

Neuronal regulation of alternative pre-mRNA splicing

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Abstract | Alternative pre-mRNA splicing has an important role in the control of neuronal gene expression. Many neuronal proteins are structurally diversified through the differential inclusion and exclusion of sequences in the final spliced mRNA. Here, we discuss common mechanisms of splicing regulation and provide examples of how alternative splicing has important roles in neuronal development and mature neuron function. Finally, we describe regulatory proteins that control the splicing of some neuronally expressed transcripts.

Pre-mRNA

The unprocessed precursor to mRNA. It contains unspliced introns.

Exon

A segment of RNA that remains in the mRNA after intron removal. Pre-mRNA splicing results in the ligation of exons into a chain.

Intron

A segment of RNA that separates two exons in the pre-mRNA and is excised during splicing. Also called an intervening sequence.

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Alternative pre-mRNA splicing is a mechanism that controls the protein output of eukaryotic genes, and a major contributor to proteomic diversity^{1,2}. This process of gene regulation is especially common for genes that are expressed in the nervous system³, where the production of particular protein isoforms helps to determine the properties of the many different types of neurons^{4,5}. Recent work has identified several important events during neuronal development that are controlled by alternative splicing, including aspects of cell-fate determination, axon guidance and synaptogenesis. In the mature nervous system, the splicing of products that determine cell excitation is dynamically regulated by various cellular inputs. There is also increasing recognition that some neurological and neuromuscular diseases are caused by errors in splicing — spinal muscular atrophy and frontotemporal dementia being two examples^{6–8}. The importance of individual splicing choices and of splicing regulators in nervous-system development and function is becoming clearer. In this Review, we describe a few of the many cellular processes in which splicing regulation is important, and discuss efforts to understand the molecular mechanisms that determine these splicing choices. We hope to provide an appreciation of the wide-ranging consequences of alternative splicing in neuronal cell biology.

General splicing mechanisms

The borders of pre-mRNA exons and introns are delineated by the 5' splice site at the upstream end of the intron and the 3' splice site at the downstream end. Alternative splicing involves changes in the choice of splice sites by the splicing apparatus and in the definition of introns⁹. Intron excision and exon ligation are catalysed by a large ribonucleoprotein (RNP) complex called the spliceosome, which is assembled onto each intron from five

small nuclear RNPs (snRNPs) and a large number of auxiliary proteins¹⁰ (BOX 1). The major class of spliceosome is made up of the U1, U2, U4, U5 and U6 snRNPs and excises nearly all introns in metazoans¹⁰. Although several interesting neuronal transcripts contain introns that are excised by the minor U12-dependent spliceosome, the two spliceosomes are very similar in their constituent parts and mechanisms of action.

Several processes alter spliceosome assembly and affect splice site choice. Spliceosome assembly initiates, and in some cases completes, intron excision during pre-mRNA synthesis, and the excision of some introns is strongly affected by transcription elongation rates¹¹. The secondary structure of a pre-mRNA can also alter the ability of the spliceosome to gain access to the splice sites^{12,13}. However, the best understood alterations in splicing are determined by proteins that bind to the pre-mRNA and enhance or repress spliceosome assembly at various steps^{9,14,15}. It is thought that even small changes in the relative rates of spliceosome assembly at different splice sites can lead to large changes in the choice of splicing pattern for a transcript¹⁵.

Pre-mRNAs can have many patterns of alternative processing, such as the controlled inclusion of a particular exon cassette or the shifting of an individual splice site⁹ (FIG. 1). Through the combinatorial assembly of multiple alternatively spliced segments, neuronally expressed genes can produce hundreds or even thousands of mRNA variants, which makes the analysis of gene function difficult. It is not currently possible to predict from sequence alone those splice sites and exons that will exhibit regulated (or 'alternative') use and those that will be constitutively spliced¹⁶. Alternative exons and splice sites often show weaker matches to the splice site consensus sequences than the average exon, and one can often identify binding sites for regulatory

Splice site

Short RNA sequences at exon–intron and intron–exon borders. They are bound by components of the spliceosome and encompass the nucleotides where the transesterification chemistry takes place.

Small nuclear ribonucleoprotein

(snRNP). A complex that is composed of a small nuclear RNA and a specific set of proteins.

Lariat RNA

A product of the first step of splicing, in which the 5' phosphate of the 5' terminal guanosine of the intron is linked to the 2' hydroxyl of an adenosine residue that lies downstream at the branchpoint. This reaction creates a branched nucleotide that has an unusual 2' to 5' phosphodiester bond in addition to the normal 3' to 5' linkages, and an unusual loop-and-3'-tail structure, hence the name.

Exon cassette

An exon that has regulated splicing, and so can be included in the mRNA or skipped.

Expressed sequence tag (EST) analysis

Sequence comparisons of EST databases. When ESTs are aligned with each other, and with mRNA and genomic sequences, regions of alternative processing can be identified.

proteins in an exon or its surrounding introns. Alternative exons and their adjacent introns can also exhibit greater sequence conservation than the constitutive exons of a gene^{17–19}. However, positions of alternative splicing are usually identified by analysis of expressed sequence tag (EST) databases or by the cloning of large numbers of cDNAs^{20,21}. Because different variants can show drastically different activities, it is important to characterize the many spliced products from a gene, to ensure that one is studying the correct variants for the cell type under study²⁰.

Altered splicing patterns can lead to functional variations in a protein. Usually, a portion of peptide sequence is added to or subtracted from the encoded protein, which can alter ligand-binding properties, subcellular localization, specific activity or regulation by signalling pathways. Non-coding sequences can be altered to affect mRNA translation, stability or localization²². By changing the reading frame or adding premature stop codons, some splicing events lead to

nonsense-mediated mRNA decay²³. This allows splicing to act as an on–off switch in gene expression. The identification of an altered transcript does not prove its functional importance, however. Besides its abundance relative to other products from the gene, one indication of the functional significance of an mRNA variant is evolutionary conservation. Many important splicing changes are highly conserved across mammals or vertebrate species¹⁹. However, new splice variants can arise through mutation, and many are species specific²⁴. Although these might just be evolutionary 'noise', it is likely that some will have important effects on gene function. Understanding the functional consequences of a splicing switch is usually much more challenging than the simple identification of the variation.

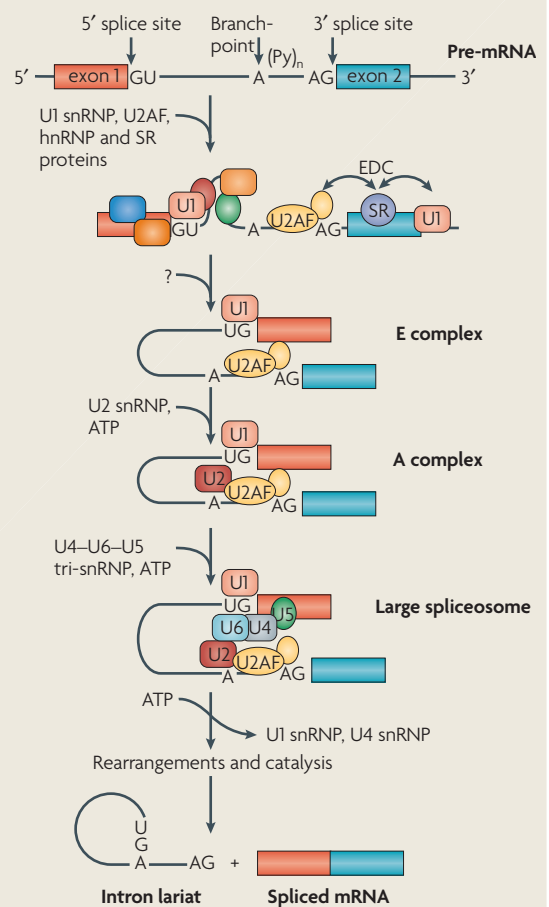
Splicing during neuronal development

Many different splicing patterns are induced during neuronal development, presumably as a result of changes in the expression of splicing regulators.

Box 1 | The pathway of spliceosome assembly *in vitro*

The assembly of the spliceosome has been characterized using *in vitro* splicing systems. The 5' splice site of an intron is recognized by the U1 small nuclear ribonucleoprotein (snRNP), through complementary pairing of the RNA bases; the 3' splice site, with its upstream polypyrimidine tract ((Py)_n), is bound by the protein U2 auxiliary factor (U2AF)¹⁰. Through mechanisms that are poorly understood, these factors, together with additional proteins, form a complex called the E (for early) or commitment complex, which bridges the intron and brings the splice sites that are to be cleaved and joined into juxtaposition. U2AF recruits the U2 snRNP, and an ATP-dependent step allows the RNA portion of the U2 snRNP to base pair with a sequence called the branchpoint, which lies upstream of the 3' splice site. The pairing of the U2 snRNA at the branchpoint completes the pre-spliceosomal A complex. Subsequent, ATP-dependent steps lead to the binding of the U4–U5–U6 tri-snRNP and the formation of the large spliceosome; rearrangements that detach the U1 and U4 snRNPs then follow. This forms the catalytic spliceosome, which performs two transesterification reactions on the splice sites. These reactions result in the ligation of the exons and the excision of the intron, in the form of a lariat RNA that has its 5' phosphate joined to the 2' hydroxyl at the branch point. Spliceosome assembly and its excision chemistry must be performed on each intron for them to be excised from an mRNA.

In cells, the early steps of spliceosome assembly on typical multi-intron RNAs are likely to differ from those that are seen on simple single-intron RNAs *in vitro*. Before the spliceosome forms across the large length of an intron (introns are typically comprised of between 500 and 50,000 nucleotides), splice sites are usually defined through interactions between splice-site-bound components across the short (typically between 50 and 300 nucleotides) exons^{18,9}. The assembly of these 'exon definition' complexes (EDCs) is stimulated by proteins that bind to special sequences in the exon itself (such as the serine–arginine (SR) proteins) and in the adjacent introns. Alternative splicing involves changes in the choice of splice sites that are assembled into a spliceosome, and this is usually controlled by the proteins that bind to these regulatory sequences⁹. These proteins can alter spliceosome assembly at various steps, but they have been most commonly studied for their effects on U1 snRNP and U2AF binding and initial complex assembly¹⁵.



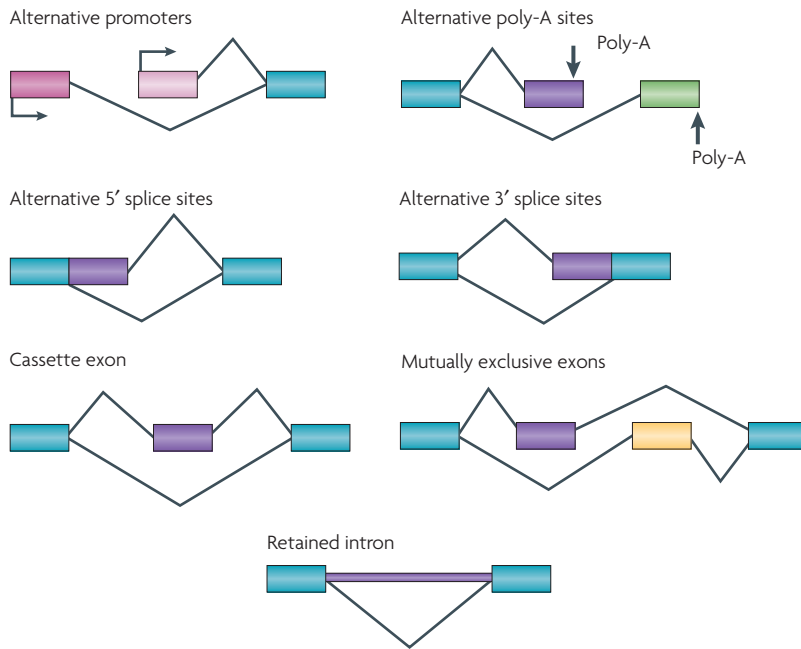


Figure 1 | Patterns of alternative splicing. Transcripts from a gene can undergo many different patterns of alternative splicing. Transcriptional initiation at different promoters (shown in two different shades of pink) generates alternative 5'-terminal exons that can be joined to a common 3' exon (shown in blue) downstream. Similarly, alternative 3' exons, with alternative polyadenylation sites, can be joined to a common upstream exon. Through the use of alternative 5' or 3' splice sites, exons can be extended or shortened in length. The most common pattern of alternative splicing is a cassette exon that can be included in the mRNA or skipped, inserting or deleting a portion of internal sequence. A special case of paired cassette exons show mutually exclusive splicing, where one exon or the other is included, but not both. Finally, the excision of an intron can be suppressed, to leave the retained intronic sequence in the mRNA that is exported to the cytoplasm. Many genes show multiple positions of alternative splicing, creating complex combinations of exons and alternative segments and a large family of encoded proteins.

Important regulatory decisions can be found at the level of splicing in nearly every step in neuronal development, from neuroblast commitment to synaptic specialization, and these decisions affect proteins ranging from transcription factors to cell-adhesion molecules. We describe only a few examples here.

FGFs and patterning of the neural tube. The anterior neural tube is organized into segments that later become forebrain, midbrain and hindbrain structures. Vertebrate midbrain development depends on fibroblast growth factor 8 (FGF8) secretion from an organizing centre at the midbrain–hindbrain boundary^{25,26}. This region expresses two FGF8 isoforms, FGF8a and FGF8b, which differ in their affinity for FGF receptors (FGFRs)^{27–30}. Compared to FGF8a, FGF8b contains an additional 11 amino acids at its amino (N) terminus — amino acids that are derived from an alternative 3' splice site in the second exon. These two FGF8 isoforms have remarkably different activities in patterning midbrain development. Ectopic expression of FGF8a expands the midbrain³¹, whereas ectopic FGF8b expression transforms midbrain tissue into cerebellar tissue^{26,32,33}. Differences in FGF8 isoform

function have also been observed in the developing forebrain³⁴. Alternative splicing also affects the mRNAs that encode FGFs 2, 5, 9 and 17 (REF. 35), and alters the ligand-binding affinity of FGF receptors³⁶. Given the important roles of the FGFs in the regulation of neurogenesis, it will be of great interest to investigate the control of their alternative splicing.

Cell-fate determination. Following neural induction, multipotent neural progenitor cells go through sequential rounds of division and differentiation to generate neurons and glia³⁷. During asymmetrical division, *numb* proteins are localized to the basal membrane and segregated to only one daughter cell, causing the two daughter cells to adopt different fates³⁷. In mammals, the *mnumb* and *numbl* homologues promote a progenitor cell fate over a neuronal fate by antagonizing notch signalling³⁸. Two protein-interaction domains are important for *numb* protein signalling and localization: a phosphotyrosine-binding domain and a proline-rich region (PRR) that interacts with SRC-homology domain 3 (SH3) domains. An alternative exon that encodes a peptide in the phosphotyrosine-binding domain is implicated in the trafficking and degradation of *numb* proteins³⁹ and might regulate the asymmetrical localization of *numb* in dividing progenitors^{40–42}. Another alternative exon that encodes part of the PRR domain modifies *numb* protein signalling properties; isoforms with this PRR insert (*numb1* and *numb3*) are expressed in undifferentiated cortical progenitors and promote proliferation, whereas isoforms that lack the exon (*numb2* and *numb4*) promote differentiation^{43–45}.

Axon guidance. Many molecules that are involved in neurite outgrowth and axon guidance are regulated by alternative splicing. These include cell-adhesion molecules, kinases, specialized cytoskeletal components and receptors for neurotrophins and guidance cues^{46,47}.

The *Drosophila* homologue of Down's syndrome cell-adhesion molecule (*DSCAM*) is required for the development of neural circuits⁴⁸. *Dscam* contains four blocks of tandemly arranged alternative exons, with each spliced mRNA containing only one alternative exon from each block. These alternative exons encode variable regions for the immunoglobulin (Ig) repeats 2, 3 and 7 of the extracellular domain, and two alternative transmembrane segments⁴⁹. The large number of alternative exons (12, 48, 33 and 2 possible choices for exons 4, 6, 9 and 17, respectively), and the apparently independent splicing in each block, lead to the potential generation of 38,016 *DSCAM* isoforms, giving *Dscam* the highest product diversity of any known gene. *DSCAM* isoforms mediate cell-surface recognition through homophilic interactions. The variable regions in the extracellular domain result in an exquisite binding specificity, such that isoforms that differ by only a few amino acids have large differences in their affinity⁵⁰. This high degree of diversity and specificity of the *DSCAM* isoforms enables developing

Asymmetrical division
A mitotic division that generates daughter cells that have different cell fates.

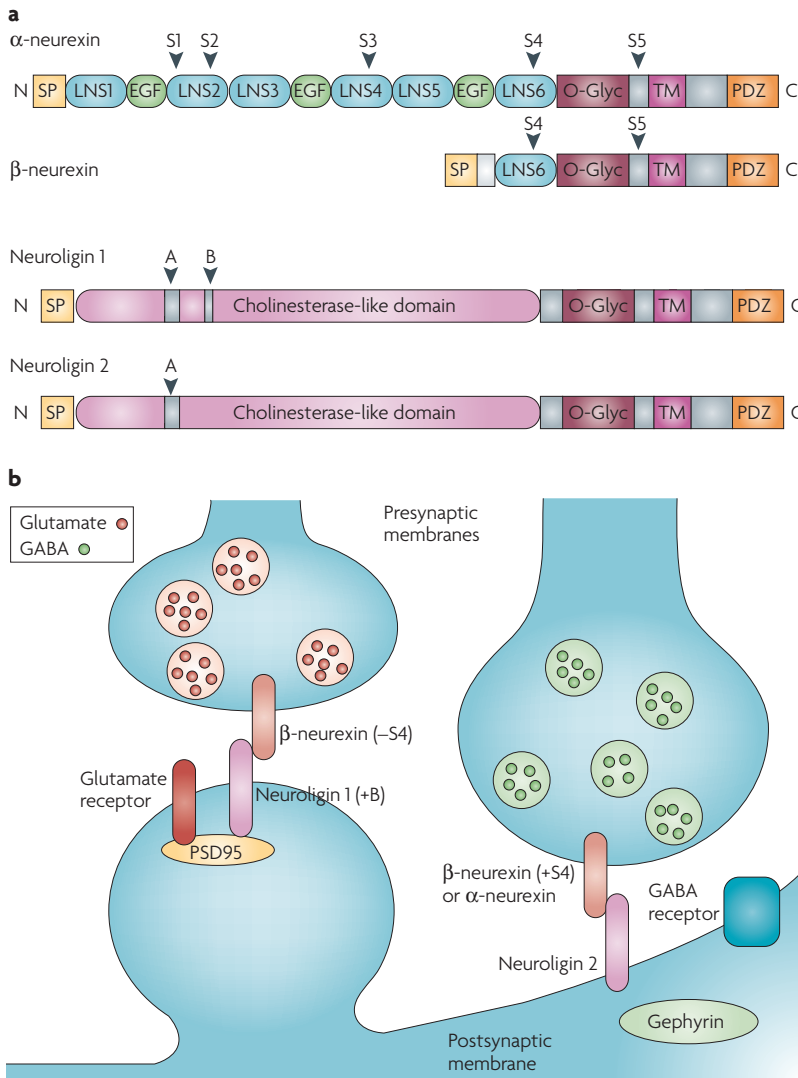


Figure 2 | Alternative splicing of neurexins and neuroligins determines the assembly of excitatory or inhibitory synapses. **a** | Neurexins and neuroligins contain complex extracellular domains that are modified at several positions by alternative splicing (indicated by the arrowheads). Alternative splicing at position 4 (S4) of β -neurexin and position B of neuroigin 1 alters the proteins' binding specificity for their neuroigin or neurexin partners and alters their ability to induce glutamatergic versus GABA (γ -aminobutyric acid)-mediated synaptogenesis. **b** | Each neurexin isoform will pair only with certain neuroigin isoforms, which leads to the formation of specialized synapses. Only β -neurexin lacking an alternative exon inserted at S4 (neurexin-1 β (-S4)) binds neuroigin 1 (REF. 190), and the neuroigin 1 splice variant must have an exon at position B (making it neuroigin 1 (+B)). Inclusion of the alternative exon at the S4 position of β -neurexin inhibits neuroigin-1-binding and reduces clustering of postsynaptic proteins that are specific to glutamatergic synapses. However, the β -neurexin (+S4) form maintains high affinity for neuroigin 2 and triggers clustering of neuroigin 2 and gephyrin at GABA-signalling synapses¹⁹¹. On the postsynaptic side, neuroigin 1 isoforms that carry the B insert (neuroigin 1 (+B)) interact with β -neurexin, but not with α -neurexin. In contrast, the -B isoforms bind both α - and β -neurexins. The alternative exon at position B inserts eight amino-acid residues that form an N-glycosylation site — this blocks α -neurexin binding and controls neuroigin localization⁶⁰. Neuroigin 1 (+B) is preferentially targeted to glutamatergic synapses. Most neuroigin 2, which does not have a corresponding B site, contains an insert at position A (neuroigin 2 (+A)) that leads to preferential localization at GABA-signalling terminals⁶¹. C, carboxyl terminus; N, amino terminus; PSD95, postsynaptic density protein 95; TM, transmembrane domain. Panel **a** modified, with permission, from REF. 192 © (2006) Birkhauser. Panel **b** modified, with permission, from REF. 193 © (2005) American Association for the Advancement of Science.

dendrites to distinguish between cells with different identities, and homophilic DSCAM interactions during dendritic branch outgrowth will repel adhesion between branches of the same cell^{51–53}. It appears that all neuronal structures in the fly express a large variety of DSCAM isoforms, but individual neurons express only a few dozen. This makes it unlikely that a neuron will encounter another cell with the same spectrum of expressed forms^{54,55}.

The mechanisms that generate this diversity of choice while maintaining the mutually exclusive pattern of splicing for each block of exons are particularly interesting. For the block of 48 copies of exon 6, exon selection seems to involve an RNA base-pairing interaction between a 'docking site' that lies downstream of exon 5 and a 'selector' element that lies upstream of the chosen exon in the block¹³. This interaction would loop out all of the exons between the selected exon 6 and the upstream exon 5, and provide a mechanism for the near-random choice of a single exon. However, the other blocks of mutually exclusive exons do not seem to use this mechanism⁵⁶. It is also unclear how exons are silenced to produce the inclusion of only a single exon in each block.

Synaptogenesis. During synapse formation, protein interactions across the synaptic cleft trigger the assembly of pre- and postsynaptic complexes. Two groups of important adhesion molecules in the trans-synaptic complex are the neurexins on the presynaptic membrane and the neuroligins on the postsynaptic membrane^{57,58} (FIG. 2). In mammals, there are three neurexin genes, each with two alternative promoters that generate a long α isoform and a truncated β isoform. Alternative splicing at 5 positions (S1–S5) in the neurexin ectodomains increases the number of potential isoforms to more than 1,000 (REF. 59). These positions of variability in the mRNA were originally called splice sites, however, we will refer to them as positions of alternative splicing, to avoid confusion with true splice sites, which define the ends of introns in pre-mRNAs. Neuroligins are also encoded by multiple genes (four in rodents and five in humans), and they contain two positions of alternative splicing (termed A and B) in their ectodomains⁵⁷. As described in FIG. 2, the regulated splicing of both neurexins and neuroligins is proposed to coordinate the appropriate excitatory or inhibitory specialization across the synaptic cleft: in glutamatergic synapses, neuroigin 1 (+B) binds β -neurexin (-S4) to trigger glutamatergic specialization; in GABA (γ -aminobutyric acid)-mediated synapses, neuroigin 2 (which does not have a potential B insert), and possibly neuroigin 1 (-B), bind both α -neurexin and β -neurexin (+S4) to promote specialization into a GABA-mediated synapse^{60,61}. The many other isoforms of these proteins are likely to direct assembly at other types of specialized synapses.

A number of other aspects of synaptogenesis are regulated by the complex alternative splicing of several interesting proteins, such as agrin, the protocadherins and components of the postsynaptic density.

SNARE

Soluble NSF (*N*-ethylmaleimide-sensitive fusion protein) accessory protein (SNAP) receptor.

Nociceptive neurons

(Also known as nociceptors.) Pain-sensitive cells that carry information about tissue damage from the body's periphery to synapses in the dorsal horn of the spinal cord.

Kindling

An experimental model of epilepsy in which an increased susceptibility to seizures arises after daily focal stimulation of specific brain areas (for example, the amygdala) — stimulation that does not by itself reach the seizure-causing threshold.

Splicing regulation in mature neurons

Some splicing events can be controlled dynamically in mature excitable cells. Proteins that are important for synaptic vesicle release, and postsynaptic density proteins, have alternatively spliced segments that can be regulated during synaptic remodelling or strengthening. Splicing switches are also common in ion channels, neurotransmitter receptors and proteins that are involved in calcium signalling for the control of membrane physiology. Many of these splicing events are themselves altered by cell excitation or by treatments that stimulate calcium signalling pathways.

Splicing regulation of presynaptic function. Proteins that are involved in neurotransmitter release are often affected by alternative splicing. The SNARE complex links proteins in the vesicle membrane (for example, *synaptobrevin*) with components at the plasma membrane (*SNAP25* and *syntaxin*) to control membrane fusion during calcium-triggered exocytosis^{62,63}. *SNAP25* consists of two SNARE domains and a linker that contains four palmitoylated cysteine residues. Alternative splicing of exon 5 of *SNAP25* generates two variants, *SNAP25a* and *SNAP25b*, which differ in their N-terminal SNARE domains and linker regions, and in their ability to stabilize vesicles that have been primed for exocytosis⁶⁴. In the mouse, expression of *SNAP25a* switches to expression of *SNAP25b* between postnatal weeks 1 and 3 (REF. 65). Disruption of this switch is lethal between weeks 3 and 5, coincident with the completion of synapse formation⁶⁶. These results point to the existence of interesting, but as-yet uncharacterized, splicing regulatory transitions during postnatal neuronal maturation.

Voltage-gated calcium channels have diverse pre- and postsynaptic roles in intracellular calcium homeostasis, regulating gene expression and coupling membrane potential changes to neurotransmitter release⁶⁷. The pore-forming $\alpha 1$ subunits of these channels are encoded by a large gene superfamily. Alternative splicing is seen in the L, N, P/Q and T-type subfamilies, with some alternative exons being conserved in the different members of a subfamily and some being present in multiple subfamilies⁶⁷.

The N-type voltage-gated calcium channels, which control neurotransmitter release at the synapse⁶⁸, contain a pair of mutually exclusive exons (37a and 37b) that modify the channel's intracellular domain. The 37a isoform is enriched in nociceptive neurons and is responsible for the characteristic large N-type currents that are seen in these cells^{68,69}. Moreover, the 37a insert mediates an unusual G-protein-dependent, voltage-independent inhibition in these cells⁷⁰. The analysis of this variant channel will be important in gaining an understanding of the action of GABA, opioids and drugs in modulating the pain pathway.

Similar examples of the role of splicing controlling membrane physiology are found for sodium-, potassium- and calcium-activated potassium channel mRNAs^{71,72}. These studies and others indicate the potential for more fruitful work that combines biophysical measurements with splice-variant analysis to understand the precise physiology of particular neurons.

Splicing changes that affect postsynaptic responses. Most major neurotransmitter receptors exhibit activities that are determined by their splicing pattern. Changes in the intra- or extracellular domains affect the receptor's trafficking and localization, gating properties, signal transduction and pharmacology^{73,74}.

Although alternative-splicing events clearly affect neuronal excitation, their role in characterized physiological models of plasticity, such as hippocampal long-term potentiation (LTP), has been difficult to assess. One example of a protein that contains an alternative exon that clearly affects LTP is apolipoprotein E receptor 2 (*APOER2*) (FIG. 3). *APOER2* functions as a receptor for *reelin*, and *reelin* signalling through *APOER2* can enhance LTP in hippocampal slices⁷⁵. The alternative exon 19 of *APOER2* encodes a 59-amino-acid proline-rich insert in *APOER2*'s intracellular domain; this insert regulates the signalling of the receptor and its interaction with postsynaptic density protein 95 (*PSD95*). In mice carrying *Apoer2* mutations, it was found that exon 19 was required for the *reelin*-mediated enhancement of LTP, and for *reelin*-stimulated tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor 2 subunit (*NMDAR2*)⁷⁶. Thus, the regulation of this exon modulates the ability of the mice to store memories. Later work demonstrated that the two isoforms of *APOER2* also promote different levels of neuronal survival in the adult brain⁷⁷. Intriguingly, exon 19 was more highly included during active periods of the sleep-wake cycle. It will be interesting to examine whether exon 19 splicing results from higher activity in *APOER2*-expressing neurons themselves, or from signalling by other cells.

The regulation of splicing by cell excitation. Many splicing events that alter neuronal activity are themselves dynamically regulated by external stimuli, such as chronic depolarization or other treatments that stimulate or mimic excitatory activity. Exons in calcium-ATPase, *SNAP25*, *NMDAR1*, the large-conductance, calcium- and voltage-gated potassium channels (BK channels) and other transcripts are repressed by shifting cells into high-potassium media^{78–80}. In many cases, inhibiting L-type calcium channels blocks this repression, indicating that a calcium signalling pathway is required, but other pathways can also be involved. The gene for *ania 6*, a transcription-associated cyclin L, produces two mRNAs that are differentially induced by glutamate, dopamine or potassium chloride stimulation of striatal cultures⁸¹, and kinase-inhibitor experiments indicate that a variety of downstream signalling pathways affect the production of these isoforms⁸². Kindling- or pilocarpine-induced seizures are associated with changes in multiple transcripts, including those for α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors and the splicing factor *TRA2 β* ^{83,84}. Similarly, the mRNA abundance and splice-variant distribution of the PSD protein *Homer 1* were altered after seizures that were induced by direct electrical stimulation⁸⁵. Changes are also observed in *syntaxin 1* splicing after perforant-path stimulation

Homeostatic plasticity

Activity-dependent changes in synaptic strength that tend to stabilize neuronal firing rates.

Heterogeneous nuclear ribonucleoprotein

(hnRNP). A protein that binds to nascent RNA polymerase II transcripts and packages pre-mRNAs into hnRNP-containing particles in the nucleus. hnRNPs have a variety of RNA-binding domains and function in various processes, including nuclear splicing, polyadenylation and export, as well as cytoplasmic mRNA translation and turnover.

SR proteins

A family of splicing regulatory proteins that bind to exonic splicing enhancer elements to stimulate exon inclusion. They contain one or more domains rich in serine (S)-arginine (R) dipeptides and one or more RNA-binding domains.

in the hippocampus, and are associated with enhanced glutamate release at mossy fibre synapses during LTP^{86,87}. In many of these systems, it is difficult to determine whether the changes in isoform ratios occur at the level of splicing, RNA stability or some other function.

The link between splicing and synaptic plasticity is particularly interesting for NMDAR1. Multiple splice variants of NMDAR1 are generated by the alternative exons 5 and 21 and alternative 3' splice sites in exon 22 (in mice). The choice of exon 22 splice site determines which of two carboxy (C)-terminal peptide cassettes, C2 or C2', is used. In cortical neuronal cultures, the level of the C2' isoform increased after neuronal activity was blocked with tetrodotoxin. Conversely, the levels of C2' decreased during bicucullin-stimulated neuronal activity⁸⁸. The switch to the C2' form was shown to accelerate receptor trafficking to the plasma membrane. It was proposed that this change in splicing could contribute to homeostatic plasticity⁸⁸⁻⁹⁰.

The roles of individual signalling pathways in regulating splicing. Changes in splicing occur on the timescale of mRNA turnover. This can make the effects of signalling pathways on the splicing apparatus difficult to distinguish from effects on transcription or mRNA stability⁹¹. A splicing effect can be confirmed by identifying the RNA-regulatory element that is involved and showing that the element can confer a splicing change on an unrelated transcript expressed from a heterologous minigene plasmid. This has been done for several

transcripts, including mRNAs that are important for neuronal excitation and calcium signalling⁹²⁻⁹⁴.

The STREX (stress axis) exon of the BK channel α subunit gene is regulated by several inputs⁹⁵⁻⁹⁷. Inclusion of the STREX exon increases the calcium and voltage sensitivity of the BK channel and alters its modulation by protein kinase A and its response to hypoxia^{72,95,98}. In primary cerebellar neurons and the GH3 pituitary cell line, the STREX exon is repressed after depolarization, through an L-type calcium channel- and calcium/calmodulin-dependent protein-kinase (CaMK)-mediated pathway^{94,99}. A CaMKIV-responsive RNA element (CaRRE) that could repress a heterologous exon upon expression of activated CaMKIV was identified in the 3' splice site of the STREX exon. A CaRRE was also found in the 3' splice site of exon 5 of NMDAR1 (which encodes the N1 peptide cassette): inclusion of this exon regulates agonist binding by the receptor^{94,99,100}.

A similar phenomenon has been studied for exon 21 of NMDAR1. The C1 peptide cassette, which is encoded by exon 21, alters the membrane trafficking of the receptor, and it has been implicated in receptor phosphorylation by protein kinases C and A, in interactions with various proteins and in activation of NMDAR-induced gene expression¹⁰¹⁻¹⁰⁶. Splicing of exon 21 is repressed by potassium-chloride-induced depolarization in differentiated P19 embryonal carcinoma cells and in primary cortical cultures^{107,108} (FIG. 4). Three potassium-chloride-responsive RNA elements were identified in the C1 cassette itself, two of which also respond to CaMKIV^{107,108}. The third depolarization-responsive RNA element was shown to be a heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) binding site¹⁰⁷ (FIG. 4). hnRNP A1 is a known splicing repressor that is affected by multiple signalling pathways^{92,109,110}. The binding of hnRNP A1 to a C1 cassette RNA probe was shown to increase in nuclear extracts from depolarized cortical cultures, but it is unclear how this change in hnRNP A1 activity is brought about¹⁰⁷. hnRNP A1 binding sites are widespread, and changes in hnRNP A1's activity will probably alter a variety of exons¹¹¹.

Consensus sequences for the CaRREs that were derived by mutagenesis were used to identify other depolarization-responsive exons. Depolarization and CaMKIV-responsive exons appear to be common in proteins that are involved in calcium signalling and homeostasis, such as the plasma membrane calcium pumps¹¹². It will be particularly interesting to learn how the splicing of a group of functionally related exons can be co-regulated by common RNA elements in response to a stimulus, and whether the phosphorylation of a protein that regulates splicing can serve as a reversible modulator of its activity.

Neuronal splicing regulators

Changes in splicing pattern are directed by regulatory proteins that bind the pre-mRNA and enhance or silence particular splicing choices^{9,14}. The best characterized positive factors are the serine-arginine-rich proteins (SR proteins) and their relatives, which bind to exonic

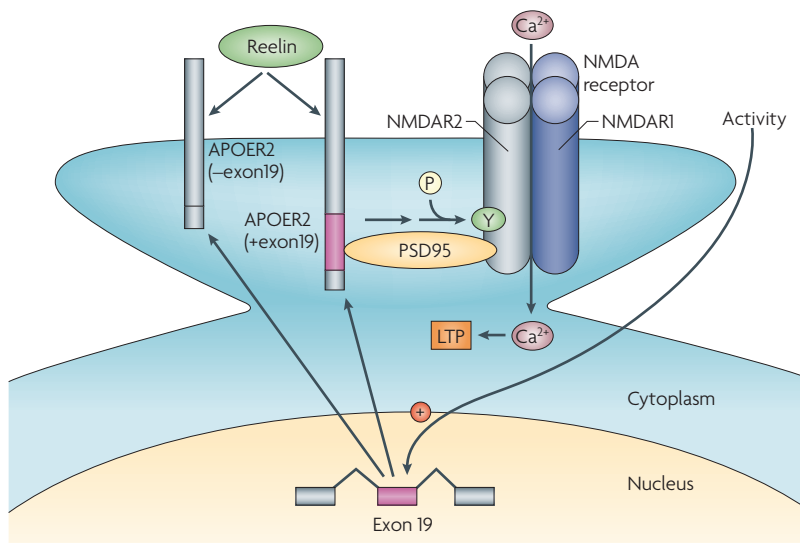


Figure 3 | Splicing of APOER2 exon 19 regulates reelin-induced enhancement of LTP. The exon-19-containing isoform of apolipoprotein E receptor 2 (APOER2), when bound by reelin protein, stimulates phosphorylation of the N-methyl-D-aspartate receptor 2 subunit (NMDAR2) (on a tyrosine residue) and enhances long-term potentiation (LTP) in the hippocampus. Exon 19 is needed for APOER2 to interact with postsynaptic density protein 95 (PSD95), and this is thought, through unknown mechanisms, to allow subsequent phosphorylation of NMDAR2. APOER2 minus the exon 19 insert binds reelin and NMDAR2, but not PSD95, and it does not mediate reelin-stimulated phosphorylation of NMDAR2. Exon 19 splicing is induced during periods of higher synaptic activity. Figure modified, with permission, from REF. 76 © (2005) Cell Press.

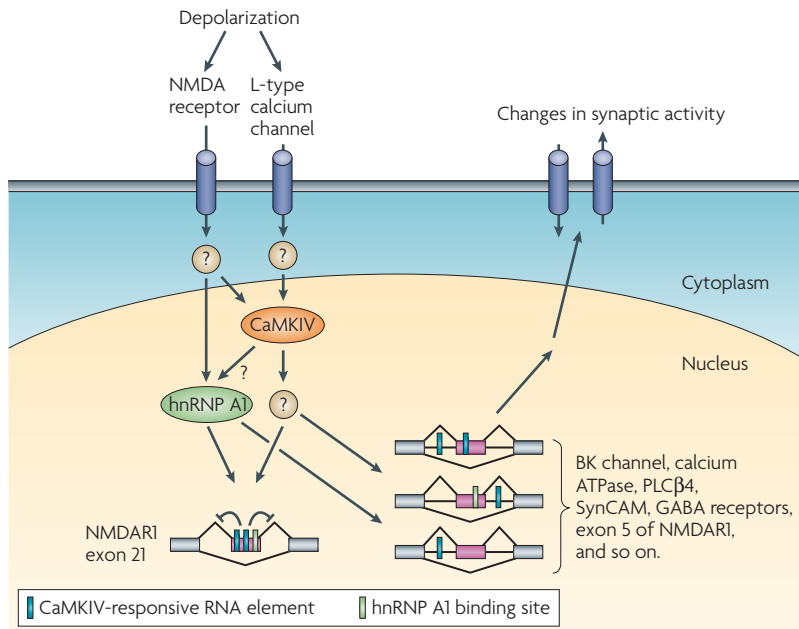


Figure 4 | The alternative splicing of ion channel transcripts is dynamically regulated by calcium signalling pathways. The inclusion of many ion channel exons, including exon 21 of *N*-methyl-D-aspartate receptor 1 (NMDAR1), in mRNA transcripts is repressed by membrane depolarization. This splicing repression is mediated by CaMKIV-responsive RNA elements (CaRREs), the binding factor of which is not known, and by binding elements for the heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1). Exon repression by hnRNP A1 is blocked by NMDA-receptor antagonists, whereas repression by the CaRREs is blocked by inhibitors of L-type calcium channels. Both types of induced exon skipping are blocked by KN93 (not shown), an inhibitor of multiple calcium/calmodulin-dependent protein kinases (CaMKs). Activated CaMKIV is known to repress splicing through CaRREs, but other CaMKs could also be involved in both pathways of repression, and the pathway from the stimulus at the plasma membrane to the nuclear CaMKIV or other component is not known. It is also not yet clear whether CaMKIV directly phosphorylates the splicing regulators or whether the regulators are affected indirectly. As shown on the right, multiple other exons contain these RNA elements and are also modulated by cell depolarization and/or CaMKIV. These include exons in the large-conductance, calcium- and voltage-gated potassium channels (BK channels), the plasma membrane calcium ATPase pumps, synaptic cell adhesion molecule (SynCAM) and other transcripts that affect membrane physiology and calcium pools. Alternative splicing of these transcripts ultimately alters synaptic activity. GABA, γ -aminobutyric acid; PLC β 4, phospholipase C- β 4.

splicing enhancer (ESE) elements and stimulate exon inclusion^{113–115}. Negative factors include members of the hnRNP group of proteins, for example, hnRNP A1 and hnRNP I (also known as polypyrimidine tract-binding protein, *PTB*). Some proteins either enhance exon inclusion or repress it, depending on the position of their binding sites relative to the target exon^{116–119}. Developmentally regulated splicing patterns are, at least in part, maintained by these RNA-binding proteins, although the proteins that mediate changes in splicing in response to neuronal activity are mostly unidentified (TABLE 1). Some splicing factors are expressed solely in neurons, others are specific to neurons in the brain but can also be found in other tissues, and still others determine neuronal splicing patterns by their absence from neurons. Although exons are regulated by multiple factors, certain proteins clearly control groups of target transcripts in a coordinated manner^{120,121}.

Individual factors bind short elements but affect large exon sets. *PTB* is a widely expressed splicing regulator that carries four RNA-binding domains of the RRM (RNA recognition motif) type and often binds multiple polypyrimidine elements that flank regulated exons or 3' splice sites^{122,123}. Splicing patterns that are specific to muscle and neurons are commonly regulated by *PTB*. During the differentiation of these cell types, *PTB* expression is lost, allowing the inclusion of exons that are normally repressed in myoblasts, neural progenitors and other cells^{120,124,125}.

PTB has a close homologue called neural *PTB* (*nPTB*; also known as *brPTB* or *PTBP2*), with which it is replaced in postmitotic neurons^{120,125–128}. Exons that are known to be affected by *PTB* and/or *nPTB* in neurons are found in the GABA_A receptor, the glycine receptor, NMDAR1, Ewing's sarcoma oncogene, *SRC* and myocyte enhancer factor 2 genes, as well as many other genes. Some exons are repressed by *PTB* and not *nPTB*, whereas others seem to be equally affected by the two proteins. During neuronal differentiation, *PTB* is downregulated and *nPTB* expression is induced. This switch in expression from *PTB* to *nPTB* reprogrammes the splicing of many different transcripts that are important for neuronal function and survival¹²⁰.

In the adult, *PTB* proteins are also found in tissues outside the brain. Other splicing regulators are expressed only in neurons, with some showing broad distribution in the brain and others having more restricted expression in neuronal subtypes. The best studied examples are the neuro-oncological ventral antigen (*Nova*) proteins¹²⁹. *NOVA1* and *NOVA2* were first identified as autoantigens in patients with paraneoplastic opsoclonus-myoclonus-ataxia (*POMA*)^{129,130}. *NOVA1* is most highly expressed in motor neurons, whereas *NOVA2* is found in neurons of the cortex, hippocampus and dorsal spinal cord^{130,131}.

Studies of the *Nova* proteins have provided important information on both their mechanisms of action and their biological roles. They recognize clusters of YCAAY elements, which are usually found in introns adjacent to regulated exons but are sometimes adjacent to the flanking exons¹¹⁶. The exons that are targeted by *Nova* proteins are especially common in genes that are involved in synaptic function. Targets are both pre- and postsynaptic and affect many aspects of synaptogenesis, synaptic vesicle release and neurotransmitter signalling, as well as the function of the postsynaptic density¹²¹. *Nova2*-mutant mice show defects in the LTP of slow inhibitory postsynaptic currents (sIPSCs) in the hippocampal CA1 region¹³² (FIG. 5). Proteins that are involved in sIPSCs include NMDAR1, CaMKII, GABA_B receptors and G-protein-activated inwardly-rectifying potassium channels (*GIRKs*), all of which are potential *NOVA2* targets^{121,132}. Interestingly, LTP of excitatory postsynaptic currents in these mutant mice was not affected, suggesting that *NOVA2* may be specifically involved in inhibitory functions. It will be interesting to examine the role of individual *Nova*-dependent splice variants in this kind of defined physiological assay.

Table 1 | **Proteins that are known to regulate splicing in the nervous system**

Neuronal splicing regulators	Homologues	Binding sites	Tissue specificity	Known targets	Refs
PTB	NA	CUCUCU, UCUUC	Widespread	Many, including α -tropomyosin, α -actinin, GABA $_{\gamma}$ 2, clathrin LCB, FGFR1 and 2, NMDAR1, SRC and tau	119,120, 123
nPTB	NA	CUCUCU	Neurons, myoblasts and testis	Many, including Bin1, GlyR α 2, PMCA1, Mef2, Nasp, Spag9 and SRC	123,126
NOVA1	NA	YCA $_{\gamma}$ *	Neurons of the hindbrain and spinal cord	Many, including GABA $_{\gamma}$ 2, GlyR α 2 and NOVA1	130
NOVA2	NA	YCA $_{\gamma}$	Neurons of the cortex, hippocampus and dorsal spinal cord	Many, including APLP2, gephyrin, JNK2, neogenin, NMDAR1 and PLC β 4	130
HuB	HuC, HuD	AU-rich elements (ARE)	Neurons	Calcitonin/CGRP	137
NAPOR	CUGBP1, CELF5	UG-rich sequences	Heart, skeletal muscle and brain	Many, including α -actinin, cTNT and NMDAR1	140,141, 144
FOX1	FOX3	(U)GCAUG	Muscle, heart and neurons	α -actinin, EWS, FGFR2, fibronectin and SRC	118,152, 154,156
FOX2	FOX3	(U)GCAUG	Muscle, heart, neurons and embryo	EWS, FGFR2, fibronectin, non-muscle myosin LC and SRC	118,152, 154,156
hnRNP H	hnRNP F, hnRNP H'	GGGA	Widespread	Many, including NMDAR1 and SRC	110,168
hnRNP A1	hnRNP A2/B1	UAGGGA/U	Widespread	Many, including NMDAR1, SMN2 and SRC	106,110, 171
Tra2 β	NA	GAA repeats, GHVVGANR*	Widespread	Clathrin LCB, SMN2, tau and Tra2 β	113,184, 187

*Y indicates C or U; R indicates A or G; V indicates A, C or G; H indicates A, C or U. APLP2, amyloid beta (A4) precursor-like protein 2; Bin1, bridging integrator 1; CELF5, CUGBP- and ETR3-like factor 5; cTNT, cardiac troponin T2; CUGBP1, CUG triplet repeat, RNA-binding protein 1; EWS, Ewing's sarcoma oncogene; FGFR, fibroblast growth factor receptor; GABA $_{\gamma}$ 2, γ -aminobutyric acid receptor $_{\gamma}$ 2 subunit; GlyR α 2, glycine receptor (α 2 subunit); hnRNP, heterogeneous nuclear ribonucleoprotein; JNK2, jun kinase 2; LC/LCB, light chain; Mef2, myocyte enhancer factor 2; NA, not applicable; NAPOR, neuroblastoma apoptosis-related RNA-binding protein; Nasp, nuclear autoantigenic sperm protein; NMDAR1, N-methyl-D-aspartate receptor 1; NOVA, neuro-oncological ventral antigen; nPTB, neural polypyrimidine tract-binding protein; PLC β 4, phospholipase C- β 4; PMCA1, plasma membrane calcium ATPase 1; PTB, polypyrimidine tract-binding protein; SMN2, survival of motor neuron 2, centromeric; Spag9, sperm-associated antigen 9.

Another set of highly neuron-specific proteins is the Hu family of triple RRM proteins, which are homologues of the *Drosophila* embryonic lethal, abnormal vision (ELAV) protein^{133–135}. The mammalian homologues include a non-tissue-specific family member, HuR, and the neuronally expressed members HuB, HuC and HuD. The mammalian proteins are mostly known for their roles in blocking targeted mRNA degradation, which they do by binding to AU-rich RNA elements (AREs)^{136,137}. However, the *Drosophila* proteins affect the splicing and polyadenylation of particular target transcripts, and it was recently shown that the mammalian Hu proteins affect the neuron-specific splicing and polyadenylation of the calcitonin/CGRP transcript^{134,138}. Like many splicing regulatory proteins, the different Hu family members shuttle between the nucleus and the cytoplasm and affect mRNA metabolism in both locations¹³⁹. There are numerous studies that implicate these proteins in neuronal differentiation^{133,140}.

Another subgroup of triple-RRM domain proteins are the CUG-binding protein (CUGBP) and ETR3-like factors (CELFs; also known as Bruno-like factors)¹⁴¹. CUGBP1 was found to bind to transcripts that contain the CUG repeat mutations that cause myotonic dystrophy, but it has higher affinity for GU repeats^{142,143}. Nevertheless, several exons that have altered splicing in

myotonic dystrophy seem to be regulated by this protein¹⁴⁴. Neuron-specific members of this family include Bruno-like 4, Bruno-like 5 (also known as CELF4 and CELF5, respectively) and NAPOR, a neuronal splice variant of CUGBP2. Exon 21 of NMDAR1 is positively regulated by NAPOR¹⁴⁵. These proteins have also been shown to antagonize splicing repression by PTB^{146,147}. Interestingly, in *Caenorhabditis elegans*, a homologue of the CUGBPs called UNC75, as well as a protein that is related to the Hu/ELAV family, EXC-7, both affect synaptic transmission in the adult worm¹⁴⁸. The mechanisms of CUGBP splicing regulation are not yet understood.

The mammalian Fox proteins are homologues of the *C. elegans* Feminizing gene on X gene product, FOX-1 (REF. 149). The worm genome also has another Fox homologue, ASD-1, and both FOX-1 and ASD-1 have a single RRM and conserved N- and C-terminal domains. A genetic screen for regulators of FGFR splicing identified ASD-1 and FOX-1 as controlling an FGFR splicing choice in worm muscle¹⁵⁰. Interestingly, mammalian FOX2 is a regulator of FGFR2 splicing, pointing to a long evolutionary history of these proteins³⁶. The vertebrate Fox proteins are specifically expressed in adult muscle and brain, and were shown to recognize the RNA element UGCAUG^{119,151,152}. The Fox proteins bind to this element to

RNA editing

Site-specific modification of mRNA sequences after transcription. The most common modification is the conversion of adenosine to inosine by adenosine deaminase enzymes (ADARs) — a conversion that changes the coding capacity of the affected mRNA.

activate or repress particular exons^{119,153–155}. A set of brain-specific exons that carry this element in their downstream intron are enhanced by the Fox proteins^{18,156,157}.

All three mammalian genes (encoding **FOX1** (*A2BP1*), **FOX2** (*RBM9*) and **FOX3** (*HRNBP3*)) are specifically expressed in neurons of the adult brain — in broadly overlapping, but not identical, sets of cells (see the [Allen Brain Atlas](#)). These genes have large and complex transcription units, with multiple promoters that encode variant N termini, alternative polyadenylation sites that encode alternative C termini, and multiple sites of alternative splicing, such that different muscle and brain isoforms are produced^{153,155}. Human FOX1 was first identified as ataxin 2-binding protein 1 (*A2BP1*), a two-hybrid interactor with *ataxin 2* (REF. 158). More recently, FOX2 (also known as RNA-binding motif protein 9 (*RBM9*)) was shown to interact with *ataxin 1* (REF. 159). Thus, these two ataxins, which are mutated in different forms of spinal cerebellar ataxia, both might alter neuronal splicing through their interactions with FOX1 and FOX2. The FOX1 gene is itself mutated in a rare inherited epilepsy and mental retardation disorder, and in some patients with autism^{108,160–162}. The characterization of this protein will be important for understanding these neurological diseases.

Combinations of factors control individual exons. Nearly all alternative-splicing events show combinatorial regulation, where the choice of splicing pattern is determined by multiple pre-mRNA-binding proteins^{9,14}. For example, exon 21 of NMDAR1 is dynamically regulated by hnRNP A1 and several unidentified factors that bind to the CaRREs, but it is also affected by PTB, NOVA1 and 2, NAPOR, hnRNP H, and probably other proteins^{107,108,111,121,145,163}.

The multiple proteins that act on the N1 exon of the SRC tyrosine kinase have been examined in detail. N1 is specifically included in neurons, where SRC modulates signalling from NMDARs and the L1-cell adhesion molecule (*L1CAM*)^{164,165}. The SRC mRNA is broadly expressed, but the N1 exon is repressed by PTB. During neuronal differentiation, the switch from PTB to nPTB expression allows the induction of N1 splicing^{120,127,166} (FIG. 6). In addition to the PTB/nPTB-binding sites that surround the N1 exon, there is a strong UGCAUG enhancer in the downstream intron that binds the Fox proteins to stimulate N1 exon splicing^{153,156,167}. There are multiple additional elements that affect N1 splicing, including binding sites for hnRNP A1 and the SR protein alternate splicing factor, splicing factor 2 (*ASF/SF2*) in the exon, and for hnRNP H, CUGBP and KH-type splicing regulatory protein (*KSRP*) in the downstream intron^{168–171}. These proteins also affect the splicing of the exon, but not as strongly as the PTB and Fox proteins. This example typifies the complexity of regulation that is seen for even a relatively simple alternative-splicing event.

In addition to regulatory proteins, special RNAs also seem to bind to pre-mRNAs and alter splicing choices by counteracting the effects of protein regulators¹⁷². The 5-hydroxytryptamine (5-HT)_{2c} receptor contains alternative 5' splice sites in exon V. Splicing at the Vb site produces the full-length, functional receptor, but use of the Va site produces an mRNA that encodes a truncated, inactive protein. A regulatory element between the two sites, which is bound by an unknown protein, silences the Vb site to induce Va splicing. RNA editing can inactivate this silencer element to allow Vb splicing. However, in the unedited RNA, the silencer can also be bound by a special complementary RNA, HBII-52, which is thought to block the silencer function, again leading to proper Vb splicing and 5-HT_{2c} receptor production. This HBII-52 RNA has several interesting features. It is similar in structure to the Box C/D family of small nucleolar RNAs, and is transcribed from an imprinted locus that is mutated in [Prader-Willi syndrome](#). The locus is most highly expressed in the brain, but only from the paternal allele. It will be interesting to examine what other splicing events HBII-52 might control, and whether other similar RNAs also function in splicing regulation.

Future directions

There are now many interesting examples of biological regulation at the level of splicing and we have described only a few here. Regulatory proteins have been implicated in some of these splicing events and, for a few,

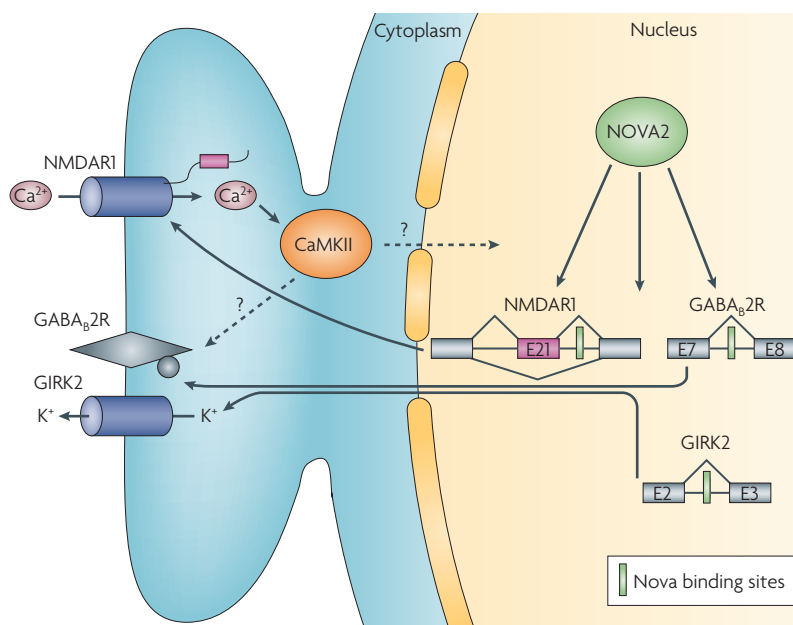


Figure 5 | LTP of sIPSCs requires the splicing regulator NOVA2. Slow inhibitory postsynaptic currents (sIPSCs) at synapses in the hippocampal CA1 region are mediated by the metabotropic GABA (γ -aminobutyric acid)_B receptor (GABA_B2R) and G-protein-activated inwardly-rectifying potassium (GIRK) channels that contain GIRK1 and GIRK2 subunits. sIPSCs undergo long-term potentiation (LTP) when stimulated by *N*-methyl-D-aspartate receptor 1 (NMDAR1) and calcium/calmodulin-dependent protein kinase II (CaMKII). NMDA stimulation is known to activate CaMKII, but how this modulates the GABA_B2R receptor and GIRK channels is unclear. LTP of sIPSCs is not seen in mice that lack NOVA2, indicating that some NOVA2-dependent gene product is required. Exon 21 of NMDAR1 is adjacent to a Nova binding site, and its splicing is reduced in mice that lack NOVA2. Both the GABA_B2R and GIRK2 transcripts also contain NOVA2 binding sites, but the functional role of NOVA2 binding at these sites is not yet known. NOVA2 might also affect splicing of these mRNAs, or might alter other aspects of GABA_B2R and GIRK2 mRNA metabolism. Figure modified, with permission, from REF. 132 © (2005) Cell Press.

there are models for their mechanism of action. The identification of new splicing regulators and their recognition sequences, combined with new tools of genomic analysis, is identifying groups of target exons for individual regulatory proteins. Nevertheless, many important questions regarding both the mechanism and biology of neuronal splicing regulation remain unanswered.

We still know little about how regulatory proteins actually affect spliceosome assembly. What contacts do the regulators make with the spliceosomal components? How do they alter the structure of the pre-mRNP? How do they cooperate with or antagonize other factors? What is the role of transcription in this function? To

understand these mechanisms, biochemical assays must be developed for each regulator and target, and this will take some time^{116,173,174}. This mechanistic knowledge will be essential for understanding the complex interplay that takes place between the positive and negative regulatory factors and the general splicing apparatus, and for developing therapeutic approaches to modifying splicing choices⁶⁻⁸.

Although the cellular function of particular spliced isoforms is increasingly well understood, the biological roles of the splicing regulators are still only vaguely defined. Alternative exon microarrays are allowing the identification of large target-exon sets for individual proteins^{18,120,121,175-178}. The analysis of the ensemble of exons that is controlled by each protein will help to implicate them in particular developmental processes. However, the combinatorial nature of the regulation complicates this analysis, as there is significant overlap between target-exon sets for individual factors. Genetic approaches, such as the analysis of splicing factor mutations in mice, will help to identify the key factors that affect a given exon, but these approaches must deal with the many paralogous genes for each type of factor^{131,179}. Studies in simpler genetic systems such as flies and worms might be faster routes to identifying the fundamental targets of a splicing regulator and the developmental events that it controls^{148,150}.

Many splicing regulators apparently control their own synthesis¹⁸⁰⁻¹⁸⁶. This limits the expression of each protein and provides a mechanism for cross-regulation between factors¹²⁰. Networks of post-transcriptional regulation by RNA-binding proteins are likely to contribute to developmental transitions both early in neuronal development and later in the postnatal period¹²⁰. It will be interesting to examine whether stable feedback loops of post-transcriptional regulation can maintain a splicing state over long periods, as seen in the *Drosophila* sex determination pathway⁹. The potential role of these autoregulatory processes in cellular homeostasis is particularly interesting.

The factors that bind to exons that are regulated by cell excitation and calcium signalling are, for the most part, unknown. However, interesting candidates are starting to emerge^{187,188}. In addition to exploring the pathways that lead from the plasma membrane to specific RNA-binding proteins and exons in the nucleus, a better understanding of the physiology of each splicing change must be developed. How do changes in ion-channel splicing contribute to homeostatic plasticity or LTP? To answer these questions, more work is needed at the single-cell level. A number of studies have combined physiology and single-cell PCR to correlate the presence of a particular gene product with particular physiological properties. This could be extended to examine the changes in splicing that occur in response to different excitatory or inhibitory inputs. What is needed is an exon that has a known effect on channel properties and that responds to a particular stimulus with consequent changes in cell physiology. These kinds of analyses will allow us to understand the role of splicing in the dynamic regulation of cell physiology.

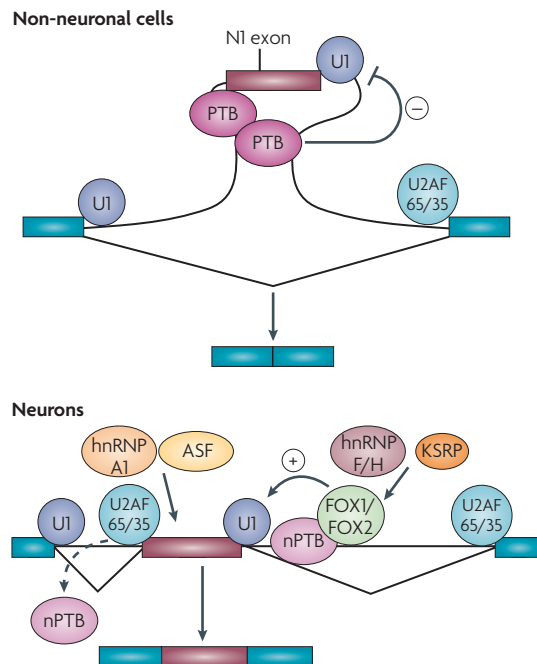


Figure 6 | Alternative exons are controlled by multiple regulators. This figure illustrates the regulation of the splicing of the N1 exon of the SRC gene. N1 is repressed in non-neuronal cells by polypyrimidine tract-binding protein (PTB), which binds to elements in the N1 3' splice site and in the downstream intron. This binding blocks the assembly of a pre-spliceosomal E complex between the N1 exon's 5' splice site and the downstream exon's 3' splice site. In neurons, PTB is replaced by neural PTB (nPTB), which binds to the PTB repressor elements but does not prevent splicing. Neurons also express splicing activators that are members of the Fox family; these bind to enhancer elements downstream of the exon to stimulate its splicing. nPTB must be displaced from the N1 3' splice site (shown as a dashed arrow) to allow its splicing, and it may or may not be displaced from its downstream binding site by the adjacent Fox protein. Other RNA-binding proteins that affect N1 exon splicing include alternate splicing factor (ASF; also known as splicing factor 2 (SF2)), which binds to the exon, and heterogeneous nuclear ribonucleoprotein H (hnRNP H), hnRNP F and KH-type splicing regulatory protein (KSRP), which bind to the downstream intron. These proteins might modulate the function of the key regulators, or might allow regulation in additional cell types.

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>

Ania-6 | APOER2 | ASD-1 | ASF/SF2 | ataxin 1 | ataxin 2 | Bruno-like 4 | Bruno-like 5 | calcitonin | CaMKIV | CUGBP1 | CUGBP2 | DSCAM | ELAV | EXC-2 | FGF8 | EGR2 | FOX-1 | FOX1 | FOX2 | FOX3 | hnRNP A1 | HuB | HuC | HuD | HuR | LICAM | mnumb | NOVA1 | NOVA2 | NMDAR1 | nPTB | numbl | PSD95 | PTB | reelin | SNAP25 | synaptobrevin | TRA2B | U2AF | UNC75
OMIM: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>
 myotonic dystrophy | Prader-Willi syndrome

FURTHER INFORMATION

Douglas L. Black's homepage:
<http://www.mimq.ucla.edu/faculty/black/>
 Allen Brain Atlas:
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