Published in final edited form as:

Curr Opin Neurobiol. 2017 August 01; 45: 162–168. doi:10.1016/j.conb.2017.05.012.

Beyond proteome diversity: alternative splicing as a regulator of neuronal transcript dynamics

Oriane Mauger, Peter Scheiffele

Biozentrum, University of Basel, 4056 Basel, Switzerland

Abstract

Brain development and function are governed by tightly controlled gene expression programs. Transcriptional repertoires in neurons are highly specific to developmental stage, neuronal cell type and can undergo rapid changes upon neuronal stimulation. Dedicated molecular mechanisms are required to achieve such fine-tuned regulation. In addition to transcriptional programs, post-transcriptional processes and notably alternative splicing substantially contribute to the elaboration of neuronal gene expression. While alternative splicing has been viewed primarily as a means for expanding proteome diversity, it recently emerged to also be major regulator of transcript levels and dynamics. In this review we will describe some of the principal alternative splicing-linked mechanisms that control neuronal transcriptomes and discuss their implications on the central nervous system.

Introduction

Brain development and function rely on sophisticated molecular programs that direct cell specification, neuronal interactions, and plasticity. The multitude of distinct neuronal cell types, the size and highly polarized morphology of individual neuronal cells, as well as the need to rapidly modify neuronal growth and function with exquisite spatio-temporal selectivity raise fascinating questions regarding the mechanisms that accomplish such regulation. Unsurprisingly, the anatomical and functional diversity of cells in the nervous system is matched by a rich repertoire of transcriptional and post-transcriptional mechanisms that direct neuronal differentiation and plasticity. Alternative splicing is more prevalent in the brain as compared to other organs. Its ability to generate numerous transcript isoforms from a finite number of genes has been proposed to encode aspects of neuronal specificity [1,2]. Disruption of post-transcriptional regulators contributing to regulation of alternative splicing, mRNA stability, mRNA transport, or mRNA translation result in severe neurological, neurodevelopmental, or neurodegenerative diseases [3–6], further highlighting the particular importance of these mechanisms for normal nervous system function.

Alternative splicing in the central nervous system has received particular attention given its well-established role in expanding proteome diversity. For example, for the neuronal cell surface receptors neurexin and DSCAM alternative splicing has been demonstrated to direct

the generation of hundreds or thousands of distinct receptors from only a few genes [7,8]. In the case of *Drosophila* DSCAM such receptor isoforms contribute to neuronal self-recognition [9] whereas for the mouse neurexin genes there are splice isoforms that govern specific forms of synaptic plasticity and are essential for specific animal behaviors [10–12]. However, many alternative splicing events modify transcripts without resulting in changes in the encoded protein sequence [2,7]. Instead, alternative splicing modifies RNA codes within a transcript and, thereby, regulates recruitment of RNA-binding proteins and/or non-coding RNAs that modify transcript stability, localization, and signaling-dependent mRNA translation. Ultimately, these RNA codes are major determinants of the spatio-temporal control of gene expression [13]. Recent genome-wide studies in neuronal as well as non-neuronal cells uncovered that such post-transcriptional roles for alternative splicing are much more widespread than previously anticipated. In this review, we will discuss several principal forms of such spatio-temporal gene regulation by alternative splicing and discuss their implications for neuronal development and function.

Alternative splicing-coupled NMD in the regulation of transcript levels

One major post-transcriptional mechanism that regulates neuronal transcript levels is alternative splicing coupled to nonsense-mediated decay (NMD) [14]. NMD was originally discovered as a quality control mechanism that triggers degradation of cytoplasmic transcripts containing a premature termination codon (PTC) positioned at least 50–55 nucleotides upstream of an exon-exon junction. Upon pre-mRNA splicing so-called exon junction complexes (EJC) are deposited on the transcripts and these EJCs are expelled from the transcripts during the pioneer round of translation. However, if EJCs are loaded downstream of the translational termination codon they remain bound to the transcript and establish stable interactions with the UPF (up frameshift) protein complex. In turn, this complex triggers degradation of the mRNA. Initially, NMD was thought to primarily act as a RNA surveillance machine by eliciting elimination of aberrant transcripts resulting from mutations or RNA processing errors. However, from more recent studies it has become clear that NMD regulates gene expression under physiological conditions. Inactivation of the NMD machinery triggers deregulation of 5–15% of cellular mRNAs [15,16]. In mice, knock-out of the NMD mediators *Upf1* or *Upf2* leads to early embryonic lethality [17,18]. *Drosophila* mutants in *Smg1* a kinase regulating NMD exhibit impairment in synaptic transmission [19] and in the human population there are strong genetic associations between genes encoding NMD components and intellectual disability [5].

There are now a number of examples for specific aspects of neuronal differentiation and growth that rely on transcript-tuning through NMD. Several mRNAs encoding synaptic proteins are expressed in neuronal precursor cells. In these precursor cells, the transcripts for post-synaptic density protein 95 (PSD95) exclude exon 18 leading to a premature termination codon and NMD-mediated degradation of the mRNA [20] (Figure 1, left). Exclusion of exon 18 is driven by the RNA-binding proteins PTBP1/2. Upon neuronal differentiation PTBP1 and 2 are down-regulated, in turn, exon 18 is included into *Psd95* mRNAs resulting in transcript stabilization and expression of the synaptic protein in the post-mitotic neurons. Similarly, expression of *Gabbr1* coding for the GABA_B receptor subunit 1 is under control of PTBP-mediated alternative splicing coupled to NMD [21]

(Figure 1, left). Only in absence of PTBP, the *Gabbr1* coding transcript is expressed. Recent genome-wide studies uncovered thousands of alternative exons and intron retentions whose inclusions regulate transcript targeting to NMD [2,22]. Many of them exhibit a neuron-specific pattern and restrict expression of coding transcript consistent with neuron biology. This work supports that alternative splicing coupled to NMD is a widespread mechanism used to elaborate neuron-specific transcriptomes.

Notably, neuronal gene regulation through alternative splicing coupled to NMD is not restricted to transitions from non-neuronal to neuronal cell fate but similarly contributes to gene regulation between distinct neuronal cell types or dynamic gene regulation in response to signaling. In the mouse hippocampus, RNA-binding protein paralogues SLM1 and SLM2 are expressed in a mutually-exclusive pattern in dentate granule cells versus *cornu ammonis* pyramidal cells. This neuronal cell type-specific expression is achieved by SLM2-dependent targeting of *Slm1* mRNA for alternative splicing coupled NMD [23]. For the neuronal RNA binding proteins NOVA1/2, a genome-wide analysis identified cryptic exons whose inclusion or skipping define whether transcripts are targeted to NMD. Interestingly, inclusion of some of these cryptic exons is dynamically regulated by neuronal stimulation. For example, *Scn9a* transcripts encoding a subunit of voltage-gated sodium channel are up-regulated upon pilocarpine-induced seizures [24] (Figure 2, left). This results from the exclusion of the Nova-dependent cryptic exon targeting *Scn9a* transcripts to NMD consequently to the cytoplasmic translocation of NOVA. Increase expression of SCN9A protein is predicted to increase neuronal activity and might contribute to the excessive spontaneous electrical discharges observed in the *Nova2* mutant mice.

Alternative splicing coupled NMD and spatio-temporal control of neuronal gene expression

The stepwise nature of the NMD mechanism, in particular the requirement for a pioneer round of mRNA translation, raises the question whether proteins encoded by the NMD-transcript might have a physiological function. The pioneer round of translation will produce a small amount of protein. It is still unclear whether in all cases NMD-mediated degradation immediately obliterates the transcript or whether in some cases additional protein copies can be generated before transcript decay. In fact, the efficiency of the NMD degradation machinery varies between cell types, developmental stages, and may be modified by signal transduction.

One interesting model for how proteins derived from NMD-transcripts might contribute to neuronal function has emerged from studies on ARC, a cytoplasmic protein that regulates trafficking of AMPA-type glutamate receptors. *Arc* transcripts are localized in dendrites and are natural targets of NMD due to the presence of two constitutively spliced introns in the 3'UTR [25]. *Arc* mRNAs are detected in neuronal dendrites where they are associated with eIF4AIII, a component of the exon junction complex. It is thought that these dendritic mRNAs are translated only in response to a synaptic stimulus. This would produce a spike in local protein production, followed by the NMD-mediated degradation of the mRNA (Figure 3).

A similar mechanism may contribute to the guidance of developing axonal growth cones at the spinal cord floor plate. After crossing the floor plate commissural axons switch their response from being attracted to being repelled. There is a specific transcript isoform encoding the axon guidance receptor ROBO3 expressed in commissural axons. This transcript retains an intron with a premature stop codon and, thus, is targeted for NMD [26] (Figure 3). In *Upf2* knockout mice, commissural axons exhibit aberrant guidance that support a role for NMD in fine tuning *Robo3* expression and function [27]. Thus, as previously demonstrated for *Arc* mRNAs NMD is indeed a key mechanism for precise spatial as well as temporal control of neuronal gene expression.

The examples of *Arc* and *Robo3* illustrate that proteins encoded by naturally-occurring NMD targets have major functions in neuronal development. This predicts that tuning the activity of the NMD-pathway up or down can be a powerful mechanism for controlling neuronal differentiation and function. Thus, with a maximal NMD efficiency a mRNA would essentially be degraded after only a single protein copy has been synthesized. By contrast, when NMD efficiency is reduced then multiple protein copies would be generated before the mRNA decays. Indeed, microRNA-dependent control of the expression levels of UPF1 has been implicated in the control of pro-neural factors [28,29]. Moreover, the paralogues UPF3A and UPF3B have antagonistic functions vis-à-vis the NMD pathway and, thereby, provide an additional means for setting NMD-activity levels in a cell and tissue-specific way [30]. Finally, in some cells intracellular calcium has been reported to modify NMD activity [31]. While the molecular mechanism is currently unknown, this observation raises the tantalizing hypothesis, that NMD activity (and thereby the protein yield from NMD-transcripts) might be controlled locally in response to synaptic and/or neuronal calcium signaling.

Alternative splicing-dependent nuclear sequestration and degradation

Targeting specific splice isoforms for cytoplasmic degradation by NMD is not the only way how alternative splicing can control overall mRNA abundance in neurons. In fact, there is a powerful mRNA degradation machinery in the cell nucleus. RNA surveillance and RNA quality control mechanisms prevent cytoplasmic export of aberrant transcripts, followed by degradation by the nuclear exosome [32]. While mainly thought to represent an error-correction mechanism, it has now emerged that the nuclear exosome machinery provides physiological regulation and buffering of transcript levels [32,33]. Mutations in genes encoding for exosome core components (*Exosc3* and *Exosc8*) are associated with spinal muscular atrophy and pontocerebellar hypoplasias [3,4]. Phenotypes in Zebrafish knock-downs for *Exosc3* and *Exosc8* reproduce neuronal defects associated with the diseases. Thus, exosome-mediated-RNA degradation indeed has an important contribution to the refinement of cellular transcriptomes for brain development.

The nuclear exosome is a versatile machine targeting various RNA substrates, but the molecular determinants responsible for the selection of RNAs for exosomal degradation remain poorly understood. The exosome complex itself is largely unspecific but its selectivity is imposed by co-factors recruited to the target transcripts [32]. AU-rich element (ARE)-binding proteins trigger exosome-mediated degradation of transcripts containing the

corresponding ARE sequences [34,35]. Moreover, incompletely spliced precursor mRNAs containing remaining introns represent an important class of exosome targets. For example, in yeast, the serine-arginine (SR) proteins Gpb2 and Hrb1 target unspliced transcripts to exosomal degradation [36]. Thus, there are RNA codes within transcripts and corresponding binding proteins that act specifically in exosomal degradation. As for NMD, the RNA codes are under direct control of alternative splicing. In particular, regulated intron retention events can serve to direct pools of transcripts to exosomal degradation. In non-neuronal cells, several transcripts encoding synaptic proteins (*Stx1b*, *Vamp2* and *Sv2a*) are significantly expressed but the mRNAs retain select introns [33]. These transcripts are sequestered in the nucleus and targeted for degradation by the nuclear exosome, thus preventing expression of the synaptic proteins (Figure 1, right). In mature neurons, these transcripts are fully spliced, the mature transcripts are exported to the cytoplasm and translated [33]. Thus, alternative splicing coupled to exosome RNA degradation controls transcriptomic programs during neuronal differentiation.

Alternative splicing coupled to nuclear storage and release

While for many transcripts intron retention results in nuclear sequestration and decay, there are some intron-containing RNAs that escape degradation. A sub-population of intron-containing transcripts exhibits surprisingly long half-lives in embryonic stem cells and in cell lines [37,38]. Genome-wide approaches in neocortical cells revealed that half of the intron-containing transcripts (about 3000) are stable for hours after completion of transcription. In fact, these transcripts are stabilized as almost mature poly-adenylated RNAs retaining only one or two introns [39]. Most of these intron retaining-transcripts are localized to the nucleus where they appear to be stored [37–39]. The mechanisms shielding the intron-containing transcripts from nuclear exosome-mediated degradation and preventing cytoplasmic export are currently unknown.

It is an attractive hypothesis that splicing of these stored transcripts could be initiated in response to cell signaling and, thereby, the transcripts exported to the cytoplasm. Under stress conditions, splicing and nuclear export rates are altered. In response to heat shock, an arrest of splicing has been described for multiple transcripts in *Drosophila melanogaster*, mouse, and human cells [40–42]. For other transcripts, osmotic stress or DNA damage have been reported to enhance pre-mRNA splicing [37,38,43,44]. Studies in cell lines uncovered transcripts encoding the kinases CLK1 and CLK4 to exhibit a substantial degree of intron-retention. Osmotic stress or heat shock resulted in intron excision [38]. Remarkably, in fern, removal of retained intron in pre-existing transcripts encoding proteins essential for gamete development have been observed during spermatogenesis [45]. Hence, by modulating splicing rates, different pools of mRNAs can be mobilized independently from transcription.

Notably, signaling-dependent splicing of stored intron retaining transcripts also contributes to physiological regulation of neuronal gene expression [39]. In neocortical cells, neuronal activity can stimulate excision of stably retained introns from certain transcripts whereas it increases retention of introns in other transcripts (Figure 2, right). Transcripts undergoing splicing upon neuronal stimulation are exported to the cytoplasm and associate with ribosomes indicating that this activity-dependent intron excision indeed produces functional

mRNAs. This mechanism relies on NMDA-type glutamate receptor function and signaling through calmodulin-dependent kinases. Multiple transcripts mobilized through this mechanism encode for cytoskeletal and signaling proteins. Thus, activity-dependent splicing of stored, intron-containing transcripts could provide a source of effectors for functional and structural plasticity.

Signaling-dependent retention or mobilization of nuclear transcripts through the attenuation or completion of intron excision may have emerged to speed up regulation of transcript levels in response to extracellular stimuli. The splicing reaction operates on a timescale of seconds to minutes [46]. By contrast, synthesis of new transcripts is limited by the RNA polymerase II elongation rate (1 - 4 kb/min) [47]. For long transcripts that are overrepresented in neurons [48,49], completion of transcription takes several hours. Notably, activity-regulated retained introns are particularly prevalent in long genes [39]. Upon signaling, the intron retaining transcripts are spliced within a few minutes, enabling the rapid transcription-independent elevation of long transcripts.

In the future, it will be interesting to investigate whether these transcription-independent nucleo-cytoplasmic RNA dynamics are restricted to intron retaining transcripts. Recent studies highlight that some fully matured RNAs are retained in the nucleus [50,51]. This nuclear retention appears to buffer gene expression variability arising from transcriptional bursting [50,51]. Such a mechanism might be repurposed for other functions to achieve rapid, transcription-independent responses to neuronal signaling.

Conclusions

The recent advances in genome-wide transcript analysis revealed that alternative splicing is a major regulator of transcript abundance. These novel roles for alternative splicing are based on the splicing-dependent integration of RNA codes that target transcripts to specific downstream pathways of RNA metabolism. These pathways – e.g. NMD or nuclear transcript sequestration - have originally been uncovered as RNA surveillance mechanisms that prevent expression of aberrant transcripts in pathological circumstances. However, the progress over the last decade shed light on the widespread usage of such mechanisms under physiological conditions. The fundamental cell biological pathways discussed here are largely shared from single cell to complex organisms. However, they are of particular interest in the central nervous system where they contribute to the molecular and functional diversification of cell types and the tight spatio-temporal control of gene expression. The recent discoveries of additional forms of RNA regulation such as circular RNAs or epitranscriptomic modifications will likely pave the way to a further, deeper understanding of the RNA-based mechanisms of neuronal identity and plasticity.

Acknowledgements

We thank Elisabetta Furlanis for constructive comments on the manuscript. O.M. was financed by a grant from the Swiss SystemsX initiative, evaluated by the Swiss National Science Foundation. Work in the laboratory was supported by funds to P.S. from the Swiss National Science Foundation, a European Research Council Advanced Grant (SPLICECODE), and the Kanton Basel-Stadt.

References

1. Barbosa-Morais NL, Irimia M, Pan Q, Xiong HY, Gueroussov S, Lee LJ, Slobodeniuc V, Kutter C, Watt S, Colak R, et al. The evolutionary landscape of alternative splicing in vertebrate species. *Science*. 2012; 338:1587–1594. [PubMed: 23258890]
2. Yan Q, Weyn-Vanhentenryck SM, Wu J, Sloan Sa, Zhang Y, Chen K, Wu JQ, Barres Ba, Zhang C. Systematic discovery of regulated and conserved alternative exons in the mammalian brain reveals NMD modulating chromatin regulators. *Proc Natl Acad Sci U S A*. 2015; 112:3445–3450. [PubMed: 25737549]
3. Wan J, Yourshaw M, Mamsa H, Rudnik-Schöneborn S, Menezes MP, Hong JE, Leong DW, Senderek J, Salman MS, Chitayat D, et al. Mutations in the RNA exosome component gene EXOSC3 cause pontocerebellar hypoplasia and spinal motor neuron degeneration. *Nat Genet*. 2012; 44:704–708. [PubMed: 22544365]
4. Boczonadi V, Müller JS, Pyle A, Munkley J, Dor T, Quartararo J, Ferrero I, Karcagi V, Giunta M, Polvikoski T, et al. EXOSC8 mutations alter mRNA metabolism and cause hypomyelination with spinal muscular atrophy and cerebellar hypoplasia. *Nat Commun*. 2014; 5
5. Sartor F, Anderson J, McCaig C, Miedzybrodzka Z, Muller B. Mutation of genes controlling mRNA metabolism and protein synthesis predisposes to neurodevelopmental disorders. *Biochem Soc Trans*. 2015; 43:1259–1265. [PubMed: 26614670]
6. De Conti L, Baralle M, Buratti E. Neurodegeneration and RNA-binding proteins. *Wiley Interdiscip Rev RNA*. 2017; 8
7. Schreiner D, Nguyen T-M, Russo G, Hebr S, Patrignani A, Ahrné E, Scheiffele P. Targeted Combinatorial Alternative Splicing Generates Brain Region-Specific Repertoires of Neurexins. *Neuron*. 2014; 84:386–398. [PubMed: 25284007]
8. Sun W, You X, Gogol-Döring A, He H, Kise Y, Sohn M, Chen T, Klebes A, Schmucker D, Chen W. Ultra-deep profiling of alternatively spliced *Drosophila* Dscam isoforms by circularization-assisted multi-segment sequencing. *EMBO J*. 2013; 32:2029–2038. [PubMed: 23792425]
9. Miura SK, Martins A, Zhang KX, Graveley BR, Zipursky SL. Probabilistic splicing of Dscam1 establishes identity at the level of single neurons. *Cell*. 2013; 155:1166–1177. [PubMed: 24267895]
10. Aoto J, Martinelli DC, Malenka RC, Tabuchi K, Südhof TC. Presynaptic neurexin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. *Cell*. 2013; 154:75–88. [PubMed: 23827676]
11. Traunmuller L, Gomez AM, Nguyen T-M, Scheiffele P. Control of neuronal synapse specification by highly dedicated alternative splicing program. *Science*. 2016; 352:982–986. [PubMed: 27174676]
12. Nguyen T-M, Schreiner D, Xiao L, Traunmuller L, Bornmann C, Scheiffele P. An alternative splicing switch shapes neurexin repertoires in principal neurons versus interneurons in the mouse hippocampus. *Elife*. 2016; 5
13. Mockenhaupt S, Makeyev EV. Non-coding functions of alternative pre-mRNA splicing in development. *Semin Cell Dev Biol*. 2015; 48:32–39.
14. Lykke-Andersen S, Jensen TH. Nonsense-mediated mRNA decay: an intricate machinery that shapes transcriptomes. *Nat Rev Mol Cell Biol*. 2015; 16:665–677. [PubMed: 26397022]
15. Mendell JT, Sharifi Na, Meyers JL, Martinez-Murillo F, Dietz HC. Nonsense surveillance regulates expression of diverse classes of mammalian transcripts and mutes genomic noise. *Nat Genet*. 2004; 36:1073–1078. [PubMed: 15448691]
16. Rehwinkel J, Letunic I, Raes J, Bork P, Izaurralde E. Nonsense-mediated mRNA decay factors act in concert to regulate common mRNA targets. *RNA*. 2005; 11:1530–1544. [PubMed: 16199763]
17. Medghalchi SM, Frischmeyer PA, Mendell JT, Kelly AG, Lawler AM, Dietz HC. Rent1, a trans-effector of nonsense-mediated mRNA decay, is essential for mammalian embryonic viability. *Hum Mol Genet*. 2001; 10:99–105. [PubMed: 11152657]
18. Weischenfeldt J, Damgaard I, Bryder D, Theilgaard-Mönch K, Thoren LA, Nielsen FC, Jacobsen SEW, Nerlov C, Porse BT. NMD is essential for hematopoietic stem and progenitor cells and for eliminating by-products of programmed DNA rearrangements. *Genes Dev*. 2008; 22:1381–1396. [PubMed: 18483223]

19. Long AA, Mahapatra CT, Woodruff EA, Rohrbough J, Leung H-T, Shino S, An L, Doerge RW, Metzstein MM, Pak WL, et al. The nonsense-mediated decay pathway maintains synapse architecture and synaptic vesicle cycle efficacy. *J Cell Sci.* 2010; 123:3303–15. [PubMed: 20826458]
20. Zheng S, Gray EE, Chawla G, Porse BT, O'Dell TJ, Black DL. PSD-95 is post-transcriptionally repressed during early neural development by PTBP1 and PTBP2. *Nat Neurosci.* 2012; 15:381–388. [PubMed: 22246437] [** This study demonstrate that neuron-specific expression of PSD-95 protein is driven by a post-transcriptional mechanism. Alternative splicing coupled to NMD regulates the abundance of *Psd95* transcripts.]
21. Makeyev EV, Zhang J, Carrasco MA, Maniatis T. The MicroRNA miR-124 Promotes Neuronal Differentiation by Triggering Brain-Specific Alternative Pre-mRNA Splicing. *Mol Cell.* 2007; 27:435–448. [PubMed: 17679093]
22. Braunschweig U, Barbosa-Morais NL, Pan Q, Nachman EN, Alipanahi B, Gonatopoulos-Pourmatzis T, Frey B, Irimia M, Blencowe BJ. Widespread intron retention in mammals functionally tunes transcriptomes. *Genome Res.* 2014; 24:1774–1786. [PubMed: 25258385] [* While intron retention was previously thought to be mainly frequent in plants, this work reveals that this class of alternative splicing is also widespread in mammals.]
23. Traunmuller L, Bornmann C, Scheiffele P. Alternative Splicing Coupled Nonsense-Mediated Decay Generates Neuronal Cell Type-Specific Expression of SLM Proteins. *J Neurosci.* 2014; 34:16755–16761. [PubMed: 25505328] [* This study sheds light on an alternative splicing program coupled to NMD allowing a neuronal-type specific expression of the RNA-binding proteins SLM1/2 in the mouse hippocampus.]
24. Eom T, Zhang C, Wang H, Lay K, Fak J, Noebels JL, Darnell RB. NOVA-dependent regulation of cryptic NMD exons controls synaptic protein levels after seizure. *Elife.* 2013; 2 [* The authors exemplify how alternative splicing coupled to NMD can modify levels of neuronal transcripts in response to seizure.]
25. Giorgi C, Yeo GW, Stone ME, Katz DB, Burge C, Turrigiano G, Moore MJ. The EJC Factor eIF4AIII Modulates Synaptic Strength and Neuronal Protein Expression. *Cell.* 2007; 130:179–191. [PubMed: 17632064] [** This work reveals that dendritic *Arc* mRNAs are natural substrates for NMD and supports a role of NMD in the spatio-temporal control of ARC protein expression.]
26. Chen Z, Gore BB, Long H, Ma L, Tessier-Lavigne M. Alternative Splicing of the Robo3 Axon Guidance Receptor Governs the Midline Switch from Attraction to Repulsion. *Neuron.* 2008; 58:325–332. [PubMed: 18466743]
27. Colak D, Ji S-J, Porse BT, Jaffrey SR. Regulation of axon guidance by compartmentalized nonsense-mediated mRNA decay. *Cell.* 2013; 153:1252–65. [PubMed: 23746841] [** This study highlights that alternative splicing coupled to NMD can control the spatio-temporal expression of ROBO3 proteins at the axonal growth cone.]
28. Bruno IG, Karam R, Huang L, Bhardwaj A, Lou CH, Shum EY, Song HW, Corbett MA, Gifford WD, Gecz J, et al. Identification of a MicroRNA that Activates Gene Expression by Repressing Nonsense-Mediated RNA Decay. *Mol Cell.* 2011; 42:500–510. [PubMed: 21596314]
29. Lou CH, Shao A, Shum EY, Espinoza JL, Huang L, Karam R, Wilkinson MF. Posttranscriptional Control of the Stem Cell and Neurogenic Programs by the Nonsense-Mediated RNA Decay Pathway. *Cell Rep.* 2014; 6:748–764. [PubMed: 24529710]
30. Shum EY, Jones SH, Shao A, Dumdie J, Krause MD, Chan WK, Lou CH, Espinoza JL, Song HW, Phan MH, et al. The Antagonistic Gene Paralogs Upf3a and Upf3b Govern Nonsense-Mediated RNA Decay. *Cell.* 2016; 165:382–395. [PubMed: 27040500]
31. Nickless A, Jackson E, Marasa J, Nugent P, Mercer RW, Piwnicka-Worms D, You Z. Intracellular calcium regulates nonsense-mediated mRNA decay. *Nat Med.* 2014; 20:961–966. [PubMed: 25064126]
32. Kilchert C, Wittmann S, Vasiljeva L. The regulation and functions of the nuclear RNA exosome complex. *Nat Rev Mol Cell Biol.* 2016; 17:227–239. [PubMed: 26726035]
33. Yap K, Lim ZQ, Khandelia P, Friedman B, Makeyev EV. Coordinated regulation of neuronal mRNA steady-state levels through developmentally controlled intron retention. *Genes Dev.* 2012; 26:1209–23. [PubMed: 22661231] [** The authors uncover an post-transcriptional program

- coupling alternative splicing and nuclear exosome-dependent degradation. This program prevents expression of transcripts encoding synaptic proteins in non-neuronal cells.]
34. Chen CY, Gherzi R, Ong SE, Chan EL, Raijmakers R, Pruijn GJM, Stoecklin G, Moroni C, Mann M, Karin M. AU binding proteins recruit the exosome to degrade ARE-containing mRNAs. *Cell*. 2001; 107:451–464. [PubMed: 11719186]
 35. Tran H, Schilling M, Wirbelauer C, Hess D, Nagamine Y. Facilitation of mRNA Deadenylation and Decay by the Exosome-Bound, DExH Protein RHAU. *Mol Cell*. 2004; 13:101–111. [PubMed: 14731398]
 36. Hackmann A, Wu H, Schneider U-M, Meyer K, Jung K, Krebber H. Quality control of spliced mRNAs requires the shuttling SR proteins Gbp2 and Hrb1. *Nat Commun*. 2014; 5:1–14.
 37. Boutz PL, Bhutkar A, Sharp PA. Detained introns are a novel, widespread class of post-transcriptionally spliced introns. *Genes Dev*. 2014; 29:63–80. [* This is the first wide scale study in mammalian cells revealing that removal of retained introns can be enhanced under cellular stress conditions.]
 38. Ninomiya K, Kataoka N, Hagiwara M. Stress-responsive maturation of Clk1/4 pre-mRNAs promotes phosphorylation of SR splicing factor. *J Cell Biol*. 2011; 195:27–40. [PubMed: 21949414] [** This study nicely demonstrates that Clk1/4 intron retaining-transcripts rapidly complete their splicing under cellular stress in mouse cell lines.]
 39. Mauger O, Lemoine F, Scheiffele P. Targeted Intron Retention and Excision for Rapid Gene Regulation in Response to Neuronal Activity. *Neuron*. 2016; 92:1266–1278. [PubMed: 28009274] [** This study reveals that neurons can rapidly regulate their gene expression in response to neuronal activity by inducing splicing of pre-existing transcripts stably retaining select introns at rest.]
 40. Bond U. Heat shock but not other stress inducers leads to the disruption of a sub-set of snRNPs and inhibition of in vitro splicing in HeLa cells. *EMBO J*. 1988; 7:3509–3518. [PubMed: 2974799]
 41. Shalgi R, Hurt JA, Lindquist S, Burge CB. Widespread Inhibition of Posttranscriptional Splicing Shapes the Cellular Transcriptome following Heat Shock. *Cell Rep*. 2014; 7:1362–70. [PubMed: 24857664]
 42. Yost HJ, Lindquist S. RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. *Cell*. 1986; 45:185–193. [PubMed: 2421918]
 43. Bergkessel M, Whitworth GB, Guthrie C. Diverse environmental stresses elicit distinct responses at the level of pre-mRNA processing in yeast. *RNA*. 2011; 17:1461–78. [PubMed: 21697354]
 44. Pleiss JA, Whitworth GB, Bergkessel M, Guthrie C. Rapid, Transcript-Specific Changes in Splicing in Response to Environmental Stress. *Mol Cell*. 2007; 27:928–937. [PubMed: 17889666]
 45. Boothby TC, Zipper RS, Van der Weele CM, Wolniak SM. Removal of Retained Introns Regulates Translation in the Rapidly Developing Gametophyte of *Marsilea vestita*. *Dev Cell*. 2013; 24:517–529. [PubMed: 23434411]
 46. Martin RM, Rino J, Carvalho C, Kirchhausen T, Carmo-Fonseca M. Live-cell visualization of pre-mRNA splicing with single-molecule sensitivity. *Cell Rep*. 2013; 4:1144–55. [PubMed: 24035393]
 47. Singh J, Padgett RA. Rates of in situ transcription and splicing in large human genes. *Nat Struct Mol Biol*. 2009; 16:1128–33. [PubMed: 19820712]
 48. Gabel HW, Kinde B, Stroud H, Gilbert CS, Harmin DA, Kastan NR, Hemberg M, Ebert DH, Greenberg ME. Disruption of DNA-methylation-dependent long gene repression in Rett syndrome. *Nature*. 2015; 522:89–93. [PubMed: 25762136]
 49. Sibley CR, Emmett W, Blazquez L, Faro A, Haberman N, Briesse M, Trabzuni D, Ryten M, Weale ME, Hardy J, et al. Recursive splicing in long vertebrate genes. *Nature*. 2015; 521:371–375. [PubMed: 25970246]
 50. Bahar Halpern K, Caspi I, Lemze D, Levy M, Landen S, Elinav E, Ulitsky I, Itzkovitz S. Nuclear Retention of mRNA in Mammalian Tissues. *Cell Rep*. 2015; 13:2653–2662. [PubMed: 26711333]
 51. Battich N, Stoeger T, Pelkmans L. Control of Transcript Variability in Single Mammalian Cells. *Cell*. 2015; 163:1596–1610. [PubMed: 26687353]

Highlights

- Function of alternative splicing in neurons is not restricted to diversification of protein families.
- Alternative splicing targets RNA populations to additional RNA metabolism pathways.
- Alternative splicing regulates spatio-temporal expression of neuronal proteins.
- Alternative splicing directs cell-type specific transcriptomes.
- Alternative splicing controls rapid modification of neuronal transcriptomes.

CELL-TYPE SPECIFICATION

Figure 1. Alternative splicing directs cell-type specific transcriptomes.

Left: Alternative splicing coupled to non-sense mediated decay (NMD) regulates stability of transcripts encoding synaptic proteins. In non-neuronal cells (top), skipping of a cassette exon (grey box) in transcripts coding for the post-synaptic density protein 95 (*Psd95*) and the GABA_B receptor subunit 1 (*Gabbr1*) leads to presence of a premature stop codon upstream of exon-junction complex (EJC) deposited on the mRNAs. The transcripts are then targeted for NMD-degradation preventing expression of the synaptic proteins in non-neuronal cells. In mature neurons (bottom), inclusion of the cassette exon in the mRNA

allows the stabilization of the transcript and thereby an efficient translation of PSD95 and GABBR1 proteins [20,21].

Right: Alternative splicing coupled to nuclear exosome-mediated degradation regulates stability of transcripts encoding synaptic proteins. In non-neuronal cells (top), intron retention events (grey line) in transcripts encoding synaptic proteins (*Vamp2*, *Stx1b*, *Sv2a*) trigger their nuclear sequestration and degradation by the nuclear exosome. In mature neurons (bottom), the final mRNAs are devoid of the retained introns allowing their cytoplasmic export and translation [33].

ACTIVITY-DEPENDENT DYNAMIC OF GENE EXPRESSION

Figure 2. Alternative splicing controls rapid modification of neuronal transcriptomes.

Left: In unstimulated neurons (bottom), inclusion of a cryptic exon (grey box) in transcript coding for the sodium channel *SCN9A* elicits degradation of the transcripts through the non-sense mediated decay (NMD). In pilocarpine-stimulated neurons (top), the cryptic exon is excluded from the *Scn9a* mRNAs allowing their efficient translation [24].

Right: In unstimulated neurons (bottom), a pool of transcripts is stored in the nucleus by stably retaining an intron (grey line) in fully transcribed RNAs. These almost mature RNAs are not targeted for degradation. In activated neurons (top), the pre-existing transcripts

undergo splicing completion. The mRNAs thereby generated are exported to the cytoplasm and associated to the translation machinery [39].

CONTROL OF SPATIO-TEMPORAL EXPRESSION

Figure 3. Alternative splicing regulates spatio-temporal expression of neuronal proteins.

Left: In mature neurons, components of the exon junction complex (EJC) and non-sense mediated decay (NMD) are found in the dendrites. *Arc* transcripts are localized in the dendrites. Presence of 2 constitutively spliced introns in the 3' untranslated region (3'UTR) and hence a stop codon upstream of the EJC makes *Arc* mRNAs natural NMD targets. It is thought that these dendritic mRNAs are translated only in response to a synaptic stimulus. This would produce a spike in local protein production, followed by the NMD-mediated degradation of the mRNA [25].

Right: In commissural neurons, *Robo3.2* mRNAs are localized in axonal growth cone. *Robo3.2* transcripts retained an intron (grey line) containing a premature stop codon. Thus,

Robo3.2 is a NMD target and this allows a fine tuned expression of the axon guidance receptor ROBO3 [26,27].