ICR The Institute of Cancer Research

PhD Project Proposal

Funder details

Studentship funded by:	ICR

Project details

Project title:	Structural investigation of spliceosomes hijacked by oncolytic viruses

Supervisory team

Primary Supervisor:	Vlad Pena
Associate Supervisor(s):	Alan Melcher
Secondary Supervisor:	Sebastian Guettler

Divisional affiliation

Primary Division:	Structural Biology
Primary Team:	Structure and mechanisms of pre-mRNA splicing
Site:	Chelsea

Project background

Splicing is an essential step of gene expression that enables the removal of the non-coding introns via two transesterification reactions. Dysregulation of splicing causes pathological conditions and is regarded as a hallmark of cancer. Splicing is executed by the spliceosome, a multimegadalton complex encompassing more than 150 proteins and five specific small nuclear RNA molecules. Spliceosomes assemble stepwise on the pre-mRNA transcripts, following a pathway of landmark intermediates referred to as E, A, B, Bact, C and P complexes. Many of these complexes, and various transition intermediates, have been characterised by cryo-EM (1).

Emerging evidence shows that viruses can influence splicing and modulate cellular metabolism (2). Of particular interest for us are picornaviruses that can be utilized as oncolytic agents for antitumor therapy (3). The virus encodes proteins that bind the spliceosome, thereby modulating splicing. The viral infection inhibits splicing preferentially for genes associated with cell growth, proliferation and differentiation. How do viral proteins interfere in the spliceosome's conformational transitions and how does it affect differentially specific genes are outstanding questions to be addressed in this project.

Our goal is to reconstitute spliceosomes stalled by oncolytic viruses, resolve the structure by cryo-EM and complement it with functional studies. Our investigation may capture novel transitions in the splicing pathway, reveal how viruses hijack and modulate spliceosomes, and pave the way to improved anti-cancer therapies based on oncolytic viruses and splicing inhibition.

Project aims

- Recombinant expression and purification of viral proteins

- Reconstitution of human spliceosomes stalled by viral proteins
- Isolation and biochemical characterisation of the stalled spliceosomes
- Characterise the 3D structure of the spliceosomes by cryo-EM
- Perform structure-guided investigation of the spliceosome in vitro and in vivo

Research proposal

Overview

The response of spliceosomes to viral infections is an emerging topic, unexplored in structural terms. We aim to reconstitute and resolve structures of spliceosomes stalled by oncolytic viruses.

The workflow's main steps (1-5) are described below. Thus, we will first express and purify the viral proteins in insect cells (1), reconstitute the stalled spliceosomes from human nuclear extracts (2), purify the spliceosomes on a large scale and characterise their biochemical properties (3), resolve the structure of spliceosomes by cryo-EM (4), complement the structure by functional assays (5). Ultimately, we will explore the potential of our findings in translational research, in close collaboration with Prof Alan Melcher, an expert in oncolytic anti-cancer viruses at our institute.

Expression of viral proteins in insect cells and chromatographic purification

We will express recombinant viral proteins in insect cells, following protocols routinely used in our lab. The strategy we are using for purification comprises several chromatographic steps, taking advantage of affinity tags fused to the target proteins. Other purification steps, such as ion-exchange chromatography or size-exclusion chromatography, follow until the protein preparation is homogenous and monodisperse. All these methods are routinely used in our lab (4),(5). We employ small-scale size-exclusion chromatography and mass photometry to assess the conformational homogeneity of the sample. We perform thermofluor assays to screen for chemical compounds that stabilise the protein.

Reconstitution of spliceosomes stalled with viral proteins

We routinely employ methodologies of engineering and isolation of spliceosomes (4,5). We assemble spliceosomes in vitro from nuclear extracts prepared in-house from HeLa cells. As RNA substrates, we will use reporter pre-mRNA molecules that are fluorescently labelled. These substrates are pre-synthesised with T7 polymerase, pre-capped and tagged with MS2 aptamers for affinity purification. To stall the spliceosome inactivated by the oncolytic virus, we will adapt protocols that proved already successful in our hands (4). Splicing inhibition and the spliceosome kinetics will be monitored by electrophoresis to identify the optimal proteins concentration required for stalling the spliceosome at a large scale.

Isolation and biochemical characterisation of the stalled spliceosomes

We will upscale the preparation of stalled spliceosomes from large amounts of HeLa nuclear extracts by chromatography and gradient ultracentrifugation (5). The composition of the complexes will be analysed by electrophoresis and mass spectrometry. The homogeneity will be assessed by mass photometry. Furthermore, we will investigate the molecular organisation of the particles by protein-protein crosslinking coupled to mass spectrometry, thus estimating proximities between the viral proteins and spliceosome components (6). If necessary, the purity and homogeneity will be enhanced at later stages by an additional affinity step. To this end, we will employ the affinity tags fused to recombinant viral proteins to purify the stalled spliceosomes.

Structure determination by cryo-EM

We will likely subject the spliceosomes to stabilisation by chemical fixation in gradient ultracentrifugation (the Grafix method) before conducting electron-microscopic investigations. Negative-staining electron microscopy will further assess the particles' homogeneity, when a preliminary 3D reconstruction will be generated at low resolution. For cryo-EM analysis, we will test various types of grids, including gold grids, which in our hands led to the best results with spliceosomal particles (5). For data collection, we will first use the Glacios cryo-electron microscope from ICR. CryoSPARK and RELION will be used for automated particle-picking and 2D classification, as well as for all the 3D classifications and 3D refinements. For structural characterisation at near-atomic resolution, we will further collect data from a Titan Krios instrument from the Crick institute, where our team has regular access. The GPU cluster from ICR will be extensively used for data processing. Strong insight into the role of these particles may become apparent as soon as the cryo-EM structures will be determined at sufficient resolution to compare them with available structures of spliceosomes.

Complementation by functional assays

To expand our understanding of spliceosome inhibition by oncolytic viruses, we will complement the structural studies with various assays in vitro and in vivo. That is, we will examine the spliceosome structure and produce recombinant viral proteins carrying mutations of residues involved in contacts with the spliceosome. The impact of

these residues will be tested in splicing assays in vitro and by way of minigenes assays in vivo. In collaboration, we will explore the potential of our findings in translational research, in collaboration with the team of Prof Alan Melcher.

Expected outcome

The proposed research may reveal the structure of a novel spliceosome intermediate, extending our understanding of the splicing pathway. Most importantly, it will show for the first time how a virus stalls a spliceosome. Accordingly, the structure may provide insight into how viruses induce alternative splicing in a differential fashion, by affecting a particular subset of genes.

Ultimately, our structural finding may suggest how to engineer oncolytic viruses to achieve higher therapeutic value. Modified viruses might be tested for anti-cancer potential either alone or in combination with antitumor compounds that inhibit splicing.

Literature references

[1] Kastner, B., Will, C. L., Stark, H., and Luhrmann, R. (2019) Structural Insights into Nuclear pre-mRNA Splicing in Higher Eukaryotes. Cold Spring Harb Perspect Biol 11

[2] Boudreault, S., Roy, P., Lemay, G., and Bisaillon, M. (2019) Viral modulation of cellular RNA alternative splicing: A new key player in virus-host interactions? Wiley Interdiscip Rev RNA 10, e1543

[3] McCarthy, C., Jayawardena, N., Burga, L. N., and Bostina, M. (2019) Developing Picornaviruses for Cancer Therapy. Cancers (Basel) 11

[4] De, I., Bessonov, S., Hofele, R., Dos Santos, K., Will, C. L., Urlaub, H., Luhrmann, R., and Pena, V. (2015) The RNA helicase Aquarius exhibits structural adaptations mediating its recruitment to spliceosomes. Nature structural & molecular biology 22, 138-144

[5] Cretu, C., Gee, P., Liu, X., Agrawal, A., Nguyen, T. V., Ghosh, A. K., Cook, A., Jurica, M., Larsen, N. A., and Pena, V. (2021) Structural basis of intron selection by U2 snRNP in the presence of covalent inhibitors. Nat Commun 12, 4491

[6] Cretu, C., Schmitzova, J., Ponce-Salvatierra, A., Dybkov, O., De Laurentiis, E. I., Sharma, K., Will, C. L., Urlaub, H., Luhrmann, R., and Pena, V. (2016) Molecular Architecture of SF3b and Structural Consequences of Its Cancer-Related Mutations. Mol Cell 64, 307-319

Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants: BSc or MSc in Molecular Biology or Biochemistry

Intended learning outcomes:

- Recombinant protein expression
- Purification of spliceosomes
- RNA structural biology
- Electron cryo-microscopy
- Protein Biochemistry

Advertising details

Project suitable for a student with a background in:

- ☒ Biological Sciences
- ☐ Physics or Engineering
- ☐ Chemistry
- ☐ Maths, Statistics or Epidemiology
- ☐ Computer Science