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Poster #40: The temporal regulation of crAss-like phage phi14:2 vRNAP across its transcription cycle

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Successful bacteriophage infection requires temporal regulations of both host and viral transcriptional machineries. To proceed through its infection cycle, crAss-like phage phi14:2 employs a virion encapsidated RNA polymerase (vRNAP) for its early genes and re-directs the host RNAP for the middle and probably late genes. The vRNAP is an evolutionarily divergent single-subunit RNAP that does not resemble T7-like RNAPs. Although the vRNAP is structurally characterized, the molecular mechanisms of its productive transcription cycle and its regulatory elements remain unknown.

Our transcriptomic analysis shows that the phage early genes are further separated into a pre-early and an early class where transcription of the pre-early genes requires only the virion packaged proteins. ONT-Cappable-seq identifies a TTS, and not a TSS that separates these temporal classes. Thus, pre-early genes probably encode an anti-terminator to facilitate vRNAP read-through over the TTS.

To map out the proteins involved in phage transcriptional regulation, we performed AP-MS with both the vRNAP and the host Cellulophaga baltica RNAP. We successfully recovered temporally resolved interactomes of both RNAPs across the infection cycle. We show that the phage replication organizer gp022, a homolog of phage λ O protein, interacts with the vRNAP at later timepoints of infection. We successfully purified a transcriptional active gp066-gp022 complex and obtained a 3.92Å Cryo-EM density map, suggesting a functional coupling of phage transcription and genome replication. We also report that gp106, a phage middle protein, can regulate both the host and viral RNAPs. The in-vitro transcription activity of the vRNAP is inhibited by gp106.

Reference #1

Reference #2

Reference #3

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