The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL : iCASE SCHEME

PROJECT DETAILS	
Project Title:	**Unravelling the molecular basis of fidelity in mRNA splicing**
Short Project Title:	**Unravelling the molecular basis of fidelity in mRNA splicing**

SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Rob van Montfort - Project Leader of a drug discovery collaboration with Merck Healthcare KGaA. - Joint Steering Committee member of multitarget ICR-Merck collaboration. - Extensive previous involvement in other drug discovery collaborations with pharma and biotech.
Associate Supervisor(s):	Dr Paul Clarke - Extensive experience of industry collaboration with Merck KGaA, Astra Zeneca, Astex, PIramed and Genentech. Collaborations discovered and developed inhibitors of HSP90, PI3K, HSF1 and WNT-pathway. On-going collaborations are developing inhibitors of WNT-pathway, protein synthesis and mRNA splicing. - Currently Project Leader of a drug discovery collaboration with Merck Healthcare KGaA and Joint Steering Committee member of multitarget ICR-Merck collaboration. - Supervisor of iCASE studentship. Dr Catarina Rodrigues - ICR structural biologist on an ongoing drug discovery collaboration with Merck Healthcare KGaA. - CryoEM expert in Dr van Montfort's team
Industry supervisor:	Dr Ulrich Grädler - Principal Scientist in protein crystallography at Merck KGaA. - Supervising one PhD student since 2017 at the iBET, Lisbon.
Secondary Supervisor:	Prof. Vlad Pena - Expertise in structural biology of pre-mRNA splicing
IRS Partner :	Not applicable

Lead contact person for the project:	Dr Rob van Montfort
DIVISIONAL AFFILIATION	
Primary Division:	Cancer Therapeutics
Primary Team:	Hit Discovery and Structural Design
Other Division:	Structural Biology
Other Team:	Vlad Pena: Mechanisms and regulation of pre-mRNA splicing Paul Clarke: Signal Transduction and Molecular Pharmacology,

PROJECT PROPOSAL

SHORT ABSTRACT

Splicing of pre-mRNA is a crucial process in gene expression in eukaryotes, linked to human diseases such as cancer. It is catalysed by the spliceosome, a large protein-RNA complex which undergoes major conformational and compositional changes to enable the splicing reactions and release of the spliced mRNA. Structural characterisation by CryoEM and X-ray crystallography have greatly enhanced the understanding of the molecular basis of splicing. However, the processes ensuring proofreading and fidelity mechanisms of the splicing remain structurally poorly understood. This project aims to elucidate these processes by the structural characterization of fidelity complexes identified in our laboratories by cryoEM.

BACKGROUND TO THE PROJECT

Removal of non-coding introns by splicing is a crucial process in gene expression in eukaryotes [1]. Approximately 95% of human genes are alternatively spliced through differences in the way exons are joined [2], thus greatly increasing the complexity of the proteome by producing multiple mature mRNA transcripts from the same gene [2]. Under normal conditions alternative splicing is tightly regulated, but erroneous changes in alternative splicing account for numerous human diseases, including cancer [3].

Splicing is catalysed by spliceosomes - dynamic protein-RNA complexes consisting of five small nuclear ribonucleoproteins and, in humans, ~200 accessory proteins [4]. At least eight RNA helicases drive the conformational changes of the spliceosomes, enabling the two catalytic splicing reactions and subsequent release of the mRNA [5]. In Yeast, two RNA helicases from the DEAH-box subfamily, named DHX38 (aka. Prp16) and DHX8 (aka. Prp22) regulate splicing fidelity. DHX8 plays a crucial role in the release of mRNA from the spliceosome [6,7] splicing fidelity and proof-reading mechanisms by promoting optimal 3' splice sites and rejecting the suboptimal ones [8]. DHX38 antagonizes suboptimal branch site substrates and promotes optimal substrates by enabling rearrangements at the 5' splice site cleavage [8,9].

The van Montfort group recently determined the crystal structure of the DHX8 helicase domain in its ADP- and RNA-bound form (Figure 1A) [10]. The Clarke laboratory has found that DHX8 siRNA silencing in human cancer cells affects the selection of 3' splice sites in cancer cells. This effect was rescued by expression of wild-type DHX8 but not by mutants defective of ATP or RNA-binding. Mass spectrometry profiling of DHX8 interactors identified a number of RNA binding proteins involved in splice junction recognition. We aim to follow-up how this DHX8 multiprotein complex (which we have termed a 'fidelity complex') regulates splicing fidelity of substrates relevant for cancer.

PROJECT AIMS

- Recombinant reconstitution of the fidelity complex
- Functional characterisation of the fidelity complex
- 3D reconstruction of the fidelity complex by cryo-electron microscopy (cryoEM)
- Expand functional and structural investigation towards larger assemblies containing the fidelity complex and RNA substrates relevant to aberrant splicing in cancer.

RESEARCH PROPOSAL

In recent years, structural characterisation of landmark spliceosome intermediates of the canonical splicing cycle (Figure 1B) has vastly increased our understanding of the molecular mechanisms of pre-mRNA splicing. However, there is still much to explore about the regulation of splicing, especially selection of splice sites and the associated mechanisms of quality control.

The current PhD proposal builds on fresh insights in splicing regulation obtained in the Clarke and van Montfort laboratories, the expertise in the structural biology of spliceosomes from Pena group, as well as the wide range of methodologies available at Merck Healthcare KGaA. Our goal is to understand how DHX8 and its protein interactors cooperate to ensure fidelity of splicing for genes implicated in cancer. As a first step, we will reconstitute the DHX8 fidelity complex (intact as subcomplex variants) by co-expression in insect cells, followed by advanced purification approaches (available in the van Montfort and Pena groups).

The PhD-student will first produce the recombinant complexes in the Pena lab. The purified complexes will be assessed for their composition, functional integrity and homogeneity using biochemical assays and characterisation methods including dynamic light scattering (DLS), and size exclusion chromatography multi-angle light scattering (SEC-MALS), thermal shift assays, and crosslinking mass spectrometry methods. The most stable and homogenous complexes will be further characterised using negative stain electron microscopy. For high quality samples, negative stain EM data sets will be collected on our in-house electron microscopes to generate a low-resolution model of the DHX8 fidelity complex. Once samples of sufficiently high quality have been identified, conditions for cryoEM sample preparation and data collection will be optimised. CryoEM data collection will be carried out using the Titan KRIOS microscopes at the Electron Bio-Imaging Centre (eBIC) at the Diamond Light Source (Oxford, UK) via the ICR EM-beamtime allocation group and at the Titan KRIOS microscope of the London CryoEM consortium, of which the Division of Structural Biology at ICR is a founding member. In addition, we will use the Glacios microscope from ICR (expected to be operational by the end of 2020).

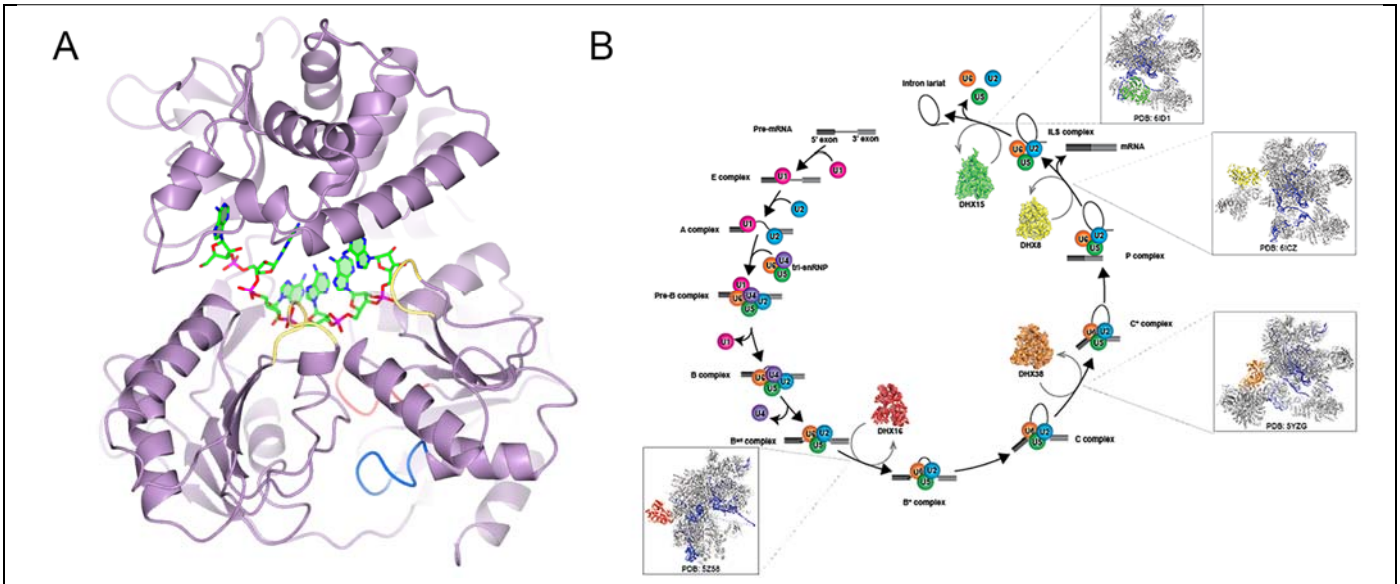

Figure 1. Structural biology of splicing. A) Crystal structure of the DHX8 helicase domain (magenta) bound to a single stranded poly-adenine substrate of 6 nucleotides (green). B) The cyclic pathway of splicing. Structures of DEAH helicases are shown as isolated components or components of the spliceosomes, resolved by crystallography and cryoEM, respectively.

Current cryo-EM structures of spliceosome have been solved using model RNA substrates. We will conduct our studies on RNA substrates relevant to alternative or aberrant splicing in cancer. We will start with the DHX8 regulated gene MCL1. In addition, we will evaluate other substrates identified in Clarke lab, via crosslinking and immunoprecipitation (eCLIP) experiments. To aid the cryoEM investigation of spliceosome complexes, we will make use of DHX8 mutations identified by our teams that modulate the ATPase and RNA-binding functions of this protein and will complement these with mutations that disrupt the functions of DHX8 interactors.

To functionally probe the DHX8 fidelity complex and its proofreading mechanism we will use the cell-based assay established in the Clarke group and at Merck Healthcare KGaA. It is envisaged that the PhD-student will carry out these experiments during a research visit of approximately 4 months at the Merck site (Darmstadt, Germany) at an appropriate time in the 2nd or 3rd year of the project.

The high resolution cryoEM elucidation of the DHX8 fidelity complex could be technically challenging. Therefore, we will complement low resolution cryoEM maps of the fidelity complex with complementary structural approaches such as Small-Angle X-ray Scattering (SAXS) experiments, molecular modelling of the individual components and potentially molecular dynamics simulations to understand conformational changes related to the function of the complex. Computational studies will be carried out in collaboration with Merck Healthcare. These studies will be further complemented by functional studies on WT and mutant fidelity complexes to put the structural data in a biological context.

The PhD-student will be co-supervised by Rob van Montfort, Paul Clarke, Vladimir Pena and Ulrich Grädler, Principal Scientist at Merck. He/she will be primarily based in the van Montfort group, but will collaborate closely with the Pena lab and at spend at least 4 months at Merck Healthcare KGaA. The PhD student and all supervisors will closely liaise with the group of Paul Clarke, as a means to embed the structural studies in a cancer biology context. We envisage that the PhD-student will acquire a skill set in state-of-the art expression, purification, data collection, data processing, and data analysis approaches in

structural biology and will become an expert in understanding the molecular basis of connections between splicing and diseases.

LITERATURE REFERENCES

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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 Hons; class 2.1 or above or MSc in Life Sciences
Intended learning outcomes (including those arising from the industry collaboration):	1) Expert in cloning and protein biochemistry 2) Expert in cryoelectron microscopy 3) Knowledge in other structural techniques such X-ray crystallography and small angle X-ray scattering (SAXS) 5) Knowledge of the application of structural biology in understanding complex biological processes such as splicing 6) Ability to present results in a coherent and analytical fashion both in person and in writing
Potential publications arising from project:	
Estimated amount and distribution of time spent with industrial partner:	Minimum of 4 months, but up to 6 months depending on the nature of the experiments and the training required.

ADVERTISING DETAILS

Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☐ Other (provide details)
Keywords:	**1. RNA splicing**
	2. cryoEM
	3. Structural Biology
	4. RNA Helicases
	5. Spliceosome
	6. Splicing and cancer