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Regulation of neuronal differentiation, function, and plasticity by alternative splicing

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Abstract

Post-transcriptional mechanisms provide powerful means to expand the coding power of genomes. In nervous systems, alternative splicing has emerged as a fundamental mechanism not only for the diversification of protein isoforms but also for the spatio-temporal control of transcripts. Thus, alternative splicing programs play instructive roles in the development of neuronal cell type-specific properties, growth, self-recognition, synapse specification, and neuronal network function. Here we discuss the most recent genome-wide efforts on mapping RNA codes and RNA binding proteins for neuronal alternative splicing regulation. We illustrate how alternative splicing shapes key steps of neuronal development, maturation, and synaptic properties. Finally, we highlight efforts on dissecting spatio-temporal dynamics of alternative splicing and their potential contribution to neuronal plasticity and the mature nervous system.

Keywords

RNA-binding proteins; splice code; recognition; neuronal specification; synaptic plasticity; cell identity

Introduction

Nervous systems have an astounding complexity and exhibit a remarkable level of functional and structural organization. This organization arises during embryonic and early postnatal development. Neuronal precursors give rise to specific neuronal cell types, characterized by their neurotransmitter phenotypes, routes of migration, morphology, as well as physiological properties. The molecular repertoires driving these differentiation steps are spatially and temporally regulated by transcription factors, with terminal selector genes ultimately driving the gene battery unique to each cell type (Hobert, 2016; Kepecs and Fishell, 2014; Lodato et al., 2015; Mi et al., 2018; Sur and Rubenstein, 2005). However, many aspects of the specification of neuronal and synaptic properties remain to be understood and recent work points to an important role for post-transcriptional mechanisms in this process.

Alternative splicing of pre-mRNAs significantly expands the coding power of genomes (Nilsen and Graveley, 2010), generating in some cases thousands of distinct transcript isoforms from single genes (Schreiner et al., 2014; Sun et al., 2013). Moreover, alternative splicing events further contribute to the spatio-temporal regulation of mRNAs by modifying the stability and localization of transcripts (Mauger and Scheiffele, 2017; Mockenhaupt and

Makeyev, 2015). Interestingly, while the number of protein coding genes is similar across vertebrate and invertebrate species, the magnitude and heterogeneity of alternative splicing appears to have greatly expanded in organisms with more complex nervous systems such as mammals. In the genome of *S. cerevisiae*, only a small subset of genes includes introns and alternative splicing is very rare (Howe et al., 2003). Instead, amongst invertebrates and mammals many alternative splicing events are evolutionarily conserved and, thus, are thought to contribute to the functional specialization of cell types and tissues (Barbosa-Morais et al., 2012; Merkin et al., 2012; Nilsen and Graveley, 2010). According to transcriptomic studies, close to 95% of human pre-mRNAs are subject to alternative splicing (Pan et al., 2008; Wang et al., 2008). There is an ongoing debate about what fraction of splice isoforms predicted based on transcript data is actually converted into proteins (Tress et al., 2017). However, both quantitative proteomics (Kim et al., 2014; Schreiner et al., 2015) as well as investigation of ribosome-associated transcripts (Weatheritt et al., 2016) confirm that there is indeed extensive production of alternative protein isoforms in mammalian organisms. The importance of alternative splicing as a regulatory mechanism is further highlighted by numerous examples where splicing alterations are associated with disease states. Dysregulation of alternative splicing has been recently related to Autism Spectrum Disorders (ASDs) (Irimia et al., 2014; Parikshak et al., 2016; Quesnel-Vallieres et al., 2016; Xiong et al., 2015). Moreover, the first splicing therapeutics are beginning to be developed. Thus, in rodent models of Spinal Muscular Atrophy (SMA), a neurodegenerative disorder, antisense oligonucleotides (Hua et al., 2011) and small molecules (Naryshkin et al., 2014) that modify splicing of the endogenous SMN2 (survival motor neuron 2) gene have shown to recover SMN2 splicing activity and provide major benefits to patients (Finkel et al., 2016).

In this review, we will focus on the physiological function of alternative splicing programs in neuronal development and plasticity. We will discuss advances from recent genome-wide efforts in mapping RNA codes and binding sites on target transcripts and in characterizing RNA-binding proteins that control alternative splicing. We will then use specific examples to illustrate general concepts of how alternative splicing contributes to the regulation of neuronal growth, maturation, and specification and the fundamental role of RNA-binding proteins in the modulation of these context- and cell type-specific splicing choices. Finally, we will discuss recent efforts in dissecting spatio-temporal dynamics of alternative splicing and their contribution to neuronal plasticity.

RNA Motifs, RNA-Binding Proteins, and Dissection of the “Splice Code”

The splicing reaction is catalyzed by the spliceosome, a dynamic macromolecular RNA-protein complex that recognizes sequence elements on target pre-mRNAs. These core splicing sequences include the 5' and 3' splice sites, branch points, and polypyrimidine tracts. Alternative splicing decisions and their regulation are largely achieved through additional *cis*-acting elements, called exonic or intronic splicing enhancers or silencers. These enhancer and silencer sequences contribute to the definition of the splice sites by recruitment of *trans*-acting splicing regulators which then increase or restrict access of the core splicing machinery. Cracking the “splice code”, i.e., being able to predict what transcript isoforms are generated within a specific cell based on the cellular repertoire of RNA-binding proteins, transcripts and their binding motifs, represents a formidable

challenge (Wang and Burge, 2008). Recent studies provided major progress towards a systematic dissection of the splicing code. There are estimates that the human genome encodes about 1'500 RNA-binding proteins (Gerstberger et al., 2014) and additional proteins continue to be discovered with experimental approaches (Castello et al., 2016) (Fig1A). While these proteins contribute to many processes besides splicing, there is a significant number of proteins that bind pre-mRNA and might contribute to alternative splicing regulation. Canonical RNA-binding proteins bind short single-stranded RNA sequences. Recent efforts began to systematically define the RNA-motifs bound by these proteins in vitro (Ray et al., 2009; Ray et al., 2013) and in eukaryotic cells (Darnell, 2013; Van Nostrand et al., 2016) resulting in high quality genome-wide maps of protein-RNA interactions (Fig.1A,B).

The transcriptome-wide mapping of binding sites for several RNA-binding proteins has been a key advance towards the understanding of the splicing code. Interestingly, the impact of RNA motifs on the splicing reaction is strongly position-dependent. Thus, the same cis-acting element can act as an enhancer or silencer depending on the position relative to the regulated exon (Fig.1C). For example, when the YCAY binding motif for the neuronal RNA-binding protein Nova is located downstream of an alternative exon, Nova frequently causes the inclusion of that particular exon, whereas it tends to promote exclusion when its binding site is positioned within 200 bps upstream of the exon (Ule et al., 2006) (Fig.1C). Many other factors have been demonstrated to act in a similar manner (Licatalosi et al., 2012; Llorian et al., 2010; Xue et al., 2009), suggesting that this mechanism may be widely used to diversify the splicing regulation driven a single splicing factor.

From Tissue to Cell Type-Specific Splicing Dissection

Most of the studies thus far geared towards dissection of the alternative splicing code have been pioneered in reductionist systems, using purified proteins and cell lines. What makes studies on post-transcriptional regulation in the nervous system particularly fascinating but also challenging is the extensive heterogeneity of the tissue. Indeed, each brain area consists of tens to possibly hundreds of molecularly and functionally distinct cell types. Early studies clearly demonstrated that there is extensive alternative splicing regulation across brain regions and across developmental stages (Dillman et al., 2013). However, it is impossible to deduce from such data whether shifts in splice isoform abundance reflect changes in alternative splicing regulation, transcript alterations in sub-populations of cells, or altered density of particular cell types in the tissue.

More recent studies pioneered approaches for mapping splicing patterns in purified preparations of eukaryotic and neuronal cells. Analysis of splicing events in immunopanning- and FACS-purified murine cell populations revealed thousands of population-specific splicing events that differ between neuronal and glial cells (Zhang et al., 2014). In the *Drosophila* visual system, purification of L1 and L2 neurons led to the discovery of cell type-specific expression of Dscam2 splice isoforms that underlie repulsive interactions and, thereby, facilitate appropriate wiring (Lah et al., 2014). Isolation of transcripts specifically from *cornis ammonis* 1 (CA1) pyramidal cells and parvalbumin (PV)-positive interneurons in the mouse hippocampus demonstrated highly differential

alternative splicing of pre-mRNAs encoding the neurexin family of synaptic adhesion molecules, and the conditional ablation of PV-cell specific exons resulted in altered hippocampal network activity (Nguyen et al., 2016). These examples illustrate that highly selective, cell type-specific alternative splicing events indeed have significant impact on neuronal circuit function.

Recent technological advances have put the in-depth genome-wide analysis of cell type-specific splicing regulation within reach. The conditional epitope-tagging initially pioneered for ribosomal proteins in the RiboTag-approach (Sanz et al., 2009) has recently been elegantly extended to generate cell population-specific CLIP(crosslinking and immunoprecipitation)-tags (Hwang et al., 2017). Another innovative method first developed in *Drosophila* for cell type-specific analysis is TRIBE (Targets of RNA-binding proteins identified by editing): Here a RNA-binding protein is fused with the catalytic domain of the RNA-editing enzyme ADAR and mRNAs associating with this fusion protein are identified based on *de novo* editing events (McMahon et al., 2016). Remarkably, when ADAR-fusions of RNA-binding proteins like Hrp-48 and dFMR1 were expressed *in vivo* in different genetically-defined neurons of the *Drosophila* nervous system, this analysis uncovered divergent interactions of these two RBPs with target mRNAs, even across transcripts that are commonly expressed in the cell populations being compared (McMahon et al., 2016). While the molecular basis of such differential RBP-mRNA interactions remains to be understood, this work provides a first glimpse that RNA-binding proteins may exhibit different specificities depending on the cellular context. Such context-dependent functions likely result from the interplay between the significant number of RBPs co-expressed in a single cell. Splicing factors frequently act synergistically on a single splice site, like in the case of Nova and Rbfox (Li et al., 2015b; Zhang et al., 2010), or antagonistically like nSR100 and Ptpb1 (Raj et al., 2014). Moreover, an additional level of regulation is exerted by accessory proteins that may enhance or reduce the function of splicing factors, as proposed for the protein Raver1 enhancing the activity of the splicing factor Ptb1 (Rideau et al., 2006).

Notably, not only the expression of RNA-binding proteins can direct splicing decisions, but in many cases RNA-binding proteins are subject to post-translational modifications e.g., phosphorylation in response to cell signaling (Feng et al., 2008; Iijima et al., 2011; Matter et al., 2002). Moreover, an additional layer of regulation is represented by the rate of RNA polymerase II progression along a gene, which has significant impact on alternative splicing regulation (Ip et al., 2011; Roberts et al., 1998): Most transcripts are thought to be spliced co-transcriptionally. Thus, the speed of transcription determines which splice donor and acceptor sites compete for the recruitment of the splicing machinery and, ultimately, influences the outcome of alternative splicing decisions. Thus, it has been proposed that chromatin structure and rearrangements that modify the kinetics of RNA polymerase progression may provide another level of alternative splicing regulation (de Almeida and Carmo-Fonseca, 2014; de la Mata and Kornblihtt, 2006; Fong et al., 2014).

Lastly, single cell studies suggest that some alternative exons may exhibit bimodal splicing patterns, such that essentially all transcripts in a single cell either include or skip the alternative insertion (Shalek et al., 2013). However, an important functional single cell splicing study demonstrated that two splice isoforms of the important signaling molecule

Cdc42 co-exist in single cells where they coordinate axonal and dendritic growth (Yap et al., 2016). The mechanisms that underlie such bimodal splicing outcomes remain unknown. In *C.elegans*, two-color reporter assay demonstrated the differential splicing regulation at single-neuron resolution, suggesting a deterministic regulation of alternative splicing at the level of single cell (Norris et al., 2014). However, in mammals, the ability to reliably quantify alternative splicing events from single cell transcriptomes remains a major challenge. Nevertheless, the continuous advances with transcript databases and analysis algorithms may change this in the coming years (Song et al., 2017; Tapial et al., 2017). This multi-layered control highlights the perplexing complexity of alternative splicing regulation and illustrates the challenge of predicting and dissecting splicing programs in the nervous system.

Neuronal Cell Type-Specific Splicing Regulators

Understanding the logic of how alternative splice isoforms are arrayed over neuronal cell types is a fundamental step towards the dissection of the properties of neuronal organization and/or function they might encode. Over the past decade, a combination of electrophysiological, anatomical, genetic, and transcriptomic studies has yielded an unprecedented insight into the molecular and functional classification of neuronal cell types (Shekhar et al., 2016; Tasic et al., 2016; Zeisel et al., 2015). Previous work mapped differential alternative splicing events in neurons versus glial cells as well as RNA-binding proteins with neuronal lineage-specific expression (Yan et al., 2015). Within the neuronal lineage, cells can be further segregated based on their neurotransmitter phenotype (e.g., glutamate and GABA). However, beyond these rather general sub-categories it is now well accepted that there are specific neuronal cell types with particular anatomical, molecular, and functional features. These neuronal cell types are specified early in development and single cell sequencing studies have provided molecular markers that unambiguously identify them (Mayer et al., 2018; Mi et al., 2018; Paul et al., 2017).

There are several RNA-binding proteins that are “neuron-specific” (e.g., Nova1/2 and nPTB), meaning that they are not expressed outside the nervous system or in the various glial lineages. However, there are currently only very few splicing factors known to exhibit highly differential expression between cardinal classes of neuronal cells or even neuronal cell types. One pair of such neuronal cell type-selective RBPs are the KH-domain proteins Slm1 and Slm2. These “Sam68-like proteins” are paralogues of the ubiquitously expressed RNA-binding protein Sam68 (Di Fruscio et al., 1999; Venables et al., 1999). In the mouse hippocampus, Slm1 and Slm2 exhibit mutually-exclusive expression in neuronal cell types due to a post-transcriptional cross-repression mechanism (Iijima et al., 2014; Stoss et al., 2004; Traunmüller et al., 2014). Thus, the Slm2 protein is highly expressed in *cornis ammonis* (CA) pyramidal cells (which are glutamatergic) and a subset of somatostatin-positive (SST) interneurons, but not in glutamatergic granule cells of the dentate gyrus or the majority of vasointestinal peptide-positive GABAergic interneurons. By contrast, Slm1 protein levels are very low in CA pyramidal and SST neurons but high in dentate granule cells and VIP interneurons (Iijima et al., 2014; Nguyen et al., 2016; Traunmüller et al., 2014). This example of neuronal cell type-selective expression also illustrates that these splicing factors do not simply segregate into glutamatergic and GABAergic lineages, but that

they provide a level of splicing regulation that is orthogonal to the developmental ontogeny of these general categories of neurons.

Alternative Splicing-Dependent Neuronal Recognition and Synapse Specification

To what extent do cell type-specific alternative splicing programs instruct specific wiring steps and neuronal circuit properties? Surface receptors and adhesion molecules have received particular attention in such functional explorations, considering that they might contribute to a neuronal chemoaffinity code. Recent studies provided evidence for key regulatory roles of alternative splicing in surface recognition events contributing to axonal guidance, neuronal self-avoidance, synapse formation as well as functional synapse specification. In the following, we discuss specific examples to illustrate the roles for alternative splice isoforms in these key steps of neuronal terminal differentiation.

Arguably, the most striking example for the control of neuronal connectivity by molecular diversification through alternative splicing is the *Drosophila* surface receptor Dscam1 (Down syndrome cell adhesion molecule 1). The single *Drosophila* Dscam1 gene encodes a transmembrane receptor with a large extracellular domain containing nine immunoglobulin (Ig) domains. Sequences in three of these (Ig2, Ig3 and Ig7) are modified by alternative splicing (Schmucker et al., 2000). Mutually exclusive selection of single alternative exons at each of these alternatively spliced segments generates receptor isoforms with 38,016 unique extracellular domains (Sun et al., 2013). Single cell analyses demonstrated that individual neurons stochastically select a subset of isoforms for expression, thereby, providing each individual cell with a unique complement of Dscam receptors (Miura et al., 2013). The stochasticity in the generation of surface receptor repertoires provide a molecular mechanism for neuronal self-recognition at the single cell level. Interestingly, Dscam proteins engage in splice isoform-specific homophilic interactions (Wojtowicz et al., 2007). During the growth of axonal and dendritic arbors, Dscam1 isoforms with the same Ig domain variants mediate repulsion of the neurites that contain identical isoforms (Fig.2A). This mechanism directs appropriate spreading of axons and dendrites in several neuronal cell types, highlighting a fundamental role for alternative splicing in neuronal self-recognition in the *Drosophila* nervous system (Chen et al., 2006; Hattori et al., 2007; Hughes et al., 2007; Soba et al., 2007; Zhan et al., 2004).

Another striking example in flies show how alternative choice of exons can modulate the high diversity of axon guidance decisions in the developing nervous system. This is the case of the transcription factor Lola, whose role is to regulate the expression levels of proteins like the middle repellent protein Slit or its receptor Robo (Crowner et al., 2002). Alternative splicing of Lola transcript generates 19 different isoforms, each with peculiar DNA-binding capacities and, possibly, distinct target genes. The complex combinatorial splicing of Lola transcript in different subset of tissues, in turn, modulate the diverse axon guidance decisions (Goeke et al., 2003).

In the developing mouse spinal cord, the axon guidance receptor DCC (Deleted in Colorectal Carcinoma) is responsible for the correct axon outgrowth and for sensing netrin-

secreting midline (Dickson and Zou, 2010; Evans and Bashaw, 2010). The alternative splicing of DCC gene by *Nova1/2* generates two protein isoforms (DCC_{short} and DCC_{long}) that differ for their capability to change conformation upon netrin binding (Leggere et al., 2016). In *Nova1/2* double-knock-out embryos, spinal commissural neurons exhibit severe defects in migration, axon growth, and guidance (Fig.2B). Notably, these anatomical defects are reminiscent of *Dcc* loss-of-function phenotypes and re-expression of the missing DCC splice isoform is sufficient to rescue some aspects of the *Nova1/2* phenotypes (Leggere et al., 2016). While the splice isoform-specific interaction partners of the Nova-dependent DCC isoform remain to be identified, this work nicely illustrates a role for alternative splicing regulators in neuronal migration and axon guidance.

Alternative splicing of ion channels has emerged as another key element for refinement of neuronal function and synaptic plasticity (Eom et al., 2013; Gehman et al., 2011). For example, voltage-gated Ca²⁺ channels (VGCCs) in the presynaptic membrane are key determinants of cell- and synapse-specific neurotransmitter release properties and the genes encoding VGCC subunits have the potential to generate thousands of splice variants (Lipscombe et al., 2013; Soong et al., 2002). The expression of different pore-forming α_1 subunits (derived from different genes) sets cell type-specific presynaptic properties. However, recent work demonstrated that neuronal network activity can acutely shift the incorporation of two mutually exclusive alternative exons (37a/37b) in the Ca_v2.1 (so-called P/Q-type) α_1 subunit (Thalhammer et al., 2017). The respective Ca_v2.1 alternative splice variants differ in an EF-hand-like domain that regulates the kinetics of Ca²⁺-influx (Chaudhuri et al., 2004). Notably, in rat hippocampal neurons the splice variants have fundamentally different impact on presynaptic plasticity: while one isoform promotes synaptic depression the other drives synaptic facilitation (Thalhammer et al., 2017). This is one example for how neuronal activity shifts alternative splicing patterns and, thereby, adjusts functional synaptic properties and circuit function. Interestingly, besides exons 37a and b, VGCC α_1 subunits undergo alternative splicing at several other sites and some of them are regulated by the neuronal RNA-binding proteins Rbfox2 and Nova1 (Allen et al., 2010; Gehman et al., 2012). Whether channel properties are mostly controlled dynamically in response to neuronal activity (as for exon 37a/b) or whether VGCC alternative splicing contributes to the specification of neuronal cell type-specific synaptic properties remains to be explored.

Finally, synapse formation and specification through cell adhesion molecules represent further steps of neuronal development where a contribution of alternative splicing has been explored in some detail. The Leukocyte common antigen-related receptor protein tyrosine phosphatases (“LAR-RPTPs”, consisting of LAR, PTP δ , and PTP σ) are a family of adhesion molecules with synaptogenic (“synapse-organizing”) activities (Takahashi and Craig, 2013). Presynaptic LAR-RPTPs interact with several postsynaptic ligands that are characterized by extracellular leucine-rich repeat domains (NGL-3, SALM3 and Sltrk1-6) as well as the Ig-domain protein IL1RAPL1. LAR-RPTPs themselves contain extracellular Ig-domains which represent the ligand interaction sites. Interestingly, these extracellular protein interactions are modified by alternative incorporation of “micro-exons” that encode 4-8 amino acid peptides. Thus, the inclusion of micro-exon B in the LAR-RPTP is required for binding to SALM3 and ILRAPL1 (two structurally different post-synaptic partners),

whereas LAR-RPTP isoforms lacking this micro-exon interact with another post-synaptic ligand (TrkC) (Li et al., 2015a; Yoshida et al., 2011) (Fig.2C). The RNA-regulators steering these important alternative splicing decisions in LAR-RPTPs or the neuronal cell types that express specific splice isoforms remain to be discovered

Neurexins represent a second class of synaptic adhesion molecules that are tightly regulated at the level of alternative splicing and that play critical roles in neuronal recognition and synapse specification. Neurexins are encoded by three genes (*Nrxn1/2/3*). Alternative promoter usage, combined with alternative splicing at up to six alternatively spliced segments (AS1-6), generates thousands of distinct neurexin isoforms which have been mapped in mice using single cDNA (PacBio) sequencing (Schreiner et al., 2014; Treutlein et al., 2014) and confirmed by targeted proteomics (Schreiner et al., 2015). Several of the alternative splicing events in neurexins control multiple biochemical interactions with a structurally diverse set of synaptic ligands (Boucard et al., 2005; Chih et al., 2006; Matsuda et al., 2016; Schreiner et al., 2015; Sugita et al., 2001; Uemura et al., 2010) and strongly impact assembly, function, and plasticity of neuronal synapses (Aoto et al., 2013; Chih et al., 2006; Graf et al., 2004; Nguyen et al., 2016; Traunmüller et al., 2016; Uemura et al., 2010). Profiling of *Nrxn* splice isoforms from genetically-defined cell populations in the mouse hippocampus revealed highly differential use of specific splice insertions in GABAergic PV-positive interneurons as compared to glutamatergic pyramidal cells in CA1. Thus, in CA1 cells the alternative exons at AS4 are almost completely skipped (AS4(-)), whereas they are highly included (AS4(+)) in PV-cells (Nguyen et al., 2016). Differential insertion rates at alternatively spliced segments are further supported by single cell PCR studies (Fuccillo et al., 2015). Conditional deletion of the *Nrxn1* and 3 AS4 alternative exons in PV-cells results in elevated neuronal network activity in the hippocampus, suggesting that these splice isoforms indeed contribute to normal network function (Nguyen et al., 2016). The alternative splicing switch at AS4 is regulated by the KH-domain-containing RNA binding protein Slm2 and its paralogues (Ehrmann et al., 2013; Iijima et al., 2014; Iijima et al., 2011). Slm2 is highly expressed in CA1 neurons but absent from the majority of PV interneurons (Nguyen et al., 2016). Remarkably, genome-wide mapping of alternative splicing in *Slm2* knock-out hippocampus revealed that Slm2 predominantly modifies alternative splicing of *Nrxns* at AS4 with only very modest changes in other mRNAs. Loss of Slm2 results in a selective deficit in post-synaptic AMPA-type glutamate receptor function and long-term potentiation at Schaffer collateral (CA3-CA1) synapses in the hippocampus, whereas many other aspects of synaptic function were unaltered (Traunmüller et al., 2016) (Fig.2D). These deficits could be rescued by genetic correction of *Nrxn1* AS4 alternative splicing in mice.

These studies clearly illustrate how the regulation of trans-synaptic adhesion complexes by alternative splicing can modulate synapse formation.

In aggregate, these examples illustrate that targeted alternative splicing programs not only control alternative splice isoforms in a tissue or cell lineage-specific manner (such as glial versus neuronal lineages), but much rather provide a powerful mechanism to drive neuronal cell type-specific splicing events. These events, in turn, define structural and functional specification of neuronal cell types, synaptic properties and, ultimately, the function of complex neuronal networks.

Control of Neuronal Transcript Dynamics by Neuronal Signaling and mRNA Processing

The cell type-specific alternative splicing programs illustrated above represent a genetically encoded blueprint for neuronal specification, migration and maturation. However, especially for more complex organisms, a significant part of nervous system organization arises from neuronal activity-dependent refinements of neuronal networks. Thus, sensory stimuli from the environment or spontaneous patterned activity control the stabilization and destabilization of synapses, the structural organization of neuronal axonal and dendritic arbors, and ultimately, the acquisition of precisely-tuned neuronal responses (Feldman and Brecht, 2005; Margolis et al., 2014; Stellwagen and Shatz, 2002; Sur and Rubenstein, 2005). In the mature nervous system, neuronal activity-dependent transcription is considered to be a major source of gene products that modify synapse formation and function (Hrvatin et al., 2018; West et al., 2002) and, thereby, contribute to learning processes and memory. A key step in such neuronal activity-dependent processes is neuronal depolarization, which triggers calcium influx and cell signaling. Interestingly, a number of alternative splicing events are modified in response to neuronal depolarization or growth factor signaling, raising the possibility that activity-dependent alternative splicing may contribute to neuronal plasticity during development and/or during learning processes.

Initial studies in cultured neuronal cells identified alternative splicing events which show regulation upon prolonged depolarization (An and Grabowski, 2007; Xie, 2008; Xie and Black, 2001). These include alternative exons in the synaptic NMDA-receptor (NR1 subunit, *Grin1*), neural cell adhesion molecule (NCAM), the synaptic scaffolding molecule Homer1, neurexins (*Nrxn1,2,3*), the apolipoprotein E receptor 2 (*Apoer2*), as well as several K⁺- and Ca²⁺- channels (An and Grabowski, 2007; Beffert et al., 2005; Iijima et al., 2011; Lee et al., 2007; Rozic-Kotliroff and Zisapel, 2007; Xie and Black, 2001). More recently, exons regulated by prolonged depolarization have been mapped with genome-wide methods. This led to the identification of more than a thousand of activity-regulated alternative exons in cultured hippocampal neurons (Quesnel-Vallieres et al., 2016).

The nuclear calmodulin-dependent kinase CaMKIV has emerged as an important signaling component in the control of depolarization-dependent alternative splicing. For several depolarization-dependent alternative exons, *cis*-acting elements in the pre-mRNAs are required for the activity-dependent splicing shift. These include UAGG motifs and a larger CaMKIV-responsive element (CaRRe) (An and Grabowski, 2007; Lee et al., 2007; Xie and Black, 2001). These *cis*-acting elements have been particularly well characterized in the so-called STREX exon in Slo potassium channels encoded by the *Kcnma1* gene. In response to depolarization and calcium influx through L-type voltage-gated calcium channels and/or NMDA- receptors, CaMKIV is activated. Consequently, the CaRRe element on the target mRNA recruits the splicing factor hnRNPL (and possibly additional factors) in a phosphorylation-dependent manner, thus providing a link from CaMKIV to the modification of the splicing step (Liu et al., 2012). Ultimately, the incorporation of the STREX exon sequences into KCNMA1 increases Ca²⁺ sensitivity of the channels and regulates burst firing of neurons.

Two further RNA-binding proteins implicated in activity-dependent alternative splicing regulation are nSR100 and Sam68. In cerebellar granule cells, Sam68 is required for a depolarization-dependent alternative splicing of neurexin mRNAs (Iijima et al., 2011). In vitro, this depolarization-dependent splicing event depends on L-type calcium channels and CaMKIV activity, which may act through phosphorylation of Sam68. The Sam68-dependent production of *Nrxn* AS4(-) splice variants shifts the responsiveness of granule cells to different sets of post-synaptic neurexin binding partners (Iijima et al., 2011), and this may contribute to a transient modification of synaptic structure and/or function. The neural-specific SR-related protein of 100 kDa (nSR100) has an important role in the alternative splicing control of microexons. Depolarization of cultured hippocampal neurons results in altered incorporation rates of a large fraction of microexons, indicating that these are particularly susceptible to this type of stimulation (Quesnel-Vallieres et al., 2016). Interestingly, prolonged depolarization also results in a decrease in nSR100 protein levels, and this auto-regulatory loop is thought to contribute to the modulation of microexon alternative splicing.

While there has been substantial progress on mapping activity-dependent splicing events and obtaining insights into the signaling mechanisms, the physiological relevance of these events is still enigmatic. Thus far, mapping of activity-dependent splicing has largely relied on prolonged chemical or pharmacological manipulation of synaptic and/or neuronal excitation. It is not understood which physiological activity patterns that drive adaptations in neuronal networks *in vivo* would in fact produce physiologically relevant changes in splice isoforms. It has been proposed that long-term changes in overall network activity that occur in disease states might result in altered activity-dependent gene expression as well as splicing. For example in autism - at least in some individuals - there might be an elevation of the excitation/inhibition ratio ("E/I balance") (Rubenstein and Merzenich, 2003) and such persistent modifications of network activity may precipitate broad changes in activity-dependent alternative splicing (Irimia et al., 2014; Quesnel-Vallieres et al., 2016).

It is difficult to assess what patterns of neuronal activity would produce persistent shifts in activity-dependent splicing *in vivo*. Activity-dependent gene expression has been extensively studied for immediate early genes which are transcribed within minutes of neuronal activation, e.g., elicited by a behavioral stimulus (Ebert and Greenberg, 2013; Nonaka et al., 2014; Spiegel et al., 2014). Many immediate early genes themselves are transcription factors – thus, a transient behavioral stimulus produces the rapid synthesis of immediate early genes, which in turn then generate a more prolonged remodeling of the neuronal transcriptome through activation of secondary target genes. For activity-dependent splicing, there is no such downstream amplification mechanism known to date. This raises the question of how such acute, possibly transient modifications of alternative splicing might functionally shape synapses and/or neuronal circuits. Transient shifts in splice isoforms may represent a milestone in neuronal development, e.g., during a critical developmental period. Thus, a neuronal or synaptic rearrangement driven at a specific developmental timepoint by an activity-dependent splicing switch may irreversibly shape the resulting neuronal network structure and/or function.

It is another puzzling question how transiently produced proteins derived from transcripts that exhibit activity-dependent alternative splicing have functional impact on the context of substantial pools of pre-existing mRNAs and proteins. Notably, the turnover of most synaptic proteins and ion channels is in the order of many hours (Cohen et al., 2013). How can an acutely produced splice variant generate functionally significant changes? One possibility is that proteins arising from acute activity-dependent alternative splicing act as dominant-negative isoforms that functionally block or interfere with the pre-existing, constitutively expressed variant. This is the case for Homer1, a cytosolic scaffolding protein that is part of postsynaptic densities and connects metabotropic glutamate receptors to the postsynaptic scaffold (Brakeman et al., 1997; Kato et al., 1998). Seizures or similar strong stimulations trigger production of a shorter Homer1a splice isoform that lacks dimerization capacity and, thereby, remodels postsynaptic protein complexes and synapses (Sala et al., 2003).

Another important parameter is the kinetics limiting production of new transcripts and alternative splice variants vis-à-vis the time window of activity-dependent modifications in developing and mature circuits. Most splicing events occur co-transcriptionally (Kornblihtt, 2015), thus the production of new splice isoforms depends on the rate of RNA polymerase II-dependent transcript production. The elongation rate of RNA polymerase II is 1-4 Kb/min (O'Brien and Lis, 1993; Tennyson et al., 1995; Darzacq et al., 2007; Singh and Padgett, 2009). Long transcripts, which are highly represented in neuronal cells (Gabel et al., 2015), can take hours to tens of hours to transcribe. In the context of activity-dependent alternative splicing, this would mean that an acute stimulus like in a single trial learning paradigm as fear conditioning, would only produce protein isoforms hours after the original stimulus. This is hard to reconcile with an important function of activity-dependent alternative splicing in such learning processes. One model for how acute splicing changes may contribute to rapid plasticity events is the activity-dependent processing of pre-existing, partially spliced transcripts. In mouse neocortex as well as cultured neocortical neurons, thousands of polyadenylated transcripts have been identified that selectively retain certain introns. Intron retention has been suggested to serve as a mechanism that targets intron-containing transcripts for degradation, thereby, tuning the transcriptional output from genes (Braunschweig et al., 2014). However, a subset of retained introns can be excised in response to stimulation (Boutz et al., 2014; Mauger et al., 2016; Ninomiya et al., 2011). In cortical neurons, approximately 200 intron-retaining transcripts are stored in the nucleus and undergo rapid intron excision in response to NMDA-receptor and CaMK-dependent stimulation. At least some of these transcripts are exported to the cytoplasm where they are translated (Mauger et al., 2016), thus providing a rapid, transcription-independent but splicing-dependent mechanism for remodeling the neuronal transcriptome in response to neuronal activity. Notably, in *Drosophila* such a regulated intron retention/excision mechanism appears to be triggered by an appetitive associative memory paradigm (Gill et al., 2017). Here, the RNA-binding protein pasilla (which shares similarity with mammalian Nova proteins) regulates dynamic intron retention in the pre-mRNAs encoding the CPEB (cytoplasmic polyadenylation element binding)-family protein Orb2A. The newly generated Orb2A protein isoform then seeds aggregation of pre-existing Orb2B isoforms, a step that is thought to be a key regulatory switch that amplifies Orb2 function and results in long-term

memory formation (Gill et al., 2017; Kruttner et al., 2015). These recent studies provide a glimpse at how splicing events triggered by physiologically relevant shifts in neuronal activity might indeed contribute to plasticity in the intact nervous system.

Conclusions

Over the past decade, there have been breathtaking advances in genome-wide transcriptomics and the systematic identification of RNA-binding proteins, their binding specificities, and RNA targets. These advances have allowed for testing hypotheses about the importance of alternative splicing in specific steps of neuronal development, maturation, and function. Recent work revealed that alternative splicing programs do not simply provide fine-tuning or “tweaking” of fine-grained details of neuronal properties. Much rather, splicing programs have emerged as instructive signals for key steps of neuronal growth, recognition, synapse specification and plasticity. New genetic tools for cell type-specific experimentation will further accelerate the progress towards the fascinating question of how genetic programs and neuronal activity-driven mechanisms synergize to achieve the functional organization of a complex tissue like the nervous system. The significant number of mis-splicing events contributing to disease risk provides an additional motivator for elucidating the molecular mechanisms and functional impact of alternative splicing regulation in neuronal development and brain function. In fact, splicing therapeutics may not just have a future to “repair” disease-causing splicing events. Instead, they may be broadly applicable approaches for the manipulation of neuron- and synapse-specific properties that determine neuronal circuit function in disease states.

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References

- Allen SE, Darnell RB, Lipscombe D. The neuronal splicing factor Nova controls alternative splicing in N-type and P-type CaV2 calcium channels. *Channels (Austin)*. 2010; 4:483–489. [PubMed: 21150296]
- An P, Grabowski PJ. Exon silencing by UAGG motifs in response to neuronal excitation. *PLoS Biol*. 2007; 5:e36. [PubMed: 17298175]
- 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]
- Barbosa-Morais NL, Irimia M, Pan Q, et al. Blencowe BJ. The evolutionary landscape of alternative splicing in vertebrate species. *Science (New York, NY)*. 2012; 338:1587–1594.
- Beffert U, Weeber EJ, Durudas A, Qiu S, Masiulis I, Sweatt JD, Li WP, Adelmann G, Frotscher M, Hammer RE, et al. Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. *Neuron*. 2005; 47:567–579. [PubMed: 16102539]
- Boucarrd AA, Chubykin AA, Comoletti D, Taylor P, Südhof TC. A splice code for trans-synaptic cell adhesion mediated by binding of neuroligin 1 to alpha- and beta-neurexins. *Neuron*. 2005; 48:229–236. [PubMed: 16242404]
- Boutz PL, Bhutkar A, Sharp PA. Detained introns are a novel, widespread class of post-transcriptionally spliced introns. *Genes & development*. 2014; 29:63–80.

- Brakeman PR, Lanahan AA, O'Brien R, Roche K, Barnes CA, Huganir RL, Worley PF. Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature*. 1997; 386:284–288. [PubMed: 9069287]
- Braunschweig U, Barbosa-Morais NL, Pan Q, Nachman EN, Alipanahi B, Gonatopoulos-Pournatzis T, Frey B, Irimia M, Blencowe BJ. Widespread intron retention in mammals functionally tunes transcriptomes. *Genome research*. 2014; 24:1774–1786. [PubMed: 25258385]
- Castello A, Fischer B, Frese CK, Horos R, Alleaume AM, Foehr S, Curk T, Krijgsvelde J, Hentze MW. Comprehensive Identification of RNA-Binding Domains in Human Cells. *Mol Cell*. 2016; 63:696–710. [PubMed: 27453046]
- Chaudhuri D, Chang SY, DeMaria CD, Alvania RS, Soong TW, Yue DT. Alternative splicing as a molecular switch for Ca²⁺/calmodulin-dependent facilitation of P/Q-type Ca²⁺ channels. *J Neurosci*. 2004; 24:6334–6342. [PubMed: 15254089]
- Chen BE, Kondo M, Garnier A, Watson FL, Puettmann-Holgado R, Lamar DR, Schmucker D. The molecular diversity of Dscam is functionally required for neuronal wiring specificity in *Drosophila*. *Cell*. 2006; 125:607–620. [PubMed: 16678102]
- Chih B, Gollan L, Scheiffele P. Alternative splicing controls selective trans-synaptic interactions of the neuroligin-neurexin complex. *Neuron*. 2006; 51:171–178. [PubMed: 16846852]
- Cohen LD, Zuchman R, Sorokina O, Muller A, Dieterich DC, Armstrong JD, Ziv T, Ziv NE. Metabolic turnover of synaptic proteins: kinetics, interdependencies and implications for synaptic maintenance. *PLoS One*. 2013; 8:e63191. [PubMed: 23658807]
- Crowner D, Madden K, Goeke S, Giniger E. Lola regulates midline crossing of CNS axons in *Drosophila*. *Development*. 2002; 129:1317–1325. [PubMed: 11880341]
- Darnell RB. RNA protein interaction in neurons. *Annu Rev Neurosci*. 2013; 36:243–270. [PubMed: 23701460]
- de Almeida SF, Carmo-Fonseca M. Reciprocal regulatory links between cotranscriptional splicing and chromatin. *Semin Cell Dev Biol*. 2014; 32:2–10. [PubMed: 24657193]
- de la Mata M, Kornblihtt AR. RNA polymerase II C-terminal domain mediates regulation of alternative splicing by SRp20. *Nat Struct Mol Biol*. 2006; 13:973–980. [PubMed: 17028590]
- Di Fruscio M, Chen T, Richard S. Characterization of Sam68-like mammalian proteins SLM-1 and SLM-2: SLM-1 is a Src substrate during mitosis. *Proc Natl Acad Sci U S A*. 1999; 96:2710–2715. [PubMed: 10077576]
- Dickson BJ, Zou Y. Navigating intermediate targets: the nervous system midline. *Cold Spring Harbor perspectives in biology*. 2010; 2:a002055. [PubMed: 20534708]
- Dillman AA, Hauser DN, Gibbs JR, Nalls MA, McCoy MK, Rudenko IN, Galter D, Cookson MR. mRNA expression, splicing and editing in the embryonic and adult mouse cerebral cortex. *Nature neuroscience*. 2013; 16:499–506. [PubMed: 23416452]
- Ebert DH, Greenberg ME. Activity-dependent neuronal signalling and autism spectrum disorder. *Nature*. 2013; 493:327–337. [PubMed: 23325215]
- Ehrmann I, Dalgliesh C, Liu Y, Danilenko M, Crosier M, Overman L, Arthur HM, Lindsay S, Clowry GJ, Venables JP, et al. The tissue-specific RNA binding protein T-STAR controls regional splicing patterns of neurexin pre-mRNAs in the brain. *PLoS Genet*. 2013; 9:e1003474. [PubMed: 23637638]
- 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:e00178. [PubMed: 23359859]
- Evans TA, Bashaw GJ. Axon guidance at the midline: of mice and flies. *Curr Opin Neurobiol*. 2010; 20:79–85. [PubMed: 20074930]
- Feldman DE, Brecht M. Map plasticity in somatosensory cortex. *Science (New York, NY)*. 2005; 310:810–815.
- Feng Y, Chen M, Manley JL. Phosphorylation switches the general splicing repressor SRp38 to a sequence-specific activator. *Nat Struct Mol Biol*. 2008; 15:1040–1048. [PubMed: 18794844]
- Finkel RS, Chiriboga CA, Vajsaar J, Day JW, Montes J, De Vivo DC, Yamashita M, Rigo F, Hung G, Schneider E, et al. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *Lancet*. 2016; 388:3017–3026. [PubMed: 27939059]

- Fong N, Kim H, Zhou Y, Ji X, Qiu J, Saldi T, Diener K, Jones K, Fu XD, Bentley DL. Pre-mRNA splicing is facilitated by an optimal RNA polymerase II elongation rate. *Genes Dev.* 2014; 28:2663–2676. [PubMed: 25452276]
- Fuccillo MV, Foldy C, Gokce O, Rothwell PE, Sun GL, Malenka RC, Sudhof TC. Single-Cell mRNA Profiling Reveals Cell-Type-Specific Expression of Neurexin Isoforms. *Neuron.* 2015; 87:326–340. [PubMed: 26182417]
- Gehman LT, Meera P, Stoilov P, Shiue L, O'Brien JE, Meisler MH, Ares M Jr, Otis TS, Black DL. The splicing regulator Rbfox2 is required for both cerebellar development and mature motor function. *Genes Dev.* 2012; 26:445–460. [PubMed: 22357600]
- Gehman LT, Stoilov P, Maguire J, Damianov A, Lin CH, Shiue L, Ares M Jr, Mody I, Black DL. The splicing regulator Rbfox1 (A2BP1) controls neuronal excitation in the mammalian brain. *Nat Genet.* 2011; 43:706–711. [PubMed: 21623373]
- Gerstberger S, Hafner M, Tuschl T. A census of human RNA-binding proteins. *Nature reviews Genetics.* 2014; 15:829–845.
- Gill J, Park Y, McGinnis JP, Perez-Sanchez C, Blanchette M, Si K. Regulated Intron Removal Integrates Motivational State and Experience. *Cell.* 2017; 169:836–848 e815. [PubMed: 28525754]
- Goeke S, Greene EA, Grant PK, Gates MA, Crowner D, Aigaki T, Giniger E. Alternative splicing of *lola* generates 19 transcription factors controlling axon guidance in *Drosophila*. *Nat Neurosci.* 2003; 6:917–924. [PubMed: 12897787]
- Graf ER, Zhang X, Jin SX, Linhoff MW, Craig AM. Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell.* 2004; 119:1013–1026. [PubMed: 15620359]
- Hattori D, Demir E, Kim HW, Viragh E, Zipursky SL, Dickson BJ. Dscam diversity is essential for neuronal wiring and self-recognition. *Nature.* 2007; 449:223–227. [PubMed: 17851526]
- Hoertel O. Terminal Selectors of Neuronal Identity. *Curr Top Dev Biol.* 2016; 116:455–475. [PubMed: 26970634]
- Howe KJ, Kane CM, Ares M Jr. Perturbation of transcription elongation influences the fidelity of internal exon inclusion in *Saccharomyces cerevisiae*. *RNA.* 2003; 9:993–1006. [PubMed: 12869710]
- Hrvatin S, Hochbaum DR, Nagy MA, Cicconet M, Robertson K, Cheadle L, Zilionis R, Ratner A, Borges-Monroy R, Klein AM, et al. Single-cell analysis of experience-dependent transcriptomic states in the mouse visual cortex. *Nature neuroscience.* 2018; 21:120–129. [PubMed: 29230054]
- Hua Y, Sahashi K, Rigo F, Hung G, Horev G, Bennett CF, Krainer AR. Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. *Nature.* 2011; 478:123–126. [PubMed: 21979052]
- Hughes ME, Bortnick R, Tsubouchi A, Baumer P, Kondo M, Uemura T, Schmucker D. Homophilic Dscam interactions control complex dendrite morphogenesis. *Neuron.* 2007; 54:417–427. [PubMed: 17481395]
- Hwang HW, Saito Y, Park CY, Blachere NE, Tajima Y, Fak JJ, Zucker-Scharff I, Darnell RB. cTag-PAPERCLIP Reveals Alternative Polyadenylation Promotes Cell-Type Specific Protein Diversity and Shifts Araf Isoforms with Microglia Activation. *Neuron.* 2017; 95:1334–1349 e1335. [PubMed: 28910620]
- Iijima T, Iijima Y, Witte H, Scheiffele P. Neuronal cell type-specific alternative splicing is regulated by the KH domain protein SLM1. *The Journal of cell biology.* 2014; 204:331–342. [PubMed: 24469635]
- Iijima T, Wu K, Witte H, Hanno-Iijima Y, Glatter T, Richard S, Scheiffele P. SAM68 regulates neuronal activity-dependent alternative splicing of neurexin-1. *Cell.* 2011; 147:1601–1614. [PubMed: 22196734]
- Ip JY, Schmidt D, Pan Q, Ramani AK, Fraser AG, Odom DT, Blencowe BJ. Global impact of RNA polymerase II elongation inhibition on alternative splicing regulation. *Genome research.* 2011; 21:390–401. [PubMed: 21163941]

- Irimia M, Weatheritt RJ, Ellis JD, Parikshak NN, Gonatopoulos-Pournatzis T, Babor M, Quesnel-Vallieres M, Tapial J, Raj B, O'Hanlon D, et al. A highly conserved program of neuronal microexons is misregulated in autistic brains. *Cell*. 2014; 159:1511–1523. [PubMed: 25525873]
- Kato A, Ozawa F, Saitoh Y, Fukazawa Y, Sugiyama H, Inokuchi K. Novel members of the Vesl/Homer family of PDZ proteins that bind metabotropic glutamate receptors. *J Biol Chem*. 1998; 273:23969–23975. [PubMed: 9727012]
- Kepecs A, Fishell G. Interneuron cell types are fit to function. *Nature*. 2014; 505:318–326. [PubMed: 24429630]
- Kim MS, Pinto SM, Getnet D, Nirujogi RS, Manda SS, Chaerkady R, Madugundu AK, Kelkar DS, Isserlin R, Jain S, et al. A draft map of the human proteome. *Nature*. 2014; 509:575–581. [PubMed: 24870542]
- Kornblihtt AR. Transcriptional control of alternative splicing along time: ideas change, experiments remain. *RNA*. 2015; 21:670–672. [PubMed: 25780184]
- Kruttner S, Traunmuller L, Dag U, Jandrasits K, Stepien B, Iyer N, Fradkin LG, Noordermeer JN, Mensh BD, Keleman K. Synaptic Orb2A Bridges Memory Acquisition and Late Memory Consolidation in *Drosophila*. *Cell reports*. 2015; 11:1953–1965. [PubMed: 26095367]
- Lah GJ, Li JS, Millard SS. Cell-specific alternative splicing of *Drosophila* dscam2 is crucial for proper neuronal wiring. *Neuron*. 2014; 83:1376–1388. [PubMed: 25175881]
- Lee JA, Xing Y, Nguyen D, Xie J, Lee CJ, Black DL. Depolarization and CaM kinase IV modulate NMDA receptor splicing through two essential RNA elements. *PLoS Biol*. 2007; 5:e40. [PubMed: 17298178]
- Leggere JC, Saito Y, Darnell RB, Tessier-Lavigne M, Junge HJ, Chen Z. NOVA regulates Dcc alternative splicing during neuronal migration and axon guidance in the spinal cord. *eLife*. 2016; 5
- Li Y, Zhang P, Choi TY, Park SK, Park H, Lee EJ, Lee D, Roh JD, Mah W, Kim R, et al. Splicing-Dependent Trans-synaptic SALM3-LAR-RPTP Interactions Regulate Excitatory Synapse Development and Locomotion. *Cell reports*. 2015a; 12:1618–1630. [PubMed: 26321637]
- Li YI, Sanchez-Pulido L, Haerty W, Ponting CP. RBFOX and PTBP1 proteins regulate the alternative splicing of micro-exons in human brain transcripts. *Genome research*. 2015b; 25:1–13.
- Licatalosi DD, Yano M, Fak JJ, Mele A, Grabinski SE, Zhang C, Darnell RB. Ptb2 represses adult-specific splicing to regulate the generation of neuronal precursors in the embryonic brain. *Genes Dev*. 2012; 26:1626–1642. [PubMed: 22802532]
- Lipscombe D, Allen SE, Toro CP. Control of neuronal voltage-gated calcium ion channels from RNA to protein. *Trends in neurosciences*. 2013; 36:598–609. [PubMed: 23907011]
- Liu G, Razanau A, Hai Y, Yu J, Sohail M, Lobo VG, Chu J, Kung SK, Xie J. A conserved serine of heterogeneous nuclear ribonucleoprotein L (hnRNP L) mediates depolarization-regulated alternative splicing of potassium channels. *J Biol Chem*. 2012; 287:22709–22716. [PubMed: 22570490]
- Llorian M, Schwartz S, Clark TA, Hollander D, Tan LY, Spellman R, Gordon A, Schweitzer AC, de la Grange P, Ast G, et al. Position-dependent alternative splicing activity revealed by global profiling of alternative splicing events regulated by PTB. *Nat Struct Mol Biol*. 2010; 17:1114–1123. [PubMed: 20711188]
- Lodato S, Shetty AS, Arlotta P. Cerebral cortex assembly: generating and reprogramming projection neuron diversity. *Trends in neurosciences*. 2015; 38:117–125. [PubMed: 25529141]
- Margolis DJ, Lutcke H, Helmchen F. Microcircuit dynamics of map plasticity in barrel cortex. *Curr Opin Neurobiol*. 2014; 24:76–81. [PubMed: 24492082]
- Matsuda K, Budisantoso T, Mitakidis N, Sugaya Y, Miura E, Kakegawa W, Yamasaki M, Konno K, Uchigashima M, Abe M, et al. Transsynaptic Modulation of Kainate Receptor Functions by C1q-like Proteins. *Neuron*. 2016; 90:752–767. [PubMed: 27133466]
- Matter N, Herrlich P, Konig H. Signal-dependent regulation of splicing via phosphorylation of Sam68. *Nature*. 2002; 420:691–695. [PubMed: 12478298]
- Mauger O, Lemoine F, Scheiffele P. Targeted Intron Retention and Excision for Rapid Gene Regulation in Response to Neuronal Activity. *Neuron*. 2016; 92
- Mauger O, Scheiffele P. Beyond proteome diversity: alternative splicing as a regulator of neuronal transcript dynamics. *Curr Opin Neurobiol*. 2017; 45:162–168. [PubMed: 28609697]

- Mayer C, Hafemeister C, Bandler RC, Machold R, Brito RB, Jaglin X, Allaway K, Butler A, Fishell G, Satija R. Developmental diversification of cortical inhibitory interneurons. *Nature*. 2018; 555:457–462. [PubMed: 29513653]
- McMahon AC, Rahman R, Jin H, Shen JL, Fieldsend A, Luo W, Rosbash M. TRIBE: Hijacking an RNA-Editing Enzyme to Identify Cell-Specific Targets of RNA-Binding Proteins. *Cell*. 2016; 165:742–753. [PubMed: 27040499]
- Merkin J, Russell C, Chen P, Burge CB. Evolutionary dynamics of gene and isoform regulation in Mammalian tissues. *Science (New York, NY)*. 2012; 338:1593–1599.
- Mi D, Li Z, Lim L, Li M, Moissidis M, Yang Y, Gao T, Hu TX, Pratt T, Price DJ, et al. Early emergence of cortical interneuron diversity in the mouse embryo. *Science (New York, NY)*. 2018
- 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]
- Mockenhaupt S, Makeyev EV. Non-coding functions of alternative pre-mRNA splicing in development. *Seminars in Cell & Developmental Biology*. 2015; 48:32–39.
- Naryshkin NA, Weetall M, Dakka A, Narasimhan J, Zhao X, Feng Z, Ling KK, Karp GM, Qi H, Woll MG, et al. Motor neuron disease. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. *Science (New York, NY)*. 2014; 345:688–693.
- Nguyen TM, 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
- Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing. *Nature*. 2010; 463:457–463. [PubMed: 20110989]
- Ninomiya K, Kataoka N, Hagiwara M. Stress-responsive maturation of Clk1/4 pre-mRNAs promotes phosphorylation of SR splicing factor. *Journal of Cell Biology*. 2011; 195:27–40. [PubMed: 21949414]
- Nonaka M, Fujii H, Kim R, Kawashima T, Okuno H, Bito H. Untangling the two-way signalling route from synapses to the nucleus, and from the nucleus back to the synapses. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2014; 369
- Norris AD, Gao S, Norris ML, Ray D, Ramani AK, Fraser AG, Morris Q, Hughes TR, Zhen M, Calarco JA. A pair of RNA-binding proteins controls networks of splicing events contributing to specialization of neural cell types. *Mol Cell*. 2014; 54:946–959. [PubMed: 24910101]
- Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet*. 2008; 40:1413–1415. [PubMed: 18978789]
- Parikshak NN, Swarup V, Belgard TG, Irimia M, Ramaswami G, Gandal MJ, Hartl C, Leppa V, Ubieta LT, Huang J, et al. Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism. *Nature*. 2016; 540:423–427. [PubMed: 27919067]
- Paul A, Crow M, Raudales R, He M, Gillis J, Huang ZJ. Transcriptional Architecture of Synaptic Communication Delineates GABAergic Neuron Identity. *Cell*. 2017; 171:522–539 e520. [PubMed: 28942923]
- Quesnel-Vallieres M, Dargaei Z, Irimia M, Gonatopoulos-Pournatzis T, Ip JY, Wu M, Sterne-Weiler T, Nakagawa S, Woodin MA, Blencowe BJ, et al. Misregulation of an Activity-Dependent Splicing Network as a Common Mechanism Underlying Autism Spectrum Disorders. *Mol Cell*. 2016; 64:1023–1034. [PubMed: 27984743]
- Raj B, Irimia M, Braunschweig U, Sterne-Weiler T, O'Hanlon D, Lin ZY, Chen GI, Easton LE, Ule J, Gingras AC, et al. A global regulatory mechanism for activating an exon network required for neurogenesis. *Mol Cell*. 2014; 56:90–103. [PubMed: 25219497]
- Ray D, Kazan H, Chan ET, Pena Castillo L, Chaudhry S, Talukder S, Blencowe BJ, Morris Q, Hughes TR. Rapid and systematic analysis of the RNA recognition specificities of RNA-binding proteins. *Nat Biotechnol*. 2009; 27:667–670. [PubMed: 19561594]
- Ray D, Kazan H, Cook KB, Weirauch MT, Najafabadi HS, Li X, Gueroussov S, Albu M, Zheng H, Yang A, et al. A compendium of RNA-binding motifs for decoding gene regulation. *Nature*. 2013; 499:172–177. [PubMed: 23846655]

- Rideau AP, Gooding C, Simpson PJ, Monie TP, Lorenz M, Huttelmaier S, Singer RH, Matthews S, Curry S, Smith CW. A peptide motif in Raver1 mediates splicing repression by interaction with the PTB RRM2 domain. *Nat Struct Mol Biol.* 2006; 13:839–848. [PubMed: 16936729]
- Roberts GC, Gooding C, Mak HY, Proudfoot NJ, Smith CW. Co-transcriptional commitment to alternative splice site selection. *Nucleic acids research.* 1998; 26:5568–5572. [PubMed: 9837984]
- Rozic-Kotliroff G, Zisapel N. Ca²⁺-dependent splicing of neurexin IIalpha. *Biochem Biophys Res Commun.* 2007; 352:226–230. [PubMed: 17107668]
- Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav.* 2003; 2:255–267. [PubMed: 14606691]
- Sala C, Futai K, Yamamoto K, Worley PF, Hayashi Y, Sheng M. Inhibition of dendritic spine morphogenesis and synaptic transmission by activity-inducible protein Homer1a. *J Neurosci.* 2003; 23:6327–6337. [PubMed: 12867517]
- Sanz E, Yang L, Su T, Morris DR, McKnight GS, Amieux PS. Cell-type-specific isolation of ribosome-associated mRNA from complex tissues. *Proc Natl Acad Sci U S A.* 2009; 106:13939–13944. [PubMed: 19666516]
- Schmucker D, Clemens JC, Shu H, Worby CA, Xiao J, Muda M, Dixon JE, Zipursky SL. *Drosophila* Dscam is an axon guidance receptor exhibiting extraordinary molecular diversity. *Cell.* 2000; 101:671–684. [PubMed: 10892653]
- 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
- Schreiner D, Simicevic J, Ahrné E, Schmidt A, Scheiffele P. Quantitative isoform-profiling of highly diversified recognition molecules. *eLife.* 2015; 4:1–17.
- Shalek AK, Satija R, Adiconis X, Gertner RS, Gaublot JM, Raychowdhury R, Schwartz S, Yosef N, Malboeuf C, Lu D, et al. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. *Nature.* 2013; 498:236–240. [PubMed: 23685454]
- Shekhar K, Lapan SW, Whitney IE, Tran NM, Macosko EZ, Kowalczyk M, Adiconis X, Levin JZ, Nemesh J, Goldman M, et al. Comprehensive Classification of Retinal Bipolar Neurons by Single-Cell Transcriptomics. *Cell.* 2016; 166:1308–1323 e1330. [PubMed: 27565351]
- Soba P, Zhu S, Emoto K, Younger S, Yang SJ, Yu HH, Lee T, Jan LY, Jan YN. *Drosophila* sensory neurons require Dscam for dendritic self-avoidance and proper dendritic field organization. *Neuron.* 2007; 54:403–416. [PubMed: 17481394]
- Song Y, Botvinnik OB, Lovci MT, Kakaradov B, Liu P, Xu JL, Yeo GW. Single-Cell Alternative Splicing Analysis with Expedition Reveals Splicing Dynamics during Neuron Differentiation. *Mol Cell.* 2017; 67:148–161 e145. [PubMed: 28673540]
- Soong TW, DeMaria CD, Alvania RS, Zweifel LS, Liang MC, Mittman S, Agnew WS, Yue DT. Systematic identification of splice variants in human P/Q-type channel alpha1(2.1) subunits: implications for current density and Ca²⁺-dependent inactivation. *J Neurosci.* 2002; 22:10142–10152. [PubMed: 12451115]
- Spiegel I, Mardinly AR, Gabel HW, Bazinet JE, Couch CH, Tzeng CP, Harmin DA, Greenberg ME. Npas4 regulates excitatory-inhibitory balance within neural circuits through cell-type-specific gene programs. *Cell.* 2014; 157:1216–1229. [PubMed: 24855953]
- Stellwagen D, Shatz CJ. An instructive role for retinal waves in the development of retinogeniculate connectivity. *Neuron.* 2002; 33:357–367. [PubMed: 11832224]
- Stoss O, Novoyatleva T, Gencheva M, Olbrich M, Benderska N, Stamm S. p59(fyn)-mediated phosphorylation regulates the activity of the tissue-specific splicing factor rSLM-1. *Mol Cell Neurosci.* 2004; 27:8–21. [PubMed: 15345239]
- Sugita S, Saito F, Tang J, Satz J, Campbell K, Sudhof TC. A stoichiometric complex of neurexins and dystroglycan in brain. *The Journal of cell biology.* 2001; 154:435–445. [PubMed: 11470830]
- Sun W, You X, Gogol-Doring 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]
- Sur M, Rubenstein JL. Patterning and plasticity of the cerebral cortex. *Science (New York, NY).* 2005; 310:805–810.

- Takahashi H, Craig AM. Protein tyrosine phosphatases PTPdelta, PTPsigma, and LAR: presynaptic hubs for synapse organization. *Trends in neurosciences*. 2013; 36:522–534. [PubMed: 23835198]
- Tapial J, Ha KCH, Sterne-Weiler T, Gohr A, Braunschweig U, Hermoso-Pulido A, Quesnel-Vallieres M, Permanyer J, Sodaei R, Marquez Y, et al. An atlas of alternative splicing profiles and functional associations reveals new regulatory programs and genes that simultaneously express multiple major isoforms. *Genome research*. 2017; 27:1759–1768. [PubMed: 28855263]
- Tasic B, Menon V, Nguyen TN, Kim TK, Jarsky T, Yao Z, Levi B, Gray LT, Sorensen SA, Dolbeare T, et al. Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nature neuroscience*. 2016; 19:335–346. [PubMed: 26727548]
- Thalhammer A, Contestabile A, Ermolyuk YS, Ng T, Volynski KE, Soong TW, Goda Y, Cingolani LA. Alternative Splicing of P/Q-Type Ca(2+) Channels Shapes Presynaptic Plasticity. *Cell reports*. 2017; 20:333–343. [PubMed: 28700936]
- Traunmüller 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]
- Traunmüller L, Gomez AM, Nguyen T-M, Scheiffele P. Control of neuronal synapse specification by highly dedicated alternative splicing program. *Science (New York, NY)*. 2016; 352:982–986.
- Tress ML, Abascal F, Valencia A. Alternative Splicing May Not Be the Key to Proteome Complexity. *Trends in biochemical sciences*. 2017; 42:98–110. [PubMed: 27712956]
- Treutlein B, Gokce O, Quake SR, Südhof TC. Cartography of neuroligin alternative splicing mapped by single-molecule long-read mRNA sequencing. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:E1291–1299. [PubMed: 24639501]
- Uemura T, Lee SJ, Yasumura M, Takeuchi T, Yoshida T, Ra M, Taguchi R, Sakimura K, Mishina M. Trans-synaptic interaction of GluRdelta2 and Neuroligin through Cbln1 mediates synapse formation in the cerebellum. *Cell*. 2010; 141:1068–1079. [PubMed: 20537373]
- Ule J, Stefani G, Mele A, Ruggiu M, Wang X, Taneri B, Gaasterland T, Blencowe BJ, Darnell RB. An RNA map predicting Nova-dependent splicing regulation. *Nature*. 2006; 444:580–586. [PubMed: 17065982]
- Van Nostrand EL, Pratt GA, Shishkin AA, Gelboin-Burkhart C, Fang MY, Sundararaman B, Blue SM, Nguyen TB, Surka C, Elkins K, et al. Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP). *Nat Methods*. 2016; 13:508–514. [PubMed: 27018577]
- Venables JP, Vernet C, Chew SL, Elliott DJ, Cowmeadow RB, Wu J, Cooke HJ, Artzt K, Eperon IC. T-STAR/ETOILE: a novel relative of SAM68 that interacts with an RNA-binding protein implicated in spermatogenesis. *Hum Mol Genet*. 1999; 8:959–969. [PubMed: 10332027]
- Wang ET, Sandberg R, Luo S, Khrebtkova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. *Nature*. 2008; 456:470–476. [PubMed: 18978772]
- Wang Z, Burge CB. Splicing regulation: from a parts list of regulatory elements to an integrated splicing code. *RNA*. 2008; 14:802–813. [PubMed: 18369186]
- Weatheritt RJ, Sterne-Weiler T, Blencowe BJ. The ribosome-engaged landscape of alternative splicing. *Nat Struct Mol Biol*. 2016; 23:1117–1123. [PubMed: 27820807]
- West AE, Griffith EC, Greenberg ME. Regulation of transcription factors by neuronal activity. *Nat Rev Neurosci*. 2002; 3:921–931. [PubMed: 12461549]
- Wojtowicz WM, Wu W, Andre I, Qian B, Baker D, Zipursky SL. A vast repertoire of Dscam binding specificities arises from modular interactions of variable Ig domains. *Cell*. 2007; 130:1134–1145. [PubMed: 17889655]
- Xie J. Control of alternative pre-mRNA splicing by Ca(++) signals. *Biochim Biophys Acta*. 2008; 1779:438–452. [PubMed: 18258215]
- Xie J, Black DL. A CaMK IV responsive RNA element mediates depolarization-induced alternative splicing of ion channels. *Nature*. 2001; 410:936–939. [PubMed: 11309619]
- Xiong HY, Alipanahi B, Lee LJ, Bretschneider H, Merico D, Yuen RK, Hua Y, Gueroussov S, Najafabadi HS, Hughes TR, et al. RNA splicing. The human splicing code reveals new insights into the genetic determinants of disease. *Science (New York, NY)*. 2015; 347

- Xue Y, Zhou Y, Wu T, Zhu T, Ji X, Kwon YS, Zhang C, Yeo G, Black DL, Sun H, et al. Genome-wide analysis of PTB-RNA interactions reveals a strategy used by the general splicing repressor to modulate exon inclusion or skipping. *Mol Cell*. 2009; 36:996–1006. [PubMed: 20064465]
- 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. *Proceedings of the National Academy of Sciences of the United States of America*. 2015; 112
- Yap K, Xiao Y, Friedman BA, Je HS, Makeyev EV. Polarizing the Neuron through Sustained Co-expression of Alternatively Spliced Isoforms. *Cell reports*. 2016; 15:1316–1328. [PubMed: 27134173]
- Yoshida T, Yasumura M, Uemura T, Lee SJ, Ra M, Taguchi R, Iwakura Y, Mishina M. IL-1 receptor accessory protein-like 1 associated with mental retardation and autism mediates synapse formation by trans-synaptic interaction with protein tyrosine phosphatase delta. *J Neurosci*. 2011; 31:13485–13499. [PubMed: 21940441]
- Zeisel A, Munoz-Manchado AB, Codeluppi S, Lonnerberg P, La Manno G, Jureus A, Marques S, Munguba H, He L, Betsholtz C, et al. Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science (New York, NY)*. 2015; 347:1138–1142.
- Zhan XL, Clemens JC, Neves G, Hattori D, Flanagan JJ, Hummel T, Vasconcelos ML, Chess A, Zipursky SL. Analysis of Dscam diversity in regulating axon guidance in *Drosophila* mushroom bodies. *Neuron*. 2004; 43:673–686. [PubMed: 15339649]
- Zhang C, Frias MA, Mele A, Ruggiu M, Eom T, Marney CB, Wang H, Licatalosi DD, Fak JJ, Darnell RB. Integrative modeling defines the Nova splicing-regulatory network and its combinatorial controls. *Science (New York, NY)*. 2010; 329:439–443.
- Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keefe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci*. 2014; 34:11929–11947. [PubMed: 25186741]

Figure 1.

Figure 2.