iCase Project / AY 2023 -2024

Understanding and manipulating rAAV DNA biology

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Department/Institute: Biochemistry

Industrial Partner: AstraZeneca

Project outline:

Recombinant adeno-associated virus (AAV) vectors (rAAV) are the leading gene delivery platform for the treatment of a variety of human diseases with several regulatory approvals in Europe and the United States.

AAV is a non-enveloped Dependoparvovirus composed of a capsid that encompasses a single-stranded DNA (ssDNA) genome. It enters cells via specific interactions with cellular receptors. Once inside the nucleus, it utilises endogenous DNA polymerases to convert its ssDNA genome into double-stranded DNA (dsDNA). In the case of rAAV, the majority of the synthesised dsDNA genomes concatemerise and form circular episomes. These episomal DNA species can persist in post-mitotic cells extrachromosomally leading to long-term therapeutic transgene expression.

This project aims to develop a deeper understanding of episomal rAAV DNA biology and its features. The student can expect to gain a multi-faceted understanding of rAAV biology and cellular proteomic machinery involved in the rAAV life cycle. The project will apply and adapt novel proteomics methodologies (doi: 10.1038/s41467-021-26000-9) and computational pipelines (doi: 10.1038/s41467-022-33570-9) developed in the laboratory of Professor Kathryn Lilley in the Cambridge Centre for Proteomics (CCP) (http://proteomics.bio.cam.ac.uk/), that allow spatiotemporal mapping of the transcriptome and proteome on a cell-wide scale to show how the cell, especially nuclear compartments response to persistent expression of rAAV transgenes. The project will also involve capturing episomes and their interacting proteins using a variety of approaches to determine the subcellular machinery that coordinates the episomes and likely candidates to target for its silencing or removal. They will also utilize cutting-edge high-resolution imaging techniques to validate their data.

BBSRC DTP main strategic theme: Understanding the rules of life