**University of Leicester**

**BBSRC MIBTP Studentship Project 2025-6 entry.**

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**Section 2 – *Project Information***

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| **Project Title** | Mechanisms of cold-dependent neuroprotection  |
| **Project Summary**  |
| Hypothermia is an important therapeutic tool for mitigating the neurological damage resulting from ischaemic brain injuries after cardiac arrest, stroke and other neurological insults. This benefit arises from increased expression of a cold-shock protein, RBM3, which is an RNA-binding protein that has effects on translation of selected mRNAs. Thus, there is considerable therapeutic interest in understanding how RBM3 is upregulated in the cold and whether it is possible to develop drugs to reproduce this effect. How does cold increase expression of RBM3? Recent research has shown that the gene for RBM3 contains a poison exon. Inclusion of this exon in the spliced product, the mRNA, leads to its degradation. However, in cold conditions this exon is missed out, or 'skipped', leading to stabilisation of the mRNA and thus increasing the levels of protein expressed. This effect seems to depend on temperature-dependent binding of hnRNPH1 to the exon, which promotes skipping (Lin et al., 2023, EMBO J. 42: e113168).  The purposes of this project are to investigate the molecular mechanisms by which temperature affects the binding of hnRNPH1 and other proteins to the exon and to establish how hnRNPH1 mediates skipping when it binds. We have recently shown that we can recapitulate the temperature-dependent skipping in vitro, when synthetic pre-mRNA is added to a nuclear extract. This means that the process is amenable to molecular methods. We will use classical methods, such as cross-linking and affinity purification to identify RNA-bound proteins, SHAPE and other analyses to map the secondary structure of the RNA, and nuclease protection to monitor the binding of splicing factors. Mutagenesis of the proteins and RNA will be used to establish whether these biochemical properties are correlated with the exon skipping effect. We have developed single molecule methods for establishing the numbers of proteins bound to the pre-mRNA (e.g., Cherny et al., 2010, EMBO J. 29, 2161-2172; Jobbins et al., 2022, EMBO J. 41: e107640). This is especially important for hnRNPH1, where there are multiple potential binding sites and the repression of the exon might be strongly dependent on the numbers bound. We can use the same methods to see whether hnRNPH1 binding excludes normal splicing factors or is correlated with the recruitment of co-repressors. Finally, single molecule FRET analyses will be done to understand whether repression of the exon at low temperatures affects the structure and dynamics of the exon and its flanking introns. These investigations may open the way to the development in future of new drugs that, for example, stabilise the interactions between specific proteins. Techniques that will be undertaken during the projectWe will use classical methods, such as cross-linking and affinity purification to identify RNA-bound proteins, SHAPE and other analyses to map the secondary structure of the RNA, and nuclease protection to monitor the binding of splicing factors. Mutagenesis of the proteins and RNA will be used to establish whether these biochemical properties are correlated with the exon skipping effect. We will use the single molecule multicolour colocalization spectroscopy methods that we have developed for establishing the numbers of proteins bound to the pre-mRNA (e.g., Cherny et al., 2010, EMBO J. 29, 2161-2172; Jobbins et al., 2022, EMBO J. 41: e107640; Wills et al., 2024, Comp. Struct. Biotech. 23, 918-928). Single molecule FRET analyses will be done to probe the structure and dynamics of the exon and its flanking introns. NMR experiments may be done in collaboration with Professor Dominguez to look at the structures and dynamics of recombinant proteins and their interactions with RNA.  |
| **References** |
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