**BBSRC MIBTP Studentship Project**

**September 2023**

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| **Project Title** | How to silence a chromosome: Molecular organization of epigenetic complexes that control X inactivation |
| **Project Summary** | |
| In early embryonic development, XX female mammals will switch off nearly 1000 genes on one of their two X chromosomes (X chromosome inactivation, XCI) to balance gene expression with XY males. The silenced state of the inactive X (Xi) will be remembered by daughter cells and play a crucial role in cell physiology.  Exit from pluripotency requires XCI, which is essential for the survival and development of female embryos. Dysregulation of XCI is involved in over 500 X-linked diseases including immune diseases, cancer and during ageing. Importantly, maintenance of XCI is necessary for the epigenetic stability of human pluripotent stem cells, which often reactivate the Xi in culture, and thus their applicability to cell-based therapies.  The non-coding (nc) RNA Xist regulates XCI by recruiting silencing proteins to the X. Many Xist-interacting proteins contain intrinsically disordered regions (IDRs) which are involved in protein-protein interactions and the formation of nuclear condensates. Using super-resolution microscopy and biochemical assays in stem cell models, we recently discovered that Xist does not bind each gene on the X chromosome subject to silencing. Instead, only 100 Xist molecules form hubs for the binding of hundreds of identified protein molecules generating a nuclear compartment where proteins interact to form supercomplexes (SMACs). Through CRISPR/Cas9 deletions we showed that the essential transcriptional repressor SPEN (SHARP) which is recruited to SMACs, functions in a concentration-dependent manner through its IDRs. Therefore, protein-protein interactions within SMACs play an essential role in epigenetic regulation.  To understand how RNA-guided molecular machines regulate gene expression it is critical to dissect their molecular organization. This project will form a unique opportunity for interdisciplinary work integrating stem cell biology, bioengineering, quantitative super-resolution microscopy and structural biology, such as small angle X-ray scattering and cryo-electron microscopy. It will span scales addressing the function of individual proteins and protein domains, the molecular organization of supercomplexes and their in situ distribution within cells at subdiffraction resolution. We will use CRISPR/Cas9-based methods to bioengineer minimal RNA-guided molecular assemblies within cells, coupled with downstream cross-linking mass spectrometry and spatial gene expression analyses with small molecule FISH probes. This platform will allow the study of RNA-protein complexes within their native environment with minimal intervention.  Ultimately, understanding the molecular mechanisms underpinning formation of Xist-complexes will allow the development of therapeutic applications to tackle dysregulation of XCI in disease or the production of epigenetically stable human pluripotent stem cells. Moreover, unravelling the function of Xist will provide insights into mechanisms of gene regulation and the role of ncRNAs implicated in embryonic development or cancer that share the same protein interactome with Xist.  Techniques that will be undertaken during the project:   * Embryonic stem cell culturing and differentiation * Cloning and other molecular biology methods * Gene editing and bioengineering techniques using CRISPR/Cas9 * RNA/DNA Fluorescence In Situ Hybridization (FISH), immunofluorescence * Super-Resolution and Confocal Laser Scanning Microscopy * Biochemical protein-RNA/protein-protein interaction assays and affinity purification * High-resolution structural studies: small angle X-ray scattering, cryo-electron microscopy and other structural biology methods * Data analysis and visualization in Fiji, R and Python   BBSRC Strategic Research Priority: Understanding the Rules of Life – Stem Cells, Structural Biology | |
| **References** | |
| 1. **Markaki Y\***, Chong JG, Wang Y, Jacobson EC, Luong C, Tan SYX, Jachowicz JW, Strehle M, Maestrini D, Dror I, Mistry BA, Schöneberg J, Banerjee A, Guttman M, Chou T**\***, Plath K**\***. *Xist nucleates local protein gradients to propagate silencing across the X chromosome*. **Cell.** 2021. 2. Pandya-Jones A, **Markaki Y**, Serizay J, Chitiashvili T, Mancia Leon WR, Damianov A, Chronis C, Papp B, Chen CK, McKee R, Wang XJ, Chau A, Sabri S, Leonhardt H, Zheng S, Guttman M, Black DL, Plath K. *A protein assembly mediates Xist localization and gene silencing*. **Nature**. 2020;587(7832):145-51.doi:10.1038/s41586-020-2703-0. 3. 3. Kraus F, Miron E, Demmerle J, Chitiashvili T, Budco A, Alle Q, Matsuda A, Leonhardt H, Schermelleh L, **Markaki Y.** *Quantitative 3D structured illumination microscopy of nuclear structures.* **Nat Protoc**. 2017;12(5):1011-28. | |