MSc in Biochemistry for Health

Dissertation Project – 2nd Cycle

Student´s Name:
Student email address: No.
Supervisor(s): Dr Colin E. McVey
Supervisor(s) email address: mcvey@itqb.unl.pt
Lab/Institution: Structural Virology Lab, ITQB AX-UNL
TITLE: Role of LANA in the establishment and maintenance of the KSHV epigenome

BACKGROUND

Herpesviruses are a leading cause of human viral disease, second only to influenza and cold viruses. They are capable of causing overt disease or remaining silent for many years only to be reactivated, for example as shingles or a cold-sore. Kaposi´s Sarcoma herpesvirus (KSHV) is one of several oncogenic viruses and has an etiology role in Kaposi´s sarcoma, the leading AIDS malignancy and in certain lymphomas. The herpesvirus infection can be lytic or latent. During latency the viral genome persists in the cell nucleus as a multicopy episome (plasmid) and only a small fraction of viral genes are expressed, including the Latency-Associated Nuclear Antigen (LANA).

LANA is a multifunctional protein with important roles during viral latency[1]. LANA is the only viral protein required for episome maintenance by supporting KSHV latent DNA replication and tethering the viral episome to cellular chromosomes. It is hypothesized that LANA plays a role in the establishment and maintenance of the KSHV epigenome[2]. LANA selectively associates with H3K4 lysine methyl-transferase hSET1 complexes which share three core components: WDR5, RbBP5, and ASH2L. WDR5 contributes to histone modification as part of the MLL1 complex and interacts with LANA via its N-terminal domain to modulate replication. Several LANA hSET1 complexes have been purified with the aim to determine their structure.

Key References

1. Crystal Structure of the Gamma-2 Herpesvirus LANA DNA Binding Domain Identifies Charged Surface Residues Which Impact Viral Latency: PLoS Pathogens 2013
2. LANA Binds to Multiple Active Viral and Cellular Promoters and Associates with the H3K4Methyltransferase hSET1 Complex. PLoS Pathogens 2014

OBJECTIVES

1) To purify and characterize LANA hSET1 complexes for binding stoichiometry using affinity and size exclusion chromatography, and analyze the stability of several LANA hSET1 complexes to select for crystallization experiments.

2) To determine binding constants and thermodynamic profiles of LANA binding to components of the hSET1 complex.

3) To obtain protein crystals of LANA-hSET1 complexes using a high throughput robotic crystallization strategy and imaging system.

The present study will aim to understand the mechanism behind LANA interaction with the hSET1 complex.

Methods

1. Modern cloning techniques (Ligation Independent Cloning, LIC) and recombinant gene expression.
2. Protein purification (affinity columns and size-exclusion chromatography)
3. Biophysical characterization
    - Native gel-shift assay – to identify complexes between protein-protein/DNA
    - Differential Scanning Fluorimetry (DSF) – to determine the stability of the proteins and/or complexes
    - Isothermal Calorimetry (ITC) – to determine the affinity and thermodynamic parameters of the complex
4. Crystallization (state-of-the-art crystallization, crystal imaging automated robot and optimization by robot or manual hanging drops/vapor diffusion methods).

Approach, expected outcome and duration

- **Task 1:** Recombinant gene expression and protein purification followed by protein stability profiling using differential scanning fluorimetry assays (duration – periodically of one or two months)
- **Task 2:** Purified proteins will be characterized for their ability to bind to their interaction partners (components of the hSET1 complex) using simple native gel-shift assays and ITC (duration – two months)
- **Task 3:** Well characterized protein and/or the complexes will be subjected to high-throughput crystallization techniques using state-of-the-art crystallization and imaging automated robots (duration – two to three months)
- **Task 4:** Given the success of the crystallization, there is a possibility to measure X-ray diffraction data on the crystals using in-house and/or synchrotron sources. (duration – two months)

Work Plan:

1) Gene expression and purification of LANA and hSET1 proteins through affinity columns and size exclusion chromatography (1 month and periodically thereafter).
2) Size exclusion analysis of LANA hSET1 complex oligomerization state and stoichiometry when in complex with components of the hSET1 complex (1-2 months).
3) Crystallization of LANA-MLL1 complexes using in-house crystallization robots, for setting up screens and automated imaging of crystallization plates and/or manual crystal optimization using vapour diffusion methods (periodically over 2-3 months).
4) Depending on the success of task 3, the student may have the opportunity of computational work and learn the basic principles of solving an X-ray crystal structure.

Other Benefits

Being part of a highly collaborative project (with both Kaye Lab at Harvard Medical School and Simas Lab Viral Pathogenesis Lab at IMM) there are several opportunities, which we strongly support and encourage, for our students to participate and present their work in national and international conferences during their thesis work.

TIMELINE

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Thesis										

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Lab/Institution: Structural Virology Lab, ITQB AX-UNL

TITLE: The effect of Ubiquitin-Specific Protease 7 on DNA binding activity of LANA.

BACKGROUND

Herpesviruses are a leading cause of human viral disease, as indicated by the severe skin lesions they can cause. Kaposi´s Sarcoma-Associated Herpesvirus (KSHV or HHV8) is the most recent human herpesvirus found to date. Its name originated from the infectious cancer called Kaposi´s Sarcoma (KS), where the virus was first discovered. KSHV is not pathogenic in healthy individuals, but is highly oncogenic in immune-suppressed individuals and is also associated with rare lymphoproliferative disorders. KSHV belongs to the γ-herpesvirus family and establish latency only in lymphocytes[1]. The murine γ-herpesvirus 68 (MHV-68 or murid herpesvirus 4) is a natural pathogen of small rodents, structurally and functionally related to human γ-herpesvirus. The MHV-68 readily infects mice, thus providing an ideal mouse model for *in vivo* studies, given that human γ-herpesviruses fail to infect small laboratory animals.

The Latency-Associated Nuclear Antigen (kLANA) is a KSHV multifunctional protein responsible for tethering the viral DNA to the chromosome, ensuring persistence of the virus during cell division. kLANA´s C-Terminal domain binds to LANA binding sites (LBS) within the viral terminal repeat (TR). The N-terminal domain and the internal repeat region are predicted to be only poorly structured, in contrast the C-terminal domain comprises a stable 3D structure with a strong hydrophobic core. The ORF73 gene from MHV-68 (mLANA) encodes a much smaller, 314 amino acids, 50 kDa nuclear protein. The C-terminal region of mLANA has structural homology to the kLANA DNA-binding domain[1] and also shown to act on TR elements of the MHV-68 genome to mediate episome maintenance and associate with mitotic chromosomes. However, kLANA and mLANA DBD interact with its respective LBS regions very differently, with a bent and linear conformation, respectively, indicating divergence within KSHV and MHV-68[2].

Besides its main role in viral maintenance, LANA also physically interacts with several host proteins to modulate cell functions. One such protein is the Ubiquitin-Specific Protease 7 (USP7), a deubiquitylation enzyme involved in regulation of several proteins including the tumor suppressor p53 and its negative regulator Mdm2. In response to genotoxic stress, USP7 binds and deubiquitylates p53 (i.e, cleaves the small protein Ubiquitin that targets substrates to degradation) thereby protecting it from proteasome-mediated degradation. The project will focus on assessing the effect of USP7 on the DNA-binding activity of LANA, from both KSHV and MHV-68 herpesviruses, using native DNA gel shift assays and to enable structural insights by crystallization of LANA-USP7 complexes.

Key References

1. Crystal Structure of the Gamma-2 Herpesvirus LANA DNA Binding Domain Identifies Charged Surface Residues Which Impact Viral Latency: PLoS Pathogens 2013
2. KSHV but not MHV-68 LANA induces a strong bend upon binding to terminal repeat viral DNA: Nucleic Acids Research 2015

OBJECTIVES

1) To purify and characterize LANA USP7 complexes for binding stoichiometry using affinity and size exclusion chromatography, and analyse the stability of several LANA-USP7 complexes to select for crystallization experiments.
2) To determine binding constants and thermodynamic profiles of LANA binding to USP7 and DNA.
3) To obtain protein crystals of LANA-USP7/DNA complexes using a high throughput robotic crystallization strategy and imaging system.

PROJECT DESCRIPTION

The present study will aim to understand the mechanism behind LANA interaction with USP7.

Methods

1. Protein purification (affinity columns and size-exclusion chromatography)
2. Biophysical characterization
    - Native gel-shift assay – to identify complexes between protein-protein/DNA
    - Differential Scanning Fluorimetry (DSF) – to determine the stability of the proteins and/or complexes
    - Isothermal Calorimetry (ITC) – to determine the affinity and thermodynamic parameters of the complex
3. Crystallization (state-of-the-art crystallization, crystal imaging automated robot and optimization by robot or manual hanging drops/vapour diffusion methods).

Approach, expected outcome and duration

- **Task 1:** Recombinant gene expression and protein purification followed by protein stability profiling using differential scanning fluorimetry assays (duration – at intervals during the first 5 months)
- **Task 2:** Purified proteins will be characterized for their ability to bind to their interaction partners (several LANA truncations in complex with USP7) using simple native gel-shift assays and ITC (duration – two months)
- **Task 3:** Well characterized protein and/or the complexes will be subjected to high-throughput crystallization techniques using state-of-the-art crystallization and imaging automated robots (duration – two to three months)
- **Task 4:** Given the success of the crystallization, there is a possibility to measure X-ray diffraction data on the crystals using in-house and/or synchrotron sources. (duration – two months)

Other Benefits

Being part of a highly collaborative project (with both Kaye Lab at Harvard medical school and Simas Lab on Viral Pathogenesis at IMM) there are several opportunities, which we strongly support and encourage, for our students to participate and present their work in national and international conferences during their thesis work.

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