**BBSRC MIBTP Studentship Project**

**September 2023**

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| **Project Title** | Roles of protein phosphorylation in coupling transcription termination and RNA maturation |
| **Project Summary** | |
| Expression of human protein-coding genes is a highly complex procedure requiring the coordination of transcription and co-transcriptional processes, including pre-mRNA splicing and mRNA cleavage and polyadenylation (CPA). Interestingly, RNA polymerase (pol) II itself plays a major role in coupling transcription and co-transcriptional processes. This function of pol II is mediated via an unstructured region called the carboxyl terminal domain (CTD), which is composed in humans of 52 repeats of the heptapeptide motif Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7. The pol II CTD can be modified by different post-translational modifications including phosphorylation. Several transcriptional kinases and phosphatases regulate the phosphorylation pattern and level of the pol II CTD but recent works have started to show the importance of protein phosphorylation on other transcriptional and co-transcriptional factors.  We were the first group to show the presence of a transcriptional checkpoint, called the poly(A)-associated checkpoint, at the 3’end of protein-coding genes, which is regulated by protein phosphorylation. Specifically, we recently demonstrated that the activities of the cyclin-dependent kinases (CDK)9, CDK12 and the phosphatase, PP2A, are essential for coordinating transcription termination of pol II with RNA maturation. As failure to pass the poly(A)-associated checkpoint promotes abortive transcription, the poly(A)-associated checkpoint could therefore play an essential role in regulation of gene expression. The presence of a 3’end checkpoint on protein-coding genes could be especially important during a cellular stress response, i.e. following UV irradiation, to ensure a quick inhibition of gene expression.  This PhD project is focused on two major questions to characterise the molecular mechanisms behind the poly(A)-associated checkpoint. The first part of the project will be to determine the targets of CDK9 and PP2A at the 3’end of protein-coding genes and investigate the effects of protein phosphorylation on the recruitment and activities of splicing and mRNA CPA factors. The second part will address how the poly(A)-associated checkpoint is defined and recognized by the elongating pol II, CDK9, and PP2A. To investigate these questions, we will use a combination of molecular biology techniques (including co-immunoprecipitation, chromatin immunoprecipitation, CRISPR/Cas9), genome-wide experiments (nascent transcription with mNET-seq, PRO-seq, and POINT-seq; ChIP-seq, Cut&Run/Cut&Tag), (phospho-)proteomics, and bioinformatics. Collectively, this PhD project aims to understand the mechanisms behind a novel transcriptional regulatory checkpoint located at the 3’end of human protein-coding genes.  Techniques that will be undertaken during the project:  Human cell culture, nascent transcription techniques (mNET-seq, PRO-seq, POINT-seq), chromosome conformation capture (ChIA-PET), chromatin immunoprecipitation (ChIP)-seq, Cut&Tag/Cut&Run, bioinformatics, ChIP-qPCR, co-immunoprecipitation, CRISPR/Cas9, targeted degradation approach (dTag, AID), cloning.  BBSRC Strategic Research Priority: Understanding the Rules of Life - Structural Biology, Systems Biology | |
| **References** | |
| 1. Tellier M, Zaborowska J, Neve J, Nojima T, Hester S, Fournier M, Furger A, Murphy S. CDK9 and PP2A regulate the link between RNA polymerase II transcription termination and RNA maturation. **EMBO Reports**, 2022.  2. Tellier M, Zaborowska J, Caizzi L, Mohammad E, Velychko T, Schwalb B, Ferrer-Vicens I, Blears D, Nojima T, Cramer P, Murphy S. CDK12 globally stimulates RNA polymerase II transcription elongation and carboxyl-terminal domain phosphorylation. **Nucleic Acids Research**, 2020.  3. Tellier M, Maudlin I, Murphy S. Transcription and splicing: A two-way street. **WIREs RNA**, 2020 | |