependent RNA Polymerase

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SUMMARY

RNA viruses have specific mutation rates that balance the conflicting needs of an evolutionary response to host antiviral defenses and avoidance of the error catastrophe. While most mutations are known to originate in replication errors, difficulties of capturing the underlying dynamics have left the mechanochemical basis of viral mutagenesis unresolved. Here, we use multiplexed magnetic tweezers to investigate error incorporation by the bacteriophage Φ6 RNA-dependent RNA polymerase. We extract large datasets fingerprinting real-time polymerase dynamics over four magnitudes in time, in the presence of nucleotide analogs, and under varying NTP and divalent cation concentrations and fork stability. Quantitative analysis reveals a new pause state that modulates polymerase fidelity and so ties viral polymerase pausing to the biological function of optimizing virulence. Adjusting the frequency of such pauses offers a target for therapeutics and may also reflect an evolutionary strategy for virus populations to track the gradual evolution of their hosts.

INTRODUCTION

RNA viruses are responsible for many human pandemics, including hepatitis C, polio, influenza, and dengue fever. Because of their high mutation rates, RNA viruses evolve rapidly and are difficult to target with vaccines (Lauring et al., 2013). On the molecular level, the dominant source of mutations is the error-prone RNA-dependent RNA polymerases (RdRPs) responsible for replicating the viral genomes (Vignuzzi et al., 2006). A high mutation rate increases evolvability, but also induces many deleterious mutations, and a delicate balance needs to be struck to ensure the pathogenicity of the viral population. Given the strict demands on the precision of mutation rates, RdRPs have become an important target for antiviral therapies that seek to either decrease (Crotty et al., 2000; Vignuzzi et al., 2005) or increase (Vignuzzi et al., 2008) RdRP replication fidelity. Understanding how RdRPs influence viral mutation rates therefore carries direct implications for human health and the development of antiviral therapies, but is also of fundamental importance for our comprehension of viral evolution. Despite this, little is known about the dynamics of RNA elongation by RdRPs (Yang et al., 2012) and in particular of the nucleotide selection process—the origin of most mutations.

Direct probing of error incorporation is challenging, as errors are infrequent random events easily masked in bulk measurements. Stop-flow and quench-flow experiments have greatly elucidated the dynamics of nucleotide addition, but such experiments often rely on nucleotide starvation conditions to induce otherwise rare error-incorporation events (Jin et al., 2011, 2012; Johnson, 2008; Yang et al., 2012); as the polymerization dynamics is severely perturbed under such conditions, it is not a priori evident that the error probabilities estimated in this way represent error rates under physiological conditions. Single-molecule experiments (Dulin et al., 2013; Geertsema and van Oijen, 2013; Larson et al., 2011) do in principle have the potential to detect error incorporation in the presence of all nucleotides, but the limited throughput of these experiments has so far precluded a detailed statistical study of such rare events.

To gain a deeper insight into the origin of viral mutagenesis, we here study error incorporation of a model RdRP at the single-molecule level. Specifically, as all the structurally characterized RNA-dependent viral polymerases share a high degree of structural conservation (Mönttinen et al., 2014; Ng et al., 2008), we use the well characterized P2 (Figure 1A)—the RdRP of the double-stranded RNA (dsRNA) bacteriophage Φ6—as a model system for viral RdRPs and RTs (Butcher et al., 2001; Makeyev and Grimes, 2004). To overcome limitations induced by bulk averaging and limited statistics, we