The focus of my research is to understand the role of ribonucleoprotein (RNP) complexes from the network perspective, and in particular to understand how RNA sequences and structures coordinate RNP assembly. We made most progress on the following topics in the last five years:

**Development of CLIP methods to study protein-RNA & RNA-RNA complexes**

We established ‘spliceosome iCLIP’ for the purpose of monitoring spliceosomal contacts and branchpoint positions on pre-mRNAs, and “purified spliceosome iCLIP” to reveal dynamic RNA binding by DEAH-box helicases before and after exon ligation.

**Mechanisms of post-transcriptional regulation**

We uncovered relationships in the positional principles for regulating alternative splicing and 3’ end processing, and discovered recursive splice sites in the longest introns of human genes that are expressed in the brain. We demonstrated that exon definition is crucial for such recursive splicing and that alternative splicing is linked to NMD competes with recursive splicing. Recently, we showed that the exon-junction complex is a potent
mechanism for regulating recursive splicing, and therefore important for proper splicing of hundreds of exons that are of particular importance for brain development.

**Regulatory functions of non-canonical RNA elements & regulatory evolution**

We demonstrated that evolution of new exons and polyA sites from retrotransposable elements (RTEs) is driven largely by gradual derepression, i.e., gradual loss of the binding motifs for the repressive RNPs. Variation in RTEs across species, individuals and somatic tissues leads to changes in RNP assembly, thereby facilitating evolutionary exploration of new gene functions. Mutations that lead to gradual derepression of RTEs in specific tissues can give rise to tissue-specific exons. In particular, we found that MATR3 plays a central role in the control of multivalent LINE-derived sequences within introns, which importantly contribute to the evolution of RNA processing in the brain.

**Protein-RNA complexes in neuronal biology and disease**

In collaboration with Rickie Patani and Nick Luscombe, we continued to unravel the transcriptome-wide functions of RBPs implicated in ALS. We showed how an RNP called “paraspeckles”, which contains these three RBPs, plays a central role in cell fate transitions. To understand links between ageing and neurodegeneration, we uncovered shifts in glial regional identity as a transcriptional hallmark of human brain ageing.

**Future work**

My future work for the remaining time at the Crick, and then later upon my return to UCL, will focus on the RNA features that promote condensation of RNP granules, which is required for mRNA localisation to distal compartments in neurons. We derived hypotheses from our iCLIP and hiCLIP studies, which indicate that long introns and 3’ UTRs form higher-order RNA structures that serve as a scaffold for RNP condensation. We will study how this allows RBPs to bind cooperatively to multiple weak sites and to each other, how the intrinsically disordered regions (IDRs) of RBPs contribute to these interactions, and how such weak interactions can be regulated for fast assembly and disassembly of the RNPs. By this work, we hope to understand how the dynamic condensation of RNPs can control mRNA localisation and other aspects of RNA metabolism.

Ultimately, we would like to contribute towards development of new potential therapeutic interventions that target specific RNP condensates in order to re-establish healthier assembly properties in ALS. This will be integrated with our translation-oriented research into Cas13 technology, which is aimed at developing new antisense RNA technologies as candidate therapies for neurologic diseases.

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**Research outputs**


We established spliceosome iCLIP to map spliceosome engagement with pre-messenger RNAs in human cell lines. We detected the use of over 40,000 branchpoints with strong sequence consensus and structural accessibility, showed how the position and strength of BPs affect the binding of spliceosomal factors, and demonstrated how the method can be used for transcriptomic studies of splicing mechanisms.

This study demonstrated that hundreds of annotated exons can be skipped from partly spliced transcripts through a mechanism called ‘recursive splicing’. Deposition of ‘exon junction complex’ represses this mechanism, which is particularly relevant in Deuterostomes and in the brain, where it can contribute to microcephaly phenotype and human disease.


One of the major surprises of our iCLIP studies was the major role that transposable elements play as hubs for RNP assembly. Here, we uncover multiple roles of LINEs in RNP assembly, and show how this helps to create a repressive environment in introns, while also driving the evolution of new tissue-specific exons.


We showed that hnRNPC maintains transcriptome integrity by repressing the exonisation of Alu elements, and we analysed how changes in the hnRNPC binding site act as a major modifier of Alu exonisation across evolution. We showed that these changes fine-tune the competition between hnRNPC and U2AF2, defining how the splicing machinery discriminates cryptic from genuine exons.


We uncovered recursive splice sites in the longest introns of human genes that are expressed in the brain, thus indicating a role for non-canonical splicing events in human transcripts. This opened the door to understanding how variation in these elements facilitates evolutionary exploration of new gene functions.