Neurogenesis: Regulation by alternative splicing and related post-transcriptional processes

Running title: Post-transcriptional regulation of neurogenesis

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ABSTRACT

The complexity of the mammalian brain requires highly specialised protein function and diversity. As neurons differentiate and the neuronal circuitry is established, several mRNAs undergo alternative splicing (AS) and other post-transcriptional changes that expand the variety of protein isoforms produced. Recent advances are beginning to shed light on the molecular mechanisms that regulate isoform switching during neurogenesis and the role played by specific RNA binding proteins in this process. Neurogenesis and neuronal wiring were recently shown to also be regulated by RNA degradation through nonsense-mediated decay. An additional layer of regulatory complexity in these biological processes is the interplay between AS and long noncoding RNAs. Dysregulation of post-transcriptional regulation results in defective neuronal differentiation and/or synaptic connections that lead to neurodevelopmental and psychiatric disorders.

MAIN TEXT

Post-transcriptional regulation generates protein diversity and specificity through a variety of integrated molecular processes (Fig. 1) (Lara-Pezzi and others 2013; Raj and Blencowe 2015). All adult tissues have a conserved and distinct expression profile that can be linked to their developmental program, but the post-transcriptional signature of the mammalian brain is unique. Tissue-dependent alternative splicing (AS) is predominant in neural tissues, and distinct splicing patterns appear to be regulated by brain-specific factors and are conserved across mammals, especially among primates (Ardlie and others 2015; Irimia and others 2014; Liu and others 2014; Merkin and others 2012; Reyes and others 2013). Neural transcriptomes also predominate in the tissue-specific expression of noncoding RNAs, which show the highest correlation between human and mouse (Necsulea and others 2014). Overall, the establishment of cellular identity in the brain appears to involve several layers of regulation and a unique differentiation program. Here we review recent findings on the regulation of neurogenesis and nervous-system development by alternative splicing (AS), nonsense-mediated decay, alternative polyadenylation, and other related post-transcriptional mechanisms and how dysregulation of these processes leads to neurodevelopmental disorders.

Regulation of neurogenesis by alternative splicing

Mechanism of alternative splicing

During maturation of pre-mRNA into mRNA, introns are removed and exons are linked together in a process known as splicing. However, splicing is not a rigid process, and different mRNAs can be generated due to alternative splicing, such as exclusion of

specific exons from the mature mRNA, partial inclusion of exons due to alternative 5' or 3' splicing sites, or intron retention (Lara-Pezzi and others 2013). AS is tightly regulated. Alternatively spliced exons and their flanking introns contain short nucleotide sequences called cis-regulatory motifs. These motifs are recognised in a sequence-specific manner by a class of RNA binding proteins (RBP) known as trans-regulatory splicing factors. Upon binding their target cis-regulatory motifs, trans-regulatory splicing factors either facilitate or preclude the assembly of the spliceosome on the exon, promoting its inclusion or exclusion (Lara-Pezzi and others 2013). Recent developments in RNA-sequencing and bioinformatics now allow deep transcriptomic analysis (Gatto and others 2014; Giudice and others 2016) that has unveiled the changes and regulation of AS and other post-transcriptional processes operating during neurogenesis (Raj and Blencowe 2015).

Contribution to neuronal wiring

The precise impact of AS on mRNA and protein function is not fully understood. AS has been shown to affect protein domains that are subject to phosphorylation, thereby altering their regulation by cellular kinases (Merkin and others 2012). In addition, recent findings show that AS increases the number of proteins with which a given protein can interact, thereby expanding its potential functionality (Theer and others 2003; Wong and others 2013; Yang and others 2016). This is particularly relevant in the context of neurogenesis, synaptic interactions, and the establishment of the neuronal network. For example, AS regulates the expression of diverse isoforms of Dscam (Down syndrome cell adhesion molecule), neurexins, and other adhesion proteins at the synapse that help to establish specific neuronal connections. In *Drosophila*, *Dscam* provides a prime example of the generation of thousands of alternative isoforms by AS. Dscam is a transmembrane protein that regulates neural circuit assembly mainly by avoiding interactions of neurons with themselves (self-avoidance). While heterophilic adhesion between different Dscam1

isoforms allows neuronal interaction, homophilic adhesion of the same isoform promotes repulsion. AS of *Dscam1* is thought to take place in a probabilistic manner, rather than in a cell-type- or spatial-specific fashion (Miura and others 2013). This mechanism allows single cells to express Dscam1 isoforms that are different from those of their neighbouring neurons. When neurites, axons or post-synaptic elements from the same neuron try to interact with each other, homophilic interaction of the expressed Dscam1 isoform in this cell promotes repulsion and self-avoidance (Wu and others 2012). Dscam2 homophilic binding also promotes repulsion in most cells (Lah and others 2014), although it facilitates interactions between dendrites of L4 lamina neurons in the visual cortex and axons in their target cells (Tadros and others 2016). Therefore, different Dscam members pattern neuronal circuits by promoting either adhesion or repulsion.

The role of Dscam in neuronal wiring has been well established in the fly; however, in mammals this role seems to be played by protocadherins, which are regulated not by AS but by alternative promoter usage (Raj and Blencowe 2015). AS also regulates neuronal interactions by controlling the expression of thousands of variants of the presynaptic adhesion protein neurexin (see below) (Reissner and others 2013; Treutlein and others 2014).

AS directs axonal guidance by generating various isoforms of the membrane receptors Robo3 and Dcc. Roundabout homologue (Robo) proteins are receptors for the secreted protein Slit, which prevents axons from crossing the midline during nervous system development. AS generates 2 Robo3 isoforms. Robo3.1 is unresponsive to Slit and inhibits expression of Robo1 and Robo2; its high expression in axons as they approach the midline allows them to enter the midline and cross it. Expression then switches to Robo3.2, which is sensitive to Slit and prevents midline re-entry (Leischner and others 2010). Robo3.2 protein is only expressed in distal axon regions. The amount

of Robo3.2 protein translated in the post-crossing growth cone is regulated by mRNA degradation through nonsense-mediated decay (NMD) (Chung and others 2013). Axon guidance is further regulated by AS of transcripts of the netrin receptor Dcc (deleted in colorectal carcinoma). Two Dcc isoforms are generated by AS, which differ in a linker sequence between two of the fibronectin repeats in its extracellular domain. Whereas both isoforms show similar affinity for netrin, binding of the ligand induces different ligand:receptor conformations for these two isoforms (Xu and others 2014). Dcc splicing is controlled, at least in part, by Nova. Knockout mice for this RBP show reduced expression of the longer Dcc isoform and a phenotype that resembles that of the Dcc knockout mice, including a delay in axon outgrowth and ventral projection to the midline (Davey and Tickle 2007). These developmental defects are rescued upon restoration of the long Dcc isoform expression, suggesting that the AS changes in this netrin receptor are responsible for the phenotype. Although the precise mechanism underlying the functional differences between both Dcc isoforms is not completely understood, the distinct conformation they adopt in response to netrin is thought to define their biological role.

Alternative-splicing-dependent gene-expression programs

AS was recently shown to control neurogenic transcription factors and epigenetic regulators. Lysine-specific demethylase 1 (LSD1) usually acts as a transcriptional co-repressor together with REST (RE1 Silencing Transcription Factor) and histone deacetylases (HDACs) 1 and 2. The regular LSD1 isoform targets histone H3K4me1/2, whereas the neuron-specific AS isoform LSD1n mediates demethylation of H3K9 and H4K20 (Sanchez and others 2016; Schindelin and others 2012). Unlike LSD1, LSDn acts as a co-activator by interacting with Supervillin to promote expression of neurogenic genes and transcription factors like Egr1 and c-Fos (Rusconi and others 2016). Neuronal

activation alters the LSD1n/LSD1 ratio, and imbalance of these isoforms leads to hypoexcitability or hyperexcitability (Schmid and others 2010). The transcriptional program controlled by LSD1n is necessary for spatial learning and long-term memory formation and for a proper anxiety response (Rusconi and others 2016; Sanchez and others 2016). H3K9 is further regulated by the methyltransferase G9a, which also undergoes AS. Inclusion of G9a exon 10 favours nuclear localisation and neuronal differentiation (Fiszbein and others 2016). In addition to regulating chromatin remodelling proteins, AS regulates the activity of transcription factors. For example, AS of Pbx1 exon 7 can either repress or promote transcription of neuronal genes as differentiation progresses (Linares and others 2015).

RNA binding proteins regulating alternative splicing during neurogenesis

Neurogenesis and brain development are regulated by a network of AS trans-regulatory factors (Fig. 2), and alterations in the expression or stoichiometry of these RBPs may lead to neuronal impairment and disease. The trans-regulatory factor polypyrimidine tract-binding protein 1 (PTBP1), expressed in neural stem cells (NSCs) and non-neuronal cells, prevents neuronal differentiation by repressing a variety of genes that regulate neural development (Zhang and others 2016). PTBP1 directly controls the expression of its paralogue PTBP2 by regulating the inclusion of exon 10. In the presence of PTBP1, this exon is skipped and a premature termination codon is introduced, resulting in PTBP2 mRNA degradation through NMD (see below) (Makeyev and others 2007). As neuronal differentiation progresses, the neuron-specific miR-124 targets and inhibits PTBP1 expression, leading to a strong increase in PTBP2 expression (Makeyev and others 2007). PBTP1 is itself regulated by AS. A newly detected brain-specific exon in PTBP1 mRNA (exon 14) introduces a premature STOP codon and promotes degradation of the mRNA through NMD (Vaquero-Garcia and others 2016). In addition, skipping of PTBP1 exon

9, a mammalian-specific splicing event, relaxes PTBP1-mediated repression of neural differentiation and activates the brain AS programme (Gueroussov and others 2015). Removal of the orthologous exon in chicken induces the expression of several isoforms previously identified in mammals.

In neural progenitors, PTBP2 regulates cell proliferation and differentiation and prevents the premature activation of adult exons in the developing brain (Licatalosi and others 2012). Both PTBP1 and PTBP2 repress splicing of exon 18 in Postsynaptic density protein 95 (Psd-95) mRNA, which results in NMD of the transcripts and reduced protein expression in the developing brain (Zheng and others 2012). The downregulation of PTBP1 and PTBP2 during development allows PSD-95 to be translated and facilitates neuronal maturation and the establishment of glutamatergic synapses. PTBP1 further regulates this process by repressing splicing of the 3' intron of several mRNAs encoding presynaptic proteins, preventing their export to the cytoplasm (Yap and others 2012). Interestingly, inhibition of PTBP1 in fibroblasts induces neuronal reprogramming (Poguzhelskaya and others 2014). This is not achieved through changes in AS, but by regulating the interaction between microRNAs and their target mRNAs. PTBP1 competes with certain microRNAs and prevents inhibition of key repressive factors. PTBP1 downregulation thus facilitates access of microRNAs to different components of the REST complex, which represses transcription of neuronal genes, thereby allowing differentiation to proceed. Full differentiation of reprogrammed cells also requires inhibition of PTBP2 by miR-9, which is induced by the transcription factor BRN2 (Susaki and others 2014).

As neurogenesis progresses, expression of the neuronal-specific trans-regulatory factor nSR100 is switched on (Raj and others 2014). nSR100 takes over the control of PTBP1-regulated exons, facilitates the formation of pre-spliceosomal complexes, and

strongly promotes exon inclusion in mRNAs encoding neural proteins. nSR100 is also the major trans-regulatory factor controlling AS of microexons, small exons of 3-27 nucleotides. Changes in microexon inclusion affect the protein-protein interaction domains of several proteins involved in neurogenesis (Irimia and others 2014). nSR100 further controls neurogenesis by promoting AS of the transcriptional repressor REST. This favours expression of a low-activity REST isoform and thereby allows the expression of genes involved in neurogenesis (Raj and others 2011).

Another major regulator of neurogenesis is RBFOX1. In NSCs, RBFOX1 controls both the expression and AS of genes involved in neuronal differentiation (Fogel and others 2012). These genes include transcription factors, other RBPs, and proteins involved in synaptic transmission and membrane excitation. In the cytoplasm, RBFOX1 binds to the 3' untranslated region (UTR) of mRNAs involved in cortical development, stabilizing them and favouring their translation (Lee and others 2016). RBFOX1 loss-of-function during development results in defective karyokinesis, which impairs radial migration and corticogenesis (Hamada and others 2015). RBFOX2, in contrast, has a more prominent role in the development of the cerebellum. Together with RBFOX1, RBFOX2 controls AS of the sodium channel Scn8a ($Na_v1.6$) and is necessary for proper Purkinje cell pacemaking (Gehman and others 2012). RBFOX2 splicing is regulated by RBFOX3, resulting either in dominant-negative RBFOX2 forms or degradation of *Rbfox2* mRNA through NMD (Dredge and Jensen 2011). RBFOX3, recently identified as the neuronal marker recognized by the anti-NeuN antibody (Dredge and Jensen 2011), promotes neuronal differentiation by regulating AS of Numb (Kim and others 2013), a cell-fate determinant protein that controls, among other processes, NSC proliferation and self-renewal through the inhibition of Notch (Aguirre and others 2010). Numb AS is also controlled by RBM4 during neuronal differentiation *in vitro* (Tarn and others 2016).

RBFOX factors further promote neurogenesis by regulating Ninein AS, which switches from a centrosomal isoform in progenitor cells to a non-centrosomal form in neurons (Zhang and others).

RBFOX1 often acts in combination with NOVA splicing factors to regulate neuronal development (Zhang and others 2010). NOVA family members regulate AS and nucleo-cytoplasmic shuttling and can be found associated with target mRNAs in dendrites (Racca and others 2010). NOVA factors also contribute to axon outgrowth and guidance in the cortex, brainstem, and spinal cord by controlling the splicing pattern of the membrane receptor *Dcc* and other mRNAs (Balaban and others 2012; Davey and Tickle 2007). In addition to genes involved in synaptic functions, NOVA regulates AS of genes encoding protein kinases and phosphatases, thus influencing the protein phosphorylation pattern in neurons (Zhang and others 2010).

Synapses are further regulated by a subset of homologous RBPs that include SAM68, SLM1, and SLM2, which regulate AS of the presynaptic receptor neurexin (Nrxn) (Ehrmann and others 2013; Iijima and others 2014; Iijima and others 2011; Traunmüller and others 2016). Neurexins are pre-synaptic adhesion proteins involved in synapse formation and neurotransmission (Reissner and others 2013). Though encoded by just 3 genes (Nrxn 1, 2, and 3), several structurally different neurexins are generated by AS. The identity of neurexin-interacting partners at the synapse is determined by inclusion or exclusion of exon 20 (termed alternatively spliced segment 4, AS4). In different neuronal subpopulations, AS4 is regulated by SAM68, SLM1, and SLM2. SLM2 knockout mice show synaptic plasticity defects that can be rescued by restoring correct AS4 splicing, underlining the functional consequences that can emerge from changes to a single AS event (Traunmüller and others 2016).

Loss of trans-regulatory splicing factors causes neurodevelopmental disorders

Disruption of key RBPs involved in neuronal differentiation and wiring impairs proper brain development and causes neurodevelopmental disorders. Reduced expression of nSR100 and misregulation of microexon splicing are associated with autism spectrum disorder (ASD). In mice, loss of function of nSR100 results in a shift towards non-neuronal splicing patterns, especially in microexons, that lead to several developmental defects in both the central and peripheral nervous system (Quesnel-Vallières and others 2015). Interestingly, the neuritogenesis defect observed in nSR100 knockout neurons can be rescued simply by restoring a single nSR100-regulated microexon in Unc13b, which is involved in vesicle fusion and neurotransmitter release at the synapse (van de Bospoort and others 2012).

ASD brains also show dysregulation of RBFOX1-controlled AS events (Voineagu and others 2011). Genetic studies in ASD patients have revealed mutations and copy number variations in *RBFOX1* and other genes (Griswold and others 2015; Turner and others 2016; Xiong and others 2015). Deletions in the *RBFOX1* gene cause predisposition to seizures and have been associated with epilepsy (Gehman and others 2011; Lal and others 2015; Lal and others 2013).

Post-transcriptional regulation of neurogenesis by the exon junction complex

The exon junction complex

After splicing, the new exon-exon junction is bound by a multiprotein complex known as the exon junction complex (EJC). The EJC is organized around four core proteins: eIF4A3, MAGOH, RBM8A/Y14, and MLN51/CASC3. The core EJC is assembled on the mRNA following the splicing reaction and is then exported with the mRNA to the cytoplasm (Hir and others 2016). The core EJC is able to interact with several proteins, forming large messenger ribonucleoproteins (mRNPs) involved in different post-transcriptional processes including mRNA transport, AS, translation and nonsense mediated decay (NMD) (Hir and others 2016; Singh and others 2012). Defects in the EJC therefore impact several biological processes and can cause a variety of developmental malformations.

The EJC is necessary for neuronal development

A regulatory role in neurogenesis has recently begun to emerge for certain EJC core components (McMahon and others). During embryonic development, RBM8A is highly expressed in the subventricular zone (SVZ) and the cerebral cortex (Mao and others 2015; Zou and others 2015). RBM8A promotes proliferation of neural progenitor cells and prevents their differentiation (Zou and others 2015). The RBM8A interacting partner MAGOH controls neural progenitor cell proliferation and differentiation by regulating the expression of the microcephaly-associated protein Lis1 (Bi and others 2009). Lis1 binds to microtubules and is necessary for the integrity of the mitotic spindle during cell division. Reduced MAGOH expression impairs the normal orientation of the mitotic division plane during NSC division, causing genomic instability and a delay in mitosis (Silver and others 2010). The resulting prolonged mitosis alters progenitor fate by promoting neurogenic or apoptotic divisions (Pilaz and others 2016). Reduced neuronal expression of another core EJC component, eIF4A3, results in defective mRNA transport to dendrites and alters the miniature excitatory postsynaptic currents at synapses (Giorgi

and others 2007). Loss-of-function of the eIF4A3-interacting factor CCDC174, on the other hand, leads to defective neuronal differentiation (Volodarsky and others 2015). Together, these results highlight the role of the EJC in the regulation of neuronal differentiation and neurogenesis.

Defects in the EJC lead to neurodevelopmental disease

Alterations to EJC components impair neurogenesis and lead to neurodevelopmental disorders. Microdeletions and duplications in the 1q21.1 region, where the *RBM8A* gene is located, are associated with different neurodevelopmental disorders, including brain size defects and autism (Brunetti-Pierri and others 2008). *Rbm8a* haploinsufficiency in the mouse brain causes a reduction in the number of progenitors and mature neurons, leading to defective cortical lamination and microcephaly (Mao and others 2015). Transcriptomic analysis revealed several genes whose expression and/or AS is regulated directly or indirectly by RBM8A, many of which have been implicated in autism and Alzheimer's disease (Zou and others 2015). Microcephaly is also a consequence of *Magoh* haploinsufficiency (Silver and others 2010), and *Magoh* heterozygous mutant mice have a lower-than-normal number of neurons and disorganized cortical layers. Interestingly, a recent study has found that haploinsufficient mice for *Eif4a3* show similar aberrant neurogenesis and microcephaly to those observed in *Magoh* and *Rbm8a* mutant mice (Mao and others 2016). The lack of any of these three EJC core components results in the alteration of common pathways at the onset of neurogenesis, which affect p53, proteasome and ribosome components as well as splicing regulators. Interestingly, loss of p53 rescues the microcephaly phenotype in each of the mutant mice by restoring neuron number and distribution (Mao and others 2016).

Regulation of neurogenesis by nonsense-mediated decay

The mechanism of nonsense-mediated decay

NMD is an essential biological pathway that controls mRNA degradation and ultimately protein expression (Lykke-Andersen and Jensen 2015). The processing of some pre-mRNAs by AS may introduce premature translation termination codons, and the resulting mRNAs are recognized by the NMD machinery and degraded (Fig. 3). The core NMD machinery includes the proteins UPF1, UPF2, and the two UPF3 paralogues UPF3A and UPF3B. The RNA helicase UPF1 recognises premature termination codons and nucleates the formation of the SURF complex. This complex is associated with the ribosome and includes eukaryotic release factors 1 and 3 (eRF1, eRF3), the RNA helicase DEAH box polypeptide 34 (DHX34), and the SMG1 complex (SMG1C, comprising SMG1, SMG8, and SMG9). When a termination codon appears upstream of the EJC bound to an exon-exon junction, the EJC facilitates the recruitment of UPF2 and UPF3 and promotes the formation of the UPF1-UPF2-UPF3 complex (Lykke-Andersen and Jensen 2015; Singh and others 2012). This complex substitutes the ribosome-associated eRF1 and eRF3 and facilitates the formation of a decay-inducing complex and degradation of the mRNA (Hug and Cáceres 2014). Disruption of NMD leads to changes in gene expression that are associated with several diseases, including various forms of intellectual disability (Nguyen and others 2012). Interestingly, in the brain these changes often affect genes encoding RBPs and chromatin regulators, connecting AS and NMD to epigenetic regulation (Yan and others 2015).

NMD is necessary for neurogenesis

The control of protein expression by NMD contributes to the regulation of neurogenesis. UPF1 has recently been shown to promote proliferation and reduce differentiation of

neural progenitor cells. This effect is achieved by promoting the decay of mRNAs encoding a number of proteins that block proliferation and favour differentiation, including p21, p27, p57, and the TGF-β signalling inhibitor SMAD7 (Lou and others 2014). Expression of UPF1 is downregulated by miR-128 (Bruno and others 2011). As neuronal differentiation progresses, miR-128 is induced, resulting in NMD repression and enhanced expression of mRNAs that would otherwise be targeted for degradation. These mRNAs are involved in neuronal development and function and can be induced in NSCs by expression of miR-128. In an interesting negative feedback loop, UPF1 reduces miR-128 expression by activating TGF-β signalling (Lou and others 2014). UPF1 represses the expression of two additional microRNAs, miR-9 and miR-124, which in turn downregulate the NMD factor UPF3B. UPF3B itself reduces the expression of miR-9 and miR-128, further reinforcing the negative feedback loop (Lou and others 2014). The role of UPF3B in neurogenesis is complex and not entirely clear. While UPF3B depletion in neural progenitor cells induces their proliferation and blocks differentiation (Jolly and others 2013), loss of this factor in NSCs results in early differentiation (Lou and others 2014). In contrast to UPF3B, its paralogue UPF3A has the opposite effect and acts as an NMD inhibitor by sequestering UPF2 (Shum and others 2016). UPF3A promotes expression of SMAD7 and other signalling intermediates, and generally has the opposite effect to UPF1.

Alterations in NMD lead to neurodevelopmental defects and disease.

Alterations of the core NMD mechanism are also associated with disease. Mutations in the UPF3B gene that cause degradation of its mRNA, and therefore reduced UPF3B protein expression, are associated with non-specific mental retardation, autism and intellectual disability (Jolly and others 2013; Laumonnier and others 2010). Abnormal

upregulation of coactivator-associated methyltransferase-1 (CARM1), which associates with UPF1 and facilitates its binding to premature stop codons on the mRNA, contributes to the development of spinal muscular atrophy by altering NMD of specific genes potentially involved in in this disease, which include ATF4 target genes, among others (Sanchez and others 2016). Loss-of-function mutations in SMG9 cause congenital anomalies that include brain atrophy and other developmental defects (Shaheen and others 2016). In contrast, NMD can be manipulated to improve neuron survival and function. The RBPs TDP-43 and FUS are abnormally upregulated in certain types of amyotrophic lateral sclerosis (ALS) due to defective NMD, causing motor neuron toxicity. In an ALS model induced by TDP-43, overexpression of UPF1 and UPF2 enhances neuron survival by promoting degradation of TDP-43 through NMD (Barmada and others 2015). Furthermore, gene therapy based on adeno-associated virus 9 (AAV9)-mediated overexpression of UPF1 improves forelimb function in a rat model of ALS induced by TDP-43. The beneficial effect of UPF1 overexpression on motor function in these animals likely derives from its neuroprotective effect, although the precise mechanism was not completely elucidated (Jackson and others 2015). Together, these results suggest that the NMD machinery is a potential therapeutic target.

Regulation of neuronal differentiation and function by alternative polyadenylation

Another post-transciptional mechanism that can impact neurogenesis is alternative polyadenylation (APA), which regulates mRNA localisation and translation and noncoding RNA activity. After transcription, the 3' end of the mRNA is cleaved and a polyA tail is added, favouring mRNA stability and export to the cytoplasm (Elkon and others 2013). The cleavage site in the mRNA is determined by the polyA signal. This

nucleotide sequence is usually found at the mRNA 3' end. However, weaker polyA signals can also be found upstream in the mRNA. Selection of these alternative polyA signals results in a shorter mRNA and often in a smaller protein.

APA is implicated in the regulation of the activity of the ubiquitin ligase Ube3a. Expression of this protein is induced by neuronal activity during the development of excitatory synapses by inducing degradation of ARC, which in turn promotes the internalisation of AMPA receptors at the synapse (Greer and others 2010). Alternative Ube3a polyadenylation results in an alternative transcript (Ube3a1) that produces a truncated protein with no catalytic activity, which regulates the switch from dendrite growth to spine maturation (Valluy and others 2015). Interestingly, this effect is not mediated by the protein itself, but by the 3'UTR of the mRNA; this region sequesters several microRNAs from the miR379-410 cluster and prevents them from binding other cellular mRNAs (Fiore and others 2009; Valluy and others 2015).

Another prime example of a protein whose expression is regulated by APA is HuR, an RBP of the ELAV/Hu family. HuR is ubiquitously expressed and mainly promotes cell proliferation. HuR mRNA in the brain is longer than the variants found in other tissues; moreover, the longer HuR mRNA is less stable and is translated into protein less efficiently than shorter HuR mRNAs (Mansfield and Keene 2012). The longer HuR mRNA is produced upon binding of the neuronal ELAV/Hu proteins HuB, HuC and HuD to the polyadenylation site in the short HuR mRNA. This promotes the generation of an extended, nontranslated 3'UTR, thereby reducing proliferation and favouring neuron differentiation.

APA is also important in the regulation of the localisation and translation of mRNAs in specific subcellular compartments. During neuronal differentiation,

muscleblind-like (Mbnl) proteins promote the use of distal polyadenylation sites that lead to alternative last exons that in turn facilitate mRNA localisation in neurites (Taliaferro and others).

Regulation of neurogenesis by the interplay between RBPs and noncoding RNAs

Long noncoding RNAs (lncRNAs) are emerging as key regulators of several biological processes. However, their role in neurogenesis is still poorly understood. The lncRNA Pinky (Pnky) is specifically expressed in the nervous system. Pnky is enriched in NSCs in their adult niche in the SVZ and in the embryonic brain, and its expression decreases during differentiation (Ramos and others 2015). Pnky prevents NSC differentiation, and its knockdown results in expansion of the transit-amplifying population and neuronal differentiation, both *in vitro* and during embryonic development. Interestingly, Pnky binds PTBP1, which regulates AS during neurogenesis as described above. Knocking down Pnky or PTBP1 results in similar AS changes, suggesting that they participate in a common regulatory pathway.

Another lncRNA involved in neurogenesis is GOMAFU/MIAT (Aprea and others 2013). GOMAFU binds the AS regulators SRSF1 and quacking (QKI), and its dysregulation induces AS changes in genes involved in brain development, such as Wnt7b (Aprea and others 2013; Barry and others 2014). In addition, GOMAFU is downregulated in schizophrenia (Barry and others 2014). The changes in the splicing pattern observed after GOMAFU manipulation resemble those associated with this condition, including those affecting the EGF receptor family member ErbB4 (Barry and others 2014; Chung and others 2016). GOMAFU is also downregulated in fear-conditioned mice: GOMAFU knockdown in the medial prefrontal cortex derepresses

beta-crystallin B1 (Crybb1) expression and induces an anxiety-like behaviour (Genina and others 2010). Repression of Crybb1 transcription by GOMAFU is mediated by interaction with the chromatin remodelling protein Bmi1, suggesting that this lncRNA regulates epigenetic changes and AS.

Conclusions and open challenges

Post-transcriptional mRNA regulation impacts the various steps that regulate neuronal differentiation and shapes the neuronal network in the brain. Recent findings have implicated dysregulation of AS, NMD, and APA in neurodevelopmental disease, particularly in ASD. However, several key questions remain unanswered and additional research is needed to fully determine the role of these processes and how they are integrated. Although roles in neuronal differentiation and brain development have been identified for some RBPs, there are hundreds RBPs whose function remains to be investigated. Importantly, only a few of these proteins show neurospecific expression, and the mechanisms by which ubiquitously expressed RBPs regulate neuronal differentiation remains unclear. Also, whereas in-depth studies are revealing the role of microRNAs in neuronal development, our knowledge of other noncoding RNAs in this process is still very limited. In particular, the study of lncRNAs should provide fresh insight into the post-transcriptional regulation of neurogenesis. The impact of AS and APA on mRNA transport and compartmentalised translation is another field deserving additional attention, offering the possibility to explain not only global expression changes but also where in the cell these changes take place. Understanding how all these processes are regulated will open the door to new therapeutic developments targeting these processes.

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FIGURE LEGENDS

Figure 1. Alternative splicing and related post-transcriptional regulatory mechanisms. During alternative splicing, inclusion or exclusion of an exon in the mature mRNA is regulated by cis-regulatory sequences and trans-regulatory factors. Cis-regulatory sequences are short nucleotide motifs found in the alternatively spliced exon or its flanking introns. These sequences can promote either inclusion of the exon (intronic or exonic splicing enhancer, ISE, ESE) or its exclusion (intronic or exonic splicing silencer, ISS, ESS). Cis-regulatory sequences are recognised by transregulatory factors such as those of the SR or hnRNP protein families, which either promote or preclude the recruitment of the spliceosome. Long noncoding RNAs (lncRNAs) can interact with trans-regulatory splicing factors and regulate their activity. Changes in alternative splicing can lead to the production of a protein with a different structure, localisation, or function. mRNAs carrying premature translation termination codons introduced by alternative splicing are degraded through nonsense-mediated decay. The use of an alternative polyadenylation site (PAS) can lead to an alternative last exon in the mRNA, which may alter the transcript's localisation or its interaction with microRNAs. Adapted from (Lara-Pezzi and others 2013).

Figure 2. Regulation of neuronal differentiation and the establishment of a neuronal network by trans-regulatory splicing factors. Trans-regulatory factors (elliptical boxes) regulate different steps of cellular differentiation and shape the brain circuitry by

regulating synapse formation through changes in AS, mRNA transport, and NMD. NSC, neural stem cells.

Figure 3. Regulation of neuronal differentiation by core nonsense-mediated decay factors. UPF1 promotes degradation through NMD of factors inhibiting neural stem and progenitor cell proliferation, such as p21, p27, p57, and the TGF-β signalling inhibitor SMAD7. In contrast, UPF3A sequesters UPF2 and inhibits NMD to promote expression of SMAD7 and genes involved in neuronal differentiation. UPF3B has been implicated in both NSC proliferation and neuronal differentiation.