UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2023-2024

Laboratory/Institute: Institut de Biologie Structurale **Director:** Winfried WEISSENHORN
Team: Viral Replication Machines **Head of the team:** Marc JAMIN

Name and status of the scientist in charge of the project: Wim BURMEISTER, Professor
HDR: yes ☑ **no** ☐

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Program of the Master's degree in Biology:

☑ Microbiology, Infectious Diseases and Immunology ☑ Structural Biology of Pathogens
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Title of the project: Structural and functional study of the telomere-binding proteins of vaccinia virus

Objectives (up to 3 lines):

The mechanism of poxvirus DNA replication, subject of our research, is not yet understood. We suppose that the telomere structure at the circularized extremities of the linear dsDNA genome is important in the initiation of viral DNA replication and we want to work on its structure and the structure of its proteins.

Abstract (up to 10 lines):

With the 2022 epidemic outbreak of mpox, poxviruses got into the headlines. A safe model system is vaccinia virus, which is 98 % identical to mpox at the amino acid level regarding the proteins involved in DNA replication. The dsDNA genome of poxviruses is circularized at the extremities with loops surrounded by a ≈35 bp telomere region with imperfect base-pairing. Three proteins are associated with the telomeres: I1, I6 and K4, where K4 has a nicking and nick-sealing activity. These proteins are packaged together with the viral DNA into viral particles and are also involved in DNA packaging. It is likely that the viral genome replication starts at the telomere as there is no proven origin of replication. The aim of the M2 internship is to produce the telomere-binding proteins in the baculovirus-insect cell system for the *in vitro* reconstitution of the telomere for structural and functional studies. We expect that this project will provide the missing information completing the current work of the team on the vaccinia virus DNA replication machinery.

Methods (up to 3 lines):

Protein production in the baculovirus-insect cell system or *E. coli*. Affinity chromatography. Characterization of the interaction with DNA by BioLayer Interferometry or Electrophoretic Mobility Shift Assays. Sample preparation for cryo-EM of complexes or macromolecular crystallography of individual proteins.

Up to 3 relevant publications of the team:

Hutin, SL, Ling, W.L., Tarbouriech, N., Schoehn, G., Grimm, C., Fischer, U. & Burmeister, W.P. (2022) The Vaccinia Virus DNA Helicase Structure from Combined Single-Particle Cryo-Electron Microscopy and AlphaFold2 Prediction. Viruses 14 (10). doi/10.3390/v14102206.

Tarbouriech N, Ducournau C, Hutin S, Mas PJ, Man P, Forest E, Hart DJ, Peyrefitte CN, Burmeister WP & Iseni F. (2017) The vaccinia virus DNA polymerase structure provides insights into the mode of processivity factor binding. Nat Commun.; 8: 1455.* doi:10.1038/s41467-017-01542-z

Bersch B, Tarbouriech N, Burmeister WP, Iseni F. (2021) Solution structure of the C-terminal domain of A20, the missing brick for the characterization of the interface between vaccinia virus DNA polymerase and its processivity factor. J Mol Biol.; 167009.* doi:10.1016/j.jmb.2021.167009

Requested domains of expertise (up to 5 keywords): Molecular biology (cloning), cell culture, protein purification, biophysical techniques, structural biology