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Supplementary information

Structural insight into arenavirus replication machinery

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Supplementary Information

Supplementary Methods

mRNA sequencing

To characterize the transcription product, the transcription reaction was performed with normal NTPs without radioactive labeling. A total of 400 μL reaction mixture was concentrated by precipitation using 100 mM sodium acetate and 70% ethanol, and then resolved by 20% polyacrylamide gels. The RNA was visualized by SYBR green staining, and the product band was cut off and crushed into powder. Then, 400 μL extraction buffer (100 mM Tris-HCl, pH 7.4, 50 mM NaCl, 10 mM EDTA and 3.5 M urea) was added into the powder and incubated at 37 ℃ for 30 min to dissolve the RNA. The supernatant was collected by centrifugation and the RNA was precipitated with 100 mM sodium acetate and 70% ethanol supplemented with ~0.03 μg/μL glycogen, which was kept at -80 ℃ overnight. The precipitant was harvested by centrifugation at 4 ℃ for 1 h, washed with 70% ethanol, and finally dissolved with 20 μL nuclease-free water.

The purified RNA was first polyadenylated with poly(A) polymerase (NEB), and then reverse-transcribed with the oligo-T primer (5'-CACGACGCTCTTCCGATCTTTTTTTTTTTTTTTTT-3'). The cDNA was turned into double-stranded DNA (dsDNA) by using the second-strand primer (5'-GTTCAGACGTGTGCTCTTCCGATCTGA+A+T+C+GC-3', here the "+" represents a locked nucleic acid (LNA) base). The dsDNA product was purified and further amplified by elongation primers (Forward primer: 5'-CAAGCAGAAGACGGCATACGAGATACTGGTGTGACTGGAGTTCAGACGTGTG

CTCTTCCGATCT-3’; Reverse primer: 5’-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3’). The final product was ligated into a pMD18-T vector for sequencing.

Supplementary Discussion

Issues for *in vitro* polymerase activity assays

In our initial attempt to conduct *in vitro* polymerase activity assays, we mainly obtained abnormal mRNA products that appeared with larger sizes than expected, as shown by autoradiographs. This posed uncertainties to our evaluation of the activity of LASV and MACV polymerases. In order to characterize the identity of these RNA products, we enriched the mRNA products and reverse-transcribed them into cDNA to enable sequencing experiments. Surprisingly, the larger mRNA products generated by both LASV and MACV polymerases were of the same sequence composition of the expected product. This observation reminded us to think the slow migration rate of these products might result from the base-pairing between RNA product and the 3’-vRNA strand which was not completely impaired in the electrophoresis conditions. As huge amount of redundant 3’-vRNA is present in the reaction system, the quite low amount of RNA product would be easily sequestered immediately after synthesis.

To test this hypothesis, we increased the incubation time for product denaturation and compared the migration profiles of the same products in gels with different urea concentrations. As shown in Extended Data Fig. 4, almost all of the mRNA products migrated much slower than expected in the gel with 1 M urea. In the gel with 7 M urea,

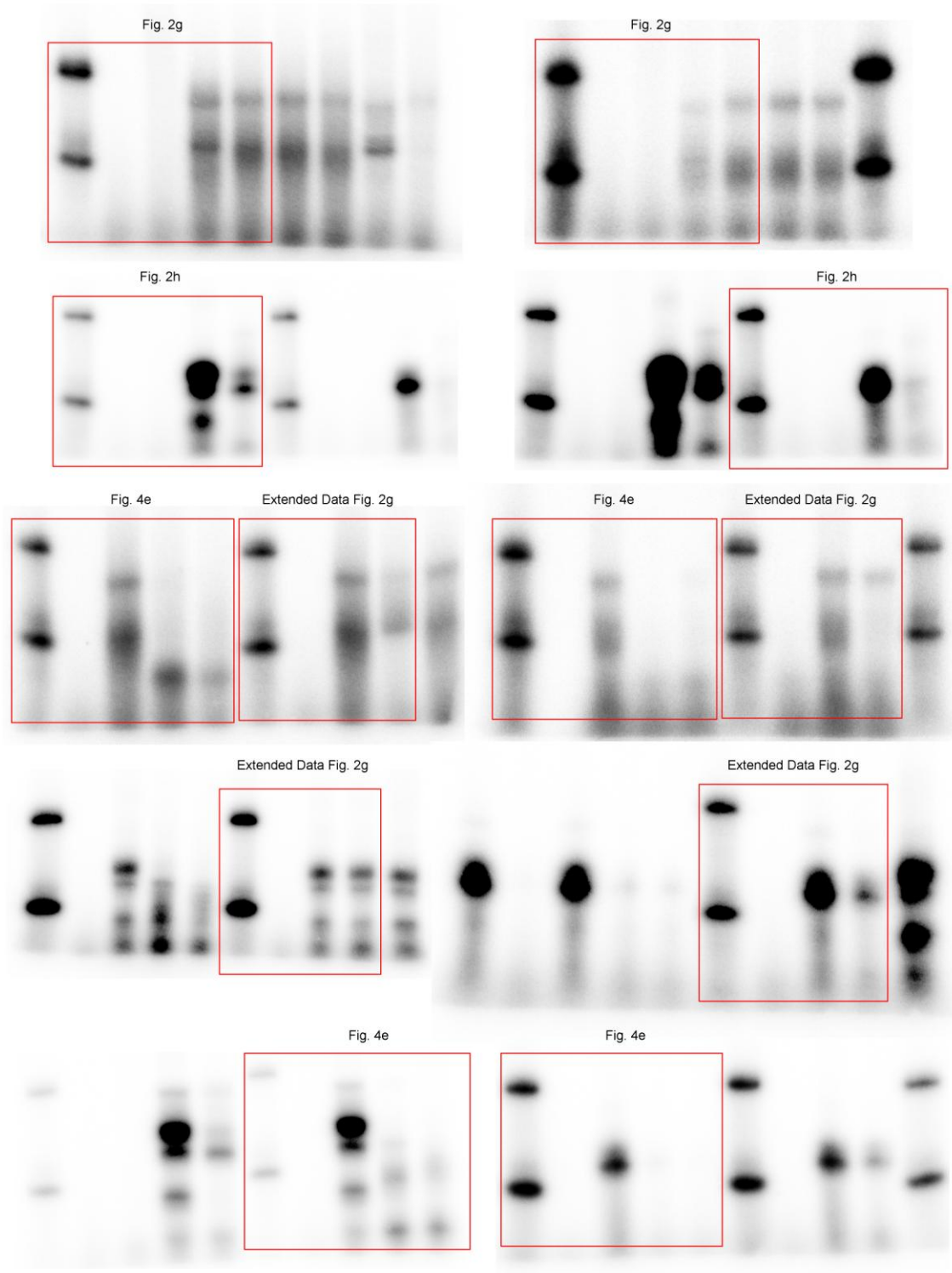
however, a fraction of the products began to reveal normal migration profiles. Further increasing the urea concentration up to 8 M in the gel rendered almost all of the products migrating to the expected position, revealing the correct molecular weight of the expected product. These evidences demonstrated that the unexpected “large products” observed in our previous experiments were results of inappropriate conditions for sample denaturation. These bands were actually the correct products with expected sequences and could be utilized to evaluate the polymerase activities. Therefore, we used PAGE gels with 8 M urea in the subsequent experiments to resolve the RNA products of all *in vitro* polymerase activity assays. In this condition, almost all of the mRNA products were completely denatured to reveal expected migration profiles. However, a small fraction (~30%) of cRNA products still retained the abnormal migration rate (Figs. 2g and 2h). Further increasing the concentration of urea seemed impractical for gel preparation as the urea and acrylamide could not be completely dissolved.

Different functional states of arenavirus polymerase for RNA synthesis

Comparing the structures of LASV and MACV L proteins, we found the main body of RdRp could be well superimposed whereas the appendage domains displayed distinct conformations, including the endonuclease, α-ribbon, pendant domain and the clamp in the PA-C-like region (Extended Data Figs. 8a and 8b). As the pendant domain in LASV L was not resolved, its location was inferred by superimposing the α-ribbon and pendant of MACV L as a rigid body with the α-ribbon domain of LASV L, which is rotated outside by ~80° relative to its position in MACV L. This rearrangement is also accompanied by the re-orientation of endonuclease domain to create an open channel

with the head lobe of the PA-C-like region (Extended Data Figs. 8a and 8b). Besides, the clamp domains in LASV and MACV L proteins also display different conformations, which might result from the variation in sequences that OW mammarenaviruses harbor two insertions as compared to NW mammarenaviruses (Extended Data Figs. 7f and 7g).

As shown by the structure of MACV L-vRNA complex, the 3'-vRNA is accommodated in a groove outside the catalytic chamber (Fig. 3a), which thus represents a pre-initiation state and structural rearrangement is required to relocate the 3'-vRNA template to reach the active site. To understand the conformation of L protein during RNA synthesis, we modeled the template and product RNAs into the catalytic center based on the structure of poliovirus elongation complex (PDB-3OLB). As shown by the model, the 3'-vRNA template binds to LASV L in the open channel right beside the α-ribbon domain from which it enters the catalytic chamber (Extended Data Fig. 8c). The partial duplex structure of 3'-vRNA template and product strands are separated by the lid domain within the PB2-N-like region that binds to the thumb domain of RdRp, thus forcing the template and product strands to go into different exit tunnels (Extended Data Figs. 8c and 8d). As the α-ribbon and pendant domains of MACV L are placed in proximity to the head lobe of PA-C-like region, the template entrance might thus be blocked in this conformation (Extended Data Figs. 8a and 8b). Conformational changes may take place for the two domains to open the channel before relocating the 3'-vRNA template for catalysis. This hypothesis could be supported by the observation that the density of α-ribbon and pendant domains became less ordered when the 3'-vRNA is bound (Extended Data Fig. 6g). Therefore, the structures of LASV and MACV L might represent two different conformational states of arenavirus polymerase for RNA synthesis.



Supplementary Fig. 1. Uncropped autoradiographs used for preparing Figs. 2 and 4, and Extended Data Fig. 2.