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# Poster #45: Regulation of Transcription by Jumbo Phage AR9 Encoding Two Multisubunit RNA Polymerases

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Group 1 jumbo phages, despite infecting diverse bacterial hosts, are unified by their multisubunit RNA polymerases (RNAPs): the virion RNA polymerase (vRNAP) and the non-virion RNA polymerase (nvRNAP). These polymerases are homologous to bacterial RNAP. Although the nvRNAP promoter recognition mechanism has been characterized, that of the vRNAP remains unknown.

Here we report the first purification of vRNAP of AR9, a Group 1 jumbo phage infecting Bacillus subtilis. This demonstrates that both AR9 RNAPs core subunits can assemble into functional complexes, without additional factors. Further, the purified vRNAP supports RNA primer extension on promoter-independent RNA–DNA scaffold templates.

Additionally, using AlphaFold predictions, we identified three candidate regulators of the vRNAP, including a putative sigma-like protein. Similar to gp226, the promoter recognition subunit of the AR9 nvRNAP, this protein also contains an N-terminal domain with three α-helices corresponding to σ region 2, the most conserved region of bacterial sigma factors. Unlike canonical $\sigma^{70}$-family of sigma factors, this protein binds DNA directly and specifically recognizes uracil-containing early promoters. Furthermore, homologs of this sigma-like protein are conserved across Group 1 jumbo phages, suggesting a conserved role in transcription regulation.

We further established crosslinking mass spectrometry workflow for B. subtilis infected with the AR9 phage, revealing interactions between the phage and the host transcription machinery and identifying the AR9 protein gp046 as a potential regulator of B. subtilis RNAP. Together, these findings provide insights into alternative strategies of transcription regulation, highlighting the diversity of phage transcription systems.

## Reference #1

Lavysh D, Sokolova M, et al., (2017) Transcription Profiling of Bacillus subtilis Cells Infected with AR9

## Reference #2

Sokolova, M., I. Misovetc, et al., (2020) ``Multisubunit RNA Polymerases of Jumbo Bacteriophages'' Viruses

## Reference #3

O'Reilly, F. J., A. Graziadei, et al., (2023) ``Protein complexes in cells by AI-assisted structural prote

**Author:** MENON, Bhavna (Research group Bacteriophages, Max Planck Institute of Biochemistry, Munich, Germany)

**Co-authors:** BEUKE, Dennis (Research group Bacteriophages, Max Planck Institute of Biochemistry, Munich, Germany); LAVRENKO, Tetiana (Research group Bacteriophages, Max Planck Institute of Biochemistry, Munich, Germany); HARTOUT, Philip Hartout (Research Department Machine Learning and Systems Biology, Max Planck Institute of Biochemistry, Munich, Germany); WYSS, Luis (Research Department Machine Learning and Systems Biology, Max Planck Institute of Biochemistry, Munich, Germany); STEIGENBERGER, Barbara (Mass Spectrometry Core Facility, Max Planck Institute of Biochemistry, Munich, Germany); BORGWARDT, Karsten (Research Department Machine Learning and Systems Biology, Max Planck Institute of Biochemistry, Munich, Germany); SOKOLOVA, Maria (Research group Bacteriophages, Max Planck Institute of Biochemistry, Munich, Germany)

**Presenter:** MENON, Bhavna (Research group Bacteriophages, Max Planck Institute of Biochemistry, Munich, Germany)

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