EPN BAG report

TiLV + vRNA (elongation) / FluPolA + vRNA + ANP32A + CTD + NP / Changping earthworm virus 2 polymerase + vRNA

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3 différents samples (3 x 6000 images)

TiLV + vRNA (elongation) (6,000 images)

Tilapia Lake Virus (TiLV) is a recently discovered pathogen affecting tilapia fish and belongs to the *Amnoonviridae* family within the *Articulavirales* order. It has 10 genome segments with distinct conserved ends that encode proteins with no known homologs, except for segment 1, which encodes an orthomyxo-like RNA-dependent-RNA polymerase core subunit. Our recent research demonstrated that segment 1 forms a heterotrimeric complex by interacting with proteins encoded in segments 2 and 3.

Based on those three CM01 cryo-EM data collections, we solved multiple high-resolution structures and found that segments 1-3 encode respectively the PB1, PB2, and PA-like subunits and form a heterotrimeric complex. Despite an unprecedented overall size reduction of 40%, TiLV polymerase still retains all the essential domains found in distantly related influenza polymerases.

In addition, we obtained multiple TiLV polymerase active states, including pre-initiation, initiation, and active elongation (with the highest resolution of 2.4Å), revealing how TiLV polymerase binds to vRNA and cRNA promoters and performs RNA synthesis. Both transcriptase and replicase configurations were characterized. However, the highly truncated endonuclease-like domain seems inactive, and the putative cap-binding domain is autoinhibited, suggesting that many functional aspects of TiLV polymerase remain to be studied.

A paper titled "**Structural and functional characterization of the minimal orthomyxovirus-like polymerase of Tilapia Lake Virus from the highly diverged *Amnoonviridae* family**" has recently been submitted. It includes 12 cryo-EM structures, 11 of which are from CM01, all with a resolution better than 3Å.

FluPolA + vRNA + ANP32A + CTD + NP (6,000 images)

The current model suggests that the influenza virus polymerase (FluPol) can bind to either host RNA polymerase II (RNAP II) or the acidic nuclear phosphoprotein 32 (ANP32), influencing its conformation and directing its activity towards viral genome transcription or replication, respectively.

Through a combination of cell-based and *in vitro* approaches, we have demonstrated that the RNAP II C-terminal domain, in conjunction with ANP32, enhances FluPol *de novo* replication activity. Based on these findings, we propose a model where the host RNAP II serves as the anchor for both transcription and replication of the viral genome.

To support our findings, we collected cryo-EM data on CM01, enabling us to obtain multiple high-resolution structures of influenza A polymerase (H7N9). Specifically, we observed FluPol in (i) a replication initiation-like state (2.5Å), (ii) a replicase conformation (2.9Å), and (iii) a stalled elongation state (2.5Å), all bound to the host RNAP II CTD.

Our research has unveiled initial evidence suggesting that the FluPol-RNAP II binding interface plays an overlooked role in the replication of the viral genome, opening up new perspectives on understanding the spatial coupling of viral transcription and replication, as well as the coordinated balance between these two critical activities.

A paper titled "**The host RNA polymerase II C-terminal domain is the anchor for replication of the influenza virus genome**" has recently been submitted.

Changping earthworm virus 2 polymerase + vRNA (6,000 images)

Changping earthworm virus 2 was recently discovered through metagenomics studies, which have unveiled a vast array of uncharacterized segmented negative-stranded RNA viruses (sNSVs). These viruses can exhibit significant differences in terms of genome organization, making them particularly intriguing. In the case of Changping earthworm virus 2, it stands out as it contains only 6 segments, in contrast to the 7-8 segments typically found in influenza viruses.

Our investigation primarily focused on the structural characterization of the viral polymerase. Among the 6 viral genome segments, segment 1 shows a clear homology to PB1 in its C-terminal region, while segment 2 does not share any homology with other known viral proteins. When considering the proteins encoded on segments 1 and 2 together, they correspond to the size of a “typical” orthomyxovirus heterotrimer, similar to that found in influenza viruses, for example.

Through co-expression and purification optimization of both proteins from segments 1 and 2, we collected cryo-EM data at CM01. These data revealed that segment 1 is a fusion of the related PA and PB1 subunits, whereas segment 2 corresponds to a PB2-like subunit. This new organization represents an intermediate between orthomyxoviruses (3 viral polymerase segments) and bunyaviruses (1 viral polymerase segment). Additionally, structures of this heterodimer revealed that the viral promoter regions bind to specific pockets characteristic of sNSVs viral polymerases.

However, there are some remaining challenges. Further structural studies will be required to unravel the complete domain organization of PB2-C, as it currently exhibits too much flexibility to be fully characterized, similarly to PA-ENDO domain.