Project Details	
Project Code	MRCNMH26Ba Vance
Title	RNA-dependent regulation of the KAP1 chromatin protein controls neuronal stem cell function
Research Theme	NMH
Project Type	Wet lab
Summary	The mammalian genome encodes hundreds of RNA binding proteins that associate with RNA using poorly defined modes of action. Mutations in these are implicated in neurologic disorders including amyotrophic lateral sclerosis and fragile X syndrome. KAP1 is an essential chromatin regulator and unconventional RNA binding protein required for neural stem cell maintenance and neuronal differentiation. KAP1-long non-coding RNA interactions broadly impact KAP1 chromatin and transcriptional regulation. This project will investigate RNA-dependent KAP1 gene expression control of neural stem cell function and nerve cell production and is important for the development of new treatments for neurological disorders.
Description	The chromatin regulatory mechanisms controlling brain development and function remain poorly understood. Recent work has shown that the mammalian genome encodes hundreds of RNA binding proteins (RBPs) and mutations in these are implicated in neurological disorders such as amyotrophic lateral sclerosis and fragile X syndrome. RBPs associate with different types of RNAs to regulate central biological processes and RNA-protein interactions are required for the function of numerous chromatin regulatory proteins. Many RBPs do not contain a known RNA binding domain (RBD) and associate with RNA using poorly defined modes of action. Understanding how RBPs and specific RNA classes interact to mediate gene and chromatin regulation in the brain is a key unanswered question. KAP1 is an essential chromatin regulatory protein required for embryonic brain development and adult brain function. Kap1-/- mice die prior to gastrulation and hypomorphic Kap1 mouse mutants have defects in the development of the nervous system. Conditional deletion of Kap1 in the adult forebrain induces behavioural vulnerability to stress whilst Kap1 depletion in post-natal neural stem cells inhibits neurogenesis and newborn neuronal maturation. Our recent work showed that KAP1 regulates the expression of genes involved in cell cycle control and critical neuronal functions to directly maintain the neural stem cell pool in post-natal and adult mice in vivo and block the differentiation of neuroblastoma cells in culture. Accumulating evidence also indicates that KAP1 is a novel RBP that associates with both mRNA and long non-coding RNA (lncRNA) targets and our work predicts that KAP1-lncRNA interactions broadly impact KAP1-dependent chromatin modification and regulation of neuronal gene expression. We have discovered that KAP1 binds a central nervous system expressed lncRNA called Paupar and that this interaction drives the assembly of a DNA bound chromatin regulatory complex containing KAP1, Paupar and the PAX6 or REST transcription factor to control the expression of key neuronal genes. However, the KAP1 RNA binding domain, the exact role of RNA in KAP1-dependent chromatin regulation and the biological function of the protein-RNA interacting regions in

	neural stem cell biology are all unknown. This work aims to address these key knowledge gaps and generate a high-resolution model of RNA-dependent KAP1 gene expression control of neural stem cell maintenance and differentiation using mouse N2A neuroblastoma cells, a neural progenitor-like cell type widely used as an in vitro model of neural differentiation, as well as primary neural stem cells derived from the SVZ and brain organoid cultures. KAP1 protein comprises several modular functional domains but does not contain any well-defined globular RNA-binding elements and its mode of RNA interaction is unknown. We expect that KAP1 uses the same interaction surface to associate with many RNAs and will thus map the KAP1 RBD by identifying the KAP1 region(s) necessary for binding to the lncRNA Paupar. **Specific objectives:** 1) We will generate KAP1 deletion mutants to systematically remove each individual modular domain and intrinsically disordered region within KAP1 and perform UV cross-linking and immunoprecipitation (CLIP)-qPCR to map of the regions of KAP1 that mediate Paupar RNA binding in N2A mouse neuroblastoma cells. 2) Site-directed mutational analysis, guided by KAP1 structural information, will then be used to identify individual amino acid residues within the KAP1 RNA interaction interface involved in RNA binding and generate a minimally altered KAP1 mutant protein that folds correctly and does not bind RNA. 3) CRISPR-Cas9 gene editing will then be performed to generate a N2A cell line endogenously expressing the KAP1 mutant protein that does not bind RNA. 4) State-of-the-art genomics technologies will be used to map wild type and mutant KAP1 chromatin occupancy along with histone modification and chromatin accessibility at key regulatory regions. This will include chromatin immunoprecipitation (ChIP-seq), Cleavage Under Targets and Tagmentation (CUT&Tag) and ATAC-seq (Assay for Transposase-Accessible Chromatin). Results will be correlated with changes in gene expression (using RNA sequencing) to determine how RNA binding controls KAP1 genome-wide recruitment and chromatin modification in neuroblastoma cells. Experimentally derived hypotheses can be further tested in SVZ-derived neural stem cells as well as human stem cell and brain organoid cultures. During Years 2 and 3 the student will be encouraged to develop and build upon these objectives, and to design their own experiments. The outputs will impact generalisable knowledge of RNA biology and epigenetic regulatory mechanisms controlling neural stem cell function and neuronal differentiation. They are a first step needed for the development of new epigenetic-based regenerative medicine treatments for neurological disorders
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