EPSRC Centre for Doctoral Training (CDT) in Molecular Sciences for Medicine (MoSMed)

Activity-based probes as chemical tool to identify protease involved in mRNA splicing

Newcastle University, School of Natural and Environmental Sciences, Chemistry

Partner: Genentech, Inc, San Francisco

Supervisory Team	• **Dr Chiara Maniaci (Newcastle University – lead supervisor),** • **Dr Ingrid Wertz (Genentech), industrial collaborator** • **Prof. Akane Kawamura (Newcastle University – co-supervisor),** • **Prof. Steven Cobb (Durham University – co-supervisor)**

Project overview/context

RNA Splicing is an essential process that allows the cell to reveal the information to make proteins, much like decrypting a code. Splicing is carried out by a multi-component protein complex called the spliceosome. Some components need to be trimmed to be incorporated into the spliceosome. Little is known about what cleaves these components, how this process is regulated, and its role on splicing. You will address these questions by using tailor-made chemical tools. Results from this project will provide important insights that could identify new targets for drug discovery.

Research Project

In human cells, most genes have multiple non-coding regions called introns. Gene expression requires for all introns to be removed to give rise to a functional protein in a process known as precursor-messenger RNA (pre-mRNA) splicing. Alternative splicing then provides a mechanism for the same gene to give rise to different proteins, for example expressed in different tissues or protein variants with different function. Splicing therefore provides an extra layer of gene regulation, and when it goes awry it can lead to disease. For example, >15,000 of cancer-specific mis-splicing events have been identified. Splicing is becoming an interesting target for the development of therapeutics, reflected by the emergence of many biotech companies targeting RNA directly, and many pharmaceutical companies interested in this area.

Due to the essential role of RNA splicing and its link to disease, it is important to understand regulatory mechanisms at the molecular level. Splicing is carried out by a large multi ribonucleoprotein complex called the spliceosome. Formation of a functional spliceosome requires the stepwise assembly of its components in a tightly regulated fashion. One example of such regulation is the post-translational proteolytic cleavage of spliceosome components. The identity and role of proteases involved in this processing are poorly understood.

Objectives
In this project, you will develop and apply chemical and biochemical tools to help to identify and characterize at the molecular level the protease(s) responsible for proteolytic processing of splicing components. This could reveal novel druggable targets and an alternative approach to modulate splicing indirectly, independent from targeting the RNA.

Experimental approach
To identify the proteases involved, you will use Activity-Based Probes (ABPs). ABPs are chemical tools that allow the specific detection of active enzyme populations under native conditions. You will design and synthesise ABPs derived from the recognition portion of the processed substrates. A range of electrophilic warheads will be explored to achieve activity-based

covalent binding to the unknown protease(s), together with various tags for visualisation and/or cellular enrichment of the labelled enzyme. You will then use the synthesised probes in cell assays to capture the protease involved in the processing of the spliceosome components. Once the proteases have been identified, you will validate their enzymatic activity using biochemical assays in vitro using recombinant proteins and tailor-made reagents, including synthetic peptides. Using CRISPR-Cas9 gene editing technologies, you will then knock out the identified protease from the cell to evaluate what are the functional consequences of impaired processing on splicing.

Novelty and impact
The findings of this project will advance our understanding on how proteases can indirectly regulate pre-mRNA splicing and drive validation of potential new drug targets in this pathway.

References: 1. Urbanski, L. M., Leclair, N. & Anczuków, O. Alternative-splicing defects in cancer: Splicing regulators and their downstream targets, guiding the way to novel cancer therapeutics. *Wiley Interdiscip. Rev. RNA* **9**, e1476 (2018). 2. Hewings, D. S. et al. Activity-based probes for the ubiquitin conjugation–deconjugation machinery: new chemistries, new tools, and new insights. *The FEBS Journal,* 284 (2017) 1555–1576.

Training & Skills

Your research will be based at the state-of-the-art Chemistry / Chemical Biology laboratories at the Bedson building, School on Natural and Environmental Sciences of the University of Newcastle, located at the heart of the city. The project is an exciting collaboration with scientists from Genentech, one of the world-leading pharmaceutical companies, located in South San Francisco, California. You will also benefit from a multidisciplinary supervisory team and a network of UK academic collaborators in Newcastle, Durham, Oxford and Dundee, and have the opportunity to interact with a large group of PhD students and post-docs. You will receive relevant training in a range of techniques and topics in chemical biology, proteomics, peptide synthesis, cell biology and biophysics. Via the Centre for Doctoral Training at Newcastle, you will also access a bespoke training programme of transferrable skills focussed on science, innovation and business skills.

This project would ideally suit candidates with a Master level qualification in Chemistry, Chemical Biology or a closely related subject, with a strong interest in Biochemistry and the Biological Sciences.

Further Information

For informal enquiries about the project, please contact Dr Chiara Maniaci (chiara.maniaci@newcastle.ac.uk)

How to Apply

You must apply through the University's online postgraduate application system

You will need to:

- Insert the **programme code 8207F** in the programme of study section
- Select '**PhD in Molecular Sciences'** as the programme of study
- Input (only) the **studentship reference code (e.g. 21_06)** that you are applying for in the studentship/partnership reference field when prompted (all codes are outlined in the individual project adverts found on the MoSMed website: https://research.ncl.ac.uk/mosmed/phdstudentships/).
- Attach all documents that are requested including a CV and cover letter. The cover letter must **clearly** state the project reference code, the full title of the studentship and set out how your interests and experience relate to the project
- Attach degree transcripts and certificates and, if English is not your first language, a copy of your English language qualifications
- Email: mosmed.cdt@ncl.ac.uk once you have submitted your application to confirm the project you have applied for. Please include the studentship reference code and full project title.

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