**BBSRC MIBTP Studentship Project**

**September 2023**

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| **Project Title** | Structural studies to investigate the mechanisms by which splice sites are selected. |
| **Project Summary** | |
| One of the biggest challenges in molecular biology is presented by the selection of sites for RNA splicing. We cannot overstate its importance or apparent complexity.  Splicing determines which mRNA and protein sequences a gene expresses. It is accurate, removing introns of 102-106 bases, but paradoxically flexible in mammals, where most genes produce multiple isoforms of mRNA. These may produce proteins with different functions (up to 1,800 functional isoforms of neurexin 3, for example) and switching is involved in memory, development, differentiation, signalling and disease. Numerous regulatory proteins and their binding sites have been identified by ensemble and ‘omic’ approaches, but our understanding of their mechanisms and functional integration remains very poor. We have recently made breakthrough findings by combining single molecule methods and chemical biology. Along with a transformative vision of the dynamics of the process, these methods leave us poised to discover the mechanisms of selection.  To meet this challenge we have formed a multi-disciplinary alliance between the Universities of Leicester, Strathclyde and Glasgow. This group brings together expertise in nano-engineering, bio-organic chemistry, photonics, structural biology, RNA splicing and molecular biology, and has been funded by a BBSRC sLOLA grant for 5 years.   This PhD project is concerned with developing a new approach, one that may have considerable impact outside the field of RNA splicing. It is not possible to purify complete complexes formed when splice sites are being selected, because many of the regulatory proteins bind transiently and would dissociate immediately. The problem, therefore, is how to visualize these complexes when they are forming in a functional environment, which in this case means in a nuclear extract replete with many other proteins and ribonucleotprotein particles. How could we visualize just those particles that contain the pre-mRNA? In single molecule microscopy, we achieve this by labelling the RNA with a fluorescent tag. How could we gain structural information, though? We propose to do this by labelling the RNA with a gold nanoparticle and using cryo-EM to deduce the structure of the RNA-protein complex attached to the nanoparticle. This would be an exciting way to obtain otherwise impossible insights into the processes of regulation. The Leicester Institute for Chemical and Structural Biology has excellent resources of expertise and equipment to back this research.  Techniques that will be undertaken during the project:  • In vitro transcription to produce RNA  • Coupling of RNA to gold beads  • Assays of splicing and complex assembly by gel electrophoresis  • Single molecule fluorescence microscopy  • Electron microscopy  • Cryo-electron microscopy and single particle analyses  • Standard molecular biology nethods, such as cloning and PCR.  BBSRC Strategic Research Priority: Understanding the Rules of Life - Structural Biology | |
| **References** | |
| 1. Cherny, D., Gooding, C., Eperon, G.E., Coelho, M.B., Bagshaw, C.R., Smith, C.W.J., & Eperon, I.C. (2010). Stoichiometry of a regulatory splicing complex revealed by single molecule analyses. **EMBO J.** 29, 2161-2172. 2. Jobbins, A.M., Reichenbach, L.F., Lucas, C.M., Hudson, A.J., Burley, G.A., & Eperon, I.C. (2018). The mechanisms of a mammalian splicing enhancer. **Nucleic Acids Research 46,**2145-2158 (*doi: 10.1093/nar/gky056*)*.* **Breakthrough article.** 3. Jobbins, A.M., Campagne, S, Weinmeister, R., Lucas, C.M., Gosliga, A.R., Clery, A.,Chen, L., Eperon, L.P., Hodson, M.J.,  Hudson, A.J.H., Allain, F.H.T. and Eperon, I.C. (2022) Exon-independent recruitment of SRSF1 is mediated by U1 snRNP stem-loop 3. **EMBO J. 41**: e107640. | |