Principal Supervisor: Dr. Dmitry Cherny

Co-supervisor: Prof Ian Eperon

**PhD project title:** Crosstalk of U1A splicing factor between transcription and splicing machineries in a transcription-splicing coupled reaction

University of Registration: University of Leicester

**Project outline**

1. Project outline describing the scientific rationale of the project

Modern views on processing of pre-mRNA imply that transcription and splicing are coupled processes. Once newly synthesized mRNA emerges from the RNA polymerase II, after around 25 nucleotides have been transcribed, splicing factors start to interact with it, but the catalytic steps that are required for RNA processing take place later. More importantly, factors controlling transcription and splicing are not entirely independent. Rather, some transcriptional factors might affect splicing reaction and vice versa, presumably via their temporal interactions.

It has been shown that some of the main splicing factors, such as U1, U2, U5 and U6 snRNPs, can be recruited by the transcription machinery in a manner depending on the intron structure of the gene, resulting in a modulation of splicing efficacy. It is believed that U1snRNP plays a major role in this cross-talk as it might interact with CTD domain of RNA polymerase II or other components of the splicing machinery.

Cross-talk between transcription and splicing components leading to the modulation of splicing activity, and alternative splicing in particular, can be explained by two models. In one, proposed by Prof Ian Eperon, the rate of transcription elongation together with the strength of splice site make a great impact on splice site selection when alternative splicing can occur. In another, a recruitment model, splicing factors being recruited by the transcription machinery affect the efficacy of selection of alternative splice sites. Both models are not exclusive, but rather they may represent two faces of the same process.

Over the last decade we have accumulated considerable experience in analysing splicing reactions using single molecule approach based on total internal reflection fluorescence (TIRF) imaging (Dr D Cherny). TIRF imaging is a modern technique that provides unique information about the structural composition of macromolecular complexes that cannot be inferred from the results of bulk measurements. Specifically, the stoichiometry of macromolecular complexes for splicing factors tagged with fluorescent protein like eGFP or mCherry can be determined with high accuracy. Recently, we developed an assay allowing us to test the impact of splicing factors like U1, U2 and U6 snRNPs upon RNA polymerase II transcription in a
transcription-splicing coupled system (Prof Ian Eperon). The results suggest a model for U1 involvement in RNA stability along with its major function that is required for 5’ splice site selection. However, little is known of the mechanisms of U1snRNP crosstalk between these two processes. Our preliminary results obtained by single molecule imaging suggest that several U1snRNP molecules are recruited into coupled transcription-splicing reactions, implying that regulation of alternative splicing can be controlled by both the recruitment of U1 and kinetics of transcription elongation. How these processes are intertwined, what transcription and splicing factors exhibit cross-talk interactions and what the stoichiometries of these coupled transcription-splicing macromolecule complexes might be are questions that will be addressed by both classical splicing assays and single molecule approaches. Furthermore, using purified components of transcription machinery that are tagged with fluorescent dyes (Dr A. Revyakin, University of Leicester), we will address the problem of their involvement in specific cross-talk with splicing factors using single molecule techniques.

References


**Relevant BBSRC Strategic Research Priority:** Bioscience for health

Techniques that will be undertaken during the project.

**Biochemical methods:** molecular cloning, DNA and RNA synthesis and purifications, design of oligonucleotides and DNA amplification by PCR, agarose and acrylamide gel electrophoresis at native and denaturing conditions, western blot analysis, transformation of competent cells, in vitro transcription, in vitro splicing assay, in vitro transcription/splicing assay, mammalian cell biology techniques, transfection of mammalian cells and isolation of nuclear extracts with over-expressed fluorescently labelled proteins.

**Biophysical methods:** purification of the samples using gel sizing chromatography, preparation liquid chambers for single molecule imaging, preparation samples for single molecule imaging, single molecule imaging using prism-based and objective-based total internal fluorescence reflection microscopes, single molecule data acquisition, multi factorial computer-assisted data analysis, super resolution imaging.

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