Splicing and Neurogenesis

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When one thinks of gene expression and regulation, transcription factors and DNA primarily come into mind but the vast potential in genomic variety produced by alternative splicing is sometimes overlooked. The various isoforms produced simply by differential joining and severing of a pre-mRNA transcript can produce a myriad of different functions from a single gene, from the large-scale diversion of the muscle proteome to the complex and intricate field of neurobiology; something hard to ignore when 90% of human genes regulated by such alternative splicing events. In this article we will look at 4 primary protein families in early neurogenesis and splicing: PTBP, SRRM, NOVA and RBFOX.

Splicing can be both constitutive and alternative. Constitutive splicing is the uniform joining of exons which involves core cis-elements (or factors) and core splicing machinery in the formation of a spliceosome; some additional regulation from SR proteins can also be required in higher eukaryotes. Alternative splicing, on the other hand, can join exons in far more ways depending on the circumstances and is a far more situational process regulated by hnRNP proteins (in conjunction with splicing enhancers and silencers), tissue specific splicing factors and SR proteins - mentioned previously.

The mammalian nervous system utilities splicing to keep up with its cellular and functional complexity. The isoforms produced are essential in neuronal development and function and thus must be specific and efficient in their function. Defects in such processes are the source of various neurodegenerative diseases and thus it is fundamental to better understand their regulatory procedures. Perhaps the most interesting regulation in splicing are its patterns and how they are employed throughout the lifecycle of an individual, most clearly in neurogenesis. Alternative splicing can even be used to regulate a cells response to a particular hormone ligand: in the thyroid alternative splicing can produce a transcription factor with a non-functional and functional ligand binding domain and via competitive inhibition the ratio of their levels determines the levels of transcription. There are two key proteins that affect neural fate/differentiation: the polypyrimidine tract binding protein family (PTBP1/2) and the serine/arginine repetitive matrix protein 4 (SRRM4 aka nSR100). The interaction of the two proteins together with miR-124 and the REST complex is the foundation of neuronal cell fate commitment. One third of *in vivo* SRRM4 targets overlap with PTBP2 targets and their relationship could provide insight into synaptogenesis and alternative splicing programmes.

Neural development begins with the PBT1 protein as the trans-differentiation of neurons is induced simply by the depletion of PTBP1 from cultured fibroblasts. The mechanism of this depletion is fundamental in neural stem cells and progenitors and its PBTP1 expression is significantly inhibited on mitotic exit due to the induction of neuronal microRNA miR-124. Furthermore, the expression of its successor PTBP2 can be controlled by PBT1 as exon 10 of PTBP2 is a target of the protein which is significant in its regulation via non-sense-mediated mRNA decay (NMD) which can control the overall abundance of gene transcripts when coupled with alternative splicing; since PTBP2 induction is fundamental in neural differentiation this is further key aspect of PBTP1's influence in development (PTBP2 is the primary protein in the PTBP family found in neurons, myoblasts and spermatocytes). Without PTBP2 a healthy wild type brain cortex at birth (initially regulated by PBTP1) undergoes mass necrosis and is degenerated. Mutated highly expressed PTBP2 is also lethal by causing premature induction of adult isoforms for proteins encoding transcription regulation and other processes which also contributes to neuronal cell death.

SRRM4 is another key protein and it is specifically found in the brain. SRRM4 promotes splicing of the REST4 isoform: its absence results inhibited neural differentiation and causes the accumulation of Pax6+ progenitor cells and depletion of differentiated cells from the cortical plate. Interestingly, these two proteins have overlapping regulatory programmes and SRRM4 can even antagonise PTBP activity; however, with both proteins serving opposing roles in the regulatory network, they have multiple fail-safe mechanisms to ensure success. Srrm4 also has a contrasting relationship with NOVA2 as lack of Srrm4 results in faulty neuronal migration cortical lamination whereas a lack of NOVA2 results in faulty subtype specification.

Alternative splicing in neuronal development is intricate and complicated where overlapping splicing regulatory programmes run in specific neuronal populations and at specific times. The aforementioned NOVA2 (neuro-

oncological ventral antigen) and RBFOX2 (RNA-binding protein fox-1 homologue 2) splicing regulators are fundamental in neuronal migration via the Reelin signalling pathway.

NOVA2 is paramount for proper cortical lamination and cortical neuronal migration. It is located at the forebrain and dorsal spinal cord where, since absence of NOVA2 has little effect on progenitor cell proliferation and radial glia morphology, its function points toward neuronal migration rather than substrate specification. *Dab1* is a component of the Reelin signalling pathway that controls cortical neuronal migration and lamination – its mis-splicing is proportional to the function of *Nova2-/-*. NOVA2'S mechanism of action is via alternative splicing of this Dab1 by forming a corresponding protein isoform DAB1Δ7bc; it does this abnormal inclusion of exon 7b and 7c of the *Dab1* transcript. NOVA2 has another form, NOVA1, and these two proteins work together in the development of the neuromuscular junction; their function is *Agrin* splicing which forms an AGRN protein that upregulates the presence of AChRs in the postsynaptic membrane – receptors are essential in muscle innervation via the neurotransmitter acetylcholine. Lack of such a protein results in paralysis due to lost skeletal muscle function.

RBFOX2, on the other hand, is essential in proper purkinje cell radial migration early in development. It's positional dependence (referred to previously) is clear as its binding upstream of an alternative exon inhibits its inclusion, whereas downstream promotes splicing. There are two other forms of RBFOX (RBFOX1 and RBFOX3) which are involved later in maturation. Lack of RBFOX2 results in hydrocephaly and a smaller cerebellum. Mutations can also occur in such proteins and (in contrast to RBFOX 2) a deletion mutation and thus lack of RBOX1 remarkably results in neuronal hyperexcitability which is common in autism and epilepsy. Although they differ, RBFOX 2 and 1 do work together to regulate mature Purkinje cell function.

These 4 protein families are both fundamental individually and collectively with various overlapping and unique targets. There are some other key families including one that could potentially have further key splicing roles in the brain roles in the brain called CUGBP, Elav-like family (CELF) proteins; however, it has not yet been studied in the mouse *in vitro*. The root of many splicing factor mutation is often unclear due to vast range of phenotypes but advancements in bioinformatics (RNA sequencing and CLIP-seq datasets) can now help identify mis-spliced targets and the corresponding mutant phenotype but due to the excessive number of targets it is still not entirely sufficient. There are examples of solutions to some deficiencies in the proteins such as in impaired synaptogenesis due NOVA1/2 defiency: Z^+ *Agrn* expression can be recovered in motor neurons lacking the protein (due to skipping of exon 32) via a transgene (alternatively spliced isoform). Finding solutions to restoring redundant function is the ultimate goal of research in this complex field of neurogenesis.