STRUCTURE, INTERACTIONS AND DYNAMICS OF AN RBM17 COMPLEX LINKED TO ALTERNATIVE SPLICING REGULATION

The CHERP, RBM17(SPF45) and SR140 proteins have been recently shown to form a stable complex that plays a key role in cell-cycle progression through a specific alternative splicing program [1]. This project combines NMR and integrative structural biology to study the structure, dynamics and molecular interactions of the CHERP/RBM17/SR140 complex, its interactions with additional proteins and the molecular effects of phosphorylation of its components linked alternative splicing. The three core proteins comprise extensive intrinsically disordered regions, that are expected to mediate protein-protein interactions within the complex and with additional factors, associated to the spliceosome. An integrative approach, centered around the use of solution-state NMR spectroscopy combined with complementary techniques, e.g. SAXS/SANS, X-ray crystallography and cryo-EM will be employed.

We have previously shown that the RBM17 UHM domain mediates key interactions for 3' splice site recognition and recently found that RBM17 plays a crucial role in the splicing of short introns with short poly-pyrimidine-tracts. This process is further modulated by an interaction of the RBM17 UHM with a ULM in SAP30BP, a component of the activated spliceosome [2]. We will combine NMR, crystallography and cryo-EM to study the assembly and protein-protein and protein-RNA interactions of the complex. Alphafold will enable initial modelling of the predicted globular domains (UHM, RRM, CID, SURP), while NMR and will be crucial for characterizing the role of the extended disordered regions. Phosphorylation of RBM17 is expected to modulate the function of the CHERP/RBM17/SR140 complex, with a potential link to DNA damage response. We will employ biochemical experiments and NMR to determine the effects of phosphorylation on the structure and dynamics of the complex (using phosphomimicking mutations and *in vitro* phosphorylation).

We will test if the complex binds RNA (motifs at the 3' splice site), and study interactions with U2 snRNP components and further factors (SAB30BP) that have been linked to mediate the splicing activity of the CHERP/RBM17/SR140 complex.

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Proposed collaboration within ArchiFun network (not mandatory at this stage): -

[1] a) Al-Ayoubi et al . (2012) Mitogen-activated protein kinase phosphorylation of splicing factor 45 (SPF45) regulates SPF45 alternative splicing site utilization, proliferation, and cell adhesion. Mol Cell Biol *32*, 2880-2893; doi: 10.1128/MCB.06327-11 b) De Maio A., et al (2018) RBM17 Interacts with U2SURP and CHERP to Regulate Expression and Splicing of RNA-Processing Proteins. Cell Rep *25*, 726-736 e727; doi: 10.1016/j.celrep.2018.09.041. c) Martin E., et al . (2021) Alternative splicing regulation of cell-cycle genes by SPF45/SR140/CHERP complex controls cell proliferation. RNA *27*, 1557-1576; doi: 10.1261/rna.078935.121

[2] a) Corsini L., et al (2007) U2AF-homology motif interactions are required for alternative splicing regulation by SPF45. Nat Struct Mol Biol *14*, 620-629; doi: 10.1038/nsmb1260. b) Fukumura K., et al. (2021) SPF45/RBM17-dependent, but not U2AF-dependent, splicing in a distinct subset of human short introns. Nat Commun *12*, 4910; doi: 10.1038/s41467-021-24879-y. c) Fukumura, et al (2023). SAP30BP Interacts with RBM17/SPF45 to Promote Splicing in a Subset of Human Short Introns. Cell Reports 42 (12): 113534. doi:10.1016/j.celrep.2023.113534.

Proposed list of secondments (not mandatory, but recommended if known already):

- European Synchrotron Radiation Facility, Grenoble, France
- Randall at King's College London

Main ArchiFun theme involved:

- Intrinsically disordered proteins
- Macromolecular complexes
- Small angle scattering