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Alternative Splicing in the Nervous System

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Overview

Soon after the discovery of split genes in 1977, evidence began to accumulate to support the hypothesis that multiple mRNAs encoding different protein products could be produced from a single precursor mRNA (pre-mRNA). It was not until the completion of sequencing the human genome, however, that the extent of alternative splicing became apparent. The results from this major undertaking indicated that the number of protein-coding genes is far fewer than expected based on phenotypic diversity. Studies over the past 30 years have revealed the complex multidimensional networks of regulation in mammalian gene expression. Regulation can occur at the level of chromatin structure, transcription, pre-mRNA splicing/alternative splicing, and alternative polyadenylation, depending on RNA stability, RNA transport, and RNA editing and posttranslational modifications. The following discussions focus on the role of alternative splicing in the nervous system and splicing mutations associated with neurodegenerative diseases.

Pre-mRNA Splicing and Alternative Splicing

Pre-mRNA splicing, the process of removing introns from the nascent transcript and splicing together of exons to produce the functional messenger RNA (mRNA), occurs in a spliceosome, a macromolecular RNA–protein machinery. In addition to the pre-mRNA and small nuclear ribonucleoprotein particles (snRNPs), the spliceosome contains over 200 protein factors. Essential to this process is the accurate recognition of the 5' splice site (5'ss) and 3' splice site (3'ss) by the spliceosome. In the assembled catalytically active spliceosome, the biochemical reactions of cleavages at splice sites and ligation of exons take place by a two-step transterification mechanism.

In higher eukaryotes, multiple splicing isoforms can be produced from a single pre-mRNA by alternative usage of different splice sites. This process of alternative splicing can occur in a variety of patterns (Figure 1), including exon skipping/inclusion (cassette alternative exon), intron retention, alternative use of 5' or 3' splice sites, mutually exclusive exons, and more complex patterns that are coupled with alternative promoter selection or alternative polyadenylation. Alternative splicing may produce mRNA species with different stability or subcellular localization and/or transcripts that encode distinct protein products. As a result, alternative splicing is an extremely powerful and versatile mechanism for generating functional diversity from a limited number of genes.

In the nervous system, the process of alternative splicing is particularly remarkable because the brain, of all tissues, has the highest frequency of alternative splicing, and it is becoming evident that alternative splicing has a significant impact on the development, function, and maintenance/repair of the nervous system. In addition, the largest group of genes that display tissue-specific splicing is expressed in the brain.

Molecular Mechanisms Regulating Alternative Splicing

In higher eukaryotes, especially mammals, pre-mRNA transcripts are usually long, containing multiple introns of variable sizes. Mammalian introns can be as large as 500 kb. In humans, the average size of exons is 150 nucleotides, and that of introns is 3500 nucleotides. The basic splicing signals in mammalian pre-mRNAs are degenerate, with only two nucleotides (5'AG at the 5'ss and 3'AUC at 3'ss) that are highly conserved. As a result, the nucleotide sequences surrounding the splice junctions usually contain only a limited amount of information, not sufficient for conferring the specificity required to achieve accurate splice site selection. Therefore, recognition not only of exon–intron junction sequences but also of the regulatory elements in intronic and exonic regions is important for defining splice junctions and maintaining splicing fidelity. This high degree of degeneracy in the splicing signals in mammalian pre-mRNAs provides the flexibility for alternative selection and pairing of different splice sites, a fundamental mechanism for regulating alternative splicing.

The intricate interactions between cis-elements in the pre-mRNA substrates and their trans-acting splicing factors determine the selective use of different splice sites. This network of interactions between pre-mRNA and trans-acting factors involves both snRNPs and non-snRNP splicing regulators. The cis-elements include splice sites and splicing regulatory sequences (splicing enhancers or silencers) inside either exons or introns. The sequences and sizes of the exons or introns, as well as secondary structures of the pre-mRNA, also influence splice site selection.
Alternative Splicing in the Nervous System

The high level of degeneracy of mammalian splicing signals and the existence of large numbers of trans-acting factors allow versatile RNA-protein and RNA–RNA interactions during different stages of spliceosome assembly. The production and delicate balance of distinct isoforms generated by the alternative splicing of pre-mRNA transcripts are determined by combinatorial effects of multisite interactions among pre-mRNA, essential spliceosomal components, and regulatory factors.

A number of splicing regulators, either positive or negative, modulate alternative splicing. These splicing regulators contain several types of protein sequence motifs, including an RNA recognition motif (RRM) and other nucleic acid binding motifs (K-homology, or zinc finger) as well as a serine/arginine (SR)-rich domain or glycine-rich or RGG-rich sequences. Many SR domain-containing factors interact with exonic splicing enhancer elements to stimulate exon inclusion, whereas proteins of the heterogeneous nuclear ribonucleoprotein (hnRNP) family containing RRM are often act as splicing repressors by interacting with intronic splicing silencer sequences. A number of bifunctional splicing factors have been identified that can act either as activators or repressors for splicing, depending on their interactions with pre-mRNAs and other proteins. The concentration, distribution, composition, and state of modification of these regulatory factors determine whether they enhance or suppress the use of a particular splice site.

The cis-acting elements that regulate splicing are enhancer or silencer sequences present in either the intronic or exonic regions. Both types of elements may regulate splicing of a single pre-mRNA. Splicing enhancer and silencer elements may also overlap with each other. The splicing enhancer sequences control well-characterized genes, including those encoding c-src, caspase-2, and the γ-aminobutyric acid (GABA)A receptor γ2 subunit, contain pyrimidine (UC)-rich elements that interact with the polypyrimidine tract binding (PTB) protein (also referred to as hnRNP I). In these examples, PTB protein binds the repressor sequences in nonneuronal cells so that splice sites are skipped. The PTB gene also undergoes alternative splicing to generate three isoforms. In addition, a neuronal or brain-enriched PTB homolog (nPTB or brPTB) has been identified, and plays a role in relieving the suppression of inclusion of a neuron-specific N1 exon in c-src in neuronal cells. Splicing regulatory elements usually contain multiple binding sites for splicing regulators and function by recruiting other spliceosomal components to form RNP-like complexes. One of the common features of these cis-regulatory elements is that they are not simple sequence elements that act independently of each other. Splicing regulatory elements have been identified that can act to stimulate the splicing of one exon but repress another exon.

Neuron-Specific Splicing Regulators

A large number of splicing regulators are expressed in a wide range of tissues or cell types. Several RNA-binding proteins have been reported that are enriched in neurons or have neuron-specific isoforms. These neuronal splicing regulators include the Nova family (Nova-1 and Nova-2), Hu/ELAV family (Hud, Hel-N), Fox family (Fox-1 and Fox-2), STAR/GSG family, and CELF family (NAPOR/CUGBP2/ETR-3). Some of these factors were originally identified because of their association with neurological disorders.

Only a few of these neuron-specific splicing factors have been well studied. These include the K-homology (KH) domain RNA-binding proteins Nova-1 and Nova-2. Nova proteins were identified by their association with paraneoplastic opsoclonus myoclonus ataxia (POMA). In this syndrome, patients develop antibodies against Nova proteins, and this autoimmune
response leads to a loss of inhibition of motor control in the spinal cord and brain stem. Nova-1 is expressed in the diencephalon, brain stem, and motor neurons of the spinal cord, whereas Nova-2 is present in the cerebral cortex, hippocampus, and dorsal spinal cord. Nova-1 binds to UCAU Y elements, and Nova-2 interacts with GAGUCAU elements in their target RNAs (Y represents a pyrimidine). Nova-1 regulates the splicing of GABA$_A$ receptor $\gamma_2$ subunit and the $\alpha_2$ subunit of the glycine receptor (GlyR $\alpha_2$), and also autoregulates its own splicing. Nova has also been reported to co-regulate the splicing of several transcripts important for synaptic function, perhaps accounting for 7% of the brain-specific alternative splicing in the neocortex.

The Hu family is another group of splicing regulators that are autoimmune targets in POMA. Hu proteins bind to mRNAs, including many that are important for neuronal function, thus stabilizing the transcripts and increasing their translation. One member of the family of proteins, HuD, is a homolog of the fruit fly splicing regulator ELAV protein, which is essential for neurogenesis and plays a role in regulating splicing in neurons. Recently, Hu was reported to regulate splicing of calcitonin/calcitonin gene-related peptide (CGRP) pre-mRNA and facilitate exon 4 skipping by inhibiting the binding of TIA-1/TIAR, two ubiquitously expressed factors that promote the nonneuronal pathway.

The neuron-specific splicing of calcitonin/CGRP is also regulated by several other splicing factors, including Fox-1 and Fox-2. These proteins are expressed in muscle, heart, and brain tissues. In the brain, the Fox proteins are neuron specific. Fox-1 and Fox-2 promote exon inclusion in NMYC-B, c-src, FGFR2, and protein 4.1R transcripts. In contrast, within neurons Fox proteins repress exon 4 inclusion in calcitonin/CGRP pre-mRNA by binding to a UGAUCAU element and blocking the constitutive splicing factor U2 auxiliary factor 65 (U2AF65) from binding to the 3' splice site upstream of exon 4. The splicing events, which result in the generation of calcitonin/CGRP, also involve several additional regulators, including Tra2B, SRp55, TIAR, SRp20, and PTB.

The STAR (signal transduction and activation of RNA)/GSG (GRP33, Sam68, GID-1) protein family includes the mammalian Sam68-like protein SLM-1, which regulates splice site selection by binding to a purine-rich enhancer. Rat SLM-1 and SLM-2 are expressed primarily in neurons. The tissue-specific splicing factor SLM-1 is present in the dentate gyrus, whereas SLM-2 is found in the pyramidal cells of the CA1, CA3, and CA4 regions. It is not known if SLM proteins regulate neuron-specific splicing, but SLM-2 regulates the splicing of CD44 and may also regulate tau and tra2B1 pre-mRNA splicing.

The neuroblastoma apoptosis-related RNA-binding protein (NAPOR)/CUG-binding protein 2 (CUGBP2)/embryonic lethal abnormal vision type RNA-binding protein 3 (ETR-3) has three splice variants; NAPOR-3 is neuron specific while the other two forms are ubiquitously expressed. NAPOR-3 binds to UG-rich regions. The concentration of NAPOR in different brain regions is important for determining its function. Within the forebrain, where it is abundantly expressed, NAPOR promotes exon 5 skipping of E5 and exon 21 inclusion of in N-methyl-D-aspartate (NMDA) receptor R1 pre-mRNA.

In addition to these neuron-specific or brain-enriched splicing factors, ubiquitously expressed splicing factors may also play a role in the regulation of splicing in neurons. For example, several splicing factors recognize pyrimidine-rich sequences within the intron similar to the sequence recognized by PTB, including U2 auxiliary factor (U2AF), CUG-binding protein (CUG-BP), and hnrNP F. Partial replacement of general splicing factors by neuron-enriched splicing factors may lead to neural specific splicing. Another example of a general splicing factor that regulates a neuronal splicing event is KSRP, a factor that binds to an intronic splicing enhancer element downstream of the neuron-specific c-src N1 exon. KSRP is present in both neuronal and nonneuronal cells, but it is more highly expressed in neuronal cells.

A good example of the complexity of neuronal gene alternative splicing regulation is that of exon 10 in the human tau gene. Mutations affecting this splicing event lead to frontotemporal lobe dementia (see later, neurological disorders). In this case, both exonic and intronic regulatory elements play important roles in controlling exon 10. In addition, both positive and negative cis-elements are involved and form a multi-domain composite regulatory element. A number of trans-acting splicing regulators have been identified that interact with the splicing regulatory element.

It is not clear yet how the extremely complex alternative splicing events of different genes in the nervous system are coordinated. In some cases, the alternative splicing events of different genes appear to be co-regulated by the same protein. For example, the splicing regulator PTB can differentially recognize neural and nonneuronal substrates. The expression pattern of PTB in various regions of the brain at different developmental stages supports a role for PTB to act as one alternative splicing coordinator for different splicing target genes. Some regulatory elements have been identified that mediate splicing responses of neurons to extracellular stimuli. In general, very little is known about the molecular mechanisms controlling changes in alternative splicing in response to environmental signals.
Role of Alternative Splicing in the Nervous System

The regulatory role that alternative splicing plays in the nervous system is multifaceted. Alternative splicing has been reported for genes involved in almost every aspect of the nervous system, from neural development to the function and maintenance of the adult nervous system. The expression of a vast number of genes important for neuronal differentiation, function, and survival undergo alternative splicing. These genes encode trophic factors, neuronal guidance molecules, guidance cue receptors, transmitter receptors, ion channels, intracellular signal transduction molecules, and synaptic components. Similar to the role of splicing in nonneuronal cells, alternative splicing may affect transcript localization/stability and generate proteins of distinct functional activities.

The variety of receptors produced by alternative splicing in neurons is a classic example of how a few genes can produce many protein products. The genes encoding receptors for major neurotransmitters, including serotonin, dopamine, glutamate, and GABA, all undergo alternative splicing. This results in the production of receptors that differ in function and/or subcellular localization. For example, the NMDA R1 receptor has an alternatively spliced C1 exon. When C1 exon is included in the mRNA, the receptor localizes to the cell surface membrane; when C1 exon is skipped, the receptor is cytoplasmic. In addition, the regulation of C1 splicing modulates binding of NMDA R1 to neurofilaments and calmodulin. The splicing regulation of mGluR1 affects both localization and receptor activity. One variant, mGluR1a, has a long C-terminus that targets the protein to dendrites, whereas mGluR1b, containing a short C-terminus, localizes to axons.

Alternative splicing may affect how a receptor responds to drugs. For example, two splice variants of the GABA_A receptor respond to benzodiazepine agonists in different manners. The splice variants of norepinephrine transporter have different rates of norepinephrine uptake and binding affinities to norepinephrine, a norepinephrine reuptake inhibitor. Additionally, the serotonin 5-HT_4 receptor has splice variants with different C-terminal domains that respond differently to agonists.

Alternative splicing is also important for the modulation of neuronal signalin. For example, alternative splicing of the gene encoding the z_1N N-type calcium channel produces a protein with a two-amino-acid insertion that results in a channel with altered kinetics. Another example is apolipoprotein E receptor 2 (Apoer2, a receptor for Reelin), which forms a functionally active complex with NMDA receptor in the postsynaptic densities of excitatory synapses. An alternatively spliced exon of Apoer2 is required for Reelin to induce phosphorylation of NMDA receptor and thus enhance long-term potentiation (LTP). Interestingly, the splicing of Apoer2 is regulated by synaptic activity.

In addition to modulating synaptic function, alternative splicing plays a role in synapse formation and plasticity by regulating the expression of proteins important for cell adhesion and cell-cell communication. Neural cell adhesion molecule NCAM1 is involved in synaptic plasticity, neurodevelopment, and neurogenesis. Single-nucleotide polymorphisms (SNPs) within NCAM1 splice sites that affect alternative splicing are associated with bipolar disorder and schizophrenia.

The role of alternative splicing in synaptic plasticity is only beginning to be understood. Recently, target genes for the splicing factor Nova were identified, including GABA_A receptors and G-protein-activated inward rectifying potassium channel 2 (GIRK2) channels. These proteins mediate LTP of the slow inhibitory postsynaptic current (sIPSC) in dendrites. In mice lacking Nova-2, the LTP of sIPSCs, but not the excitatory postsynaptic currents, is absent. The current model is that Nova proteins control the splicing of a number of pre-mRNAs, resulting in the regulation of LTP of the sIPSCs. The application of new techniques such as cross-linking immunoprecipitation (CLIP) and microarray assays may reveal additional examples of splicing factors regulating networks that control synaptic plasticity.

Neurological Disorders Associated with Dysregulation and Abnormalities of pre-mRNA Splicing

Human diseases may result from defective or aberrant splicing caused by mutations in regulatory pre-mRNA sequences and splicing factors. Recent data suggest that approximately 31% of human diseases are caused by mistakes in pre-mRNA splicing, and 15% of disease-causing point mutations affect pre-mRNA splicing. These statistics most likely underestimate the extent of human diseases associated with abnormalities in splicing, because splicing enhancer or silencer sequences, very few of which have been clearly identified, may carry point mutations that disrupt the balance of splice variants and thus result in disease. In addition, environmental stimuli such as stress may change intracellular distribution or post-translational modifications of splicing factors and thus affect pre-mRNA splicing. To demonstrate the role of splicing dysregulation in human diseases, a
few well-studied examples are described in the following sections. In general, these diseases can be classified in two categories: those caused by cis-acting mutations in the affected genes and those associated with secondary splicing defects resulting from trans-acting mutations.

**Neurological Diseases caused by Cis-Acting Splicing Mutations**

Dementia Splicing mutations and aberrant pre-mRNA splicing have been associated with both sporadic and familial forms of dementia, including Alzheimer’s disease. For example, splicing mutations have been identified in both presenilin-1 and presenilin-2 genes in Alzheimer’s disease. Frontotemporal dementia and parkinsonism linked to chromosome some 17 (FTDP-17) and related tauopathies are characterized by intraneuronal tau-containing deposits in affected brain regions. Microtubule-binding protein tau, a key player in microtubule stability, is critical for neuronal function. Mutations in human tau genes have been identified in FTDP-17 patients, including not only missense mutations affecting tau protein function but also splicing mutations causing imbalance of different tau splicing isoforms. Three exons in the human tau gene are alternatively spliced. In particular, exon 10 encodes one of four microtubule-binding repeat (R) domains, and alternative inclusion or exclusion of exon 10 leads to the formation of tau4R or tau3R, respectively. Exonic or intronic mutations that disrupt the balance of tau4R versus tau3R splicing isoforms lead to FTDP-17 and other forms of tauopathy with neurofibrillary tangles. In addition, splicing mutations altering exon 2 and exon 3 inclusion are associated with glioopathy and spinal cord degeneration. These observations demonstrate that an imbalance of different splicing isoforms can result in disease development or progression.

Muscular dystrophy Various mutations that affect alternative splicing of the dystrophin gene lead to Duchenne muscular dystrophy (DMD). Both exonic and intronic splicing mutations have been reported in DMD patients, including those inducing exon skipping and cryptic splice site activation. These mutations act either by disrupting splicing enhancers or by creating splicing silencers. In some cases, nonsense mutation(s) can also affect splicing by generating splicing silencer binding sites. For example, nonsense mutations in the dystrophin gene of some patients results in a binding site for hnRNP A1 (UAGACA) within the resultant pre-mRNA. These recent observations highlight the complexity of splicing errors and suggest that disease-causing splicing mutations may be underestimated.

Ataxia–telangiectasia and neurofibromatosis Ataxia–telangiectasia and neurofibromatosis type 1 are autosomal dominant neurological diseases with malignancy predisposition. Genetic studies have identified splicing mutations as common genetic defects in these patients, with approximately 50% patients carrying splicing mutations. These studies suggest the significant contribution of splicing defects to the pathogenesis of neurological disorders.

Spinal muscular atrophy Spinal muscular atrophy (SMA) is characterized by selective degeneration of motor neurons that leads to progressive paralysis. SMA is an autosomal recessive disease caused by loss of or mutations in the telomeric survival of motor neuron 1 (SMN1) gene. The centromeric SMN2 gene regulates the severity of the disease. Although SMN1 and SMN2 are almost identical, a single translationally silent nucleotide change in SMN2 disrupts an exonic splicing enhancer and results in exon 7 skipping, with only about 20% of the SMN2 transcripts producing a functional full-length SMN2 protein. This low level of full-length SMN2 protein is not sufficient to compensate for the loss of SMN1. A splice variant that retains intron 3 encodes an axonally localized truncated form of the protein called α-SMN. During development, α-SMN is expressed mainly in the axons of motor neurons and is only produced from the SMN1 gene. It remains to be elucidated why the loss of SMN1 primarily affects motor neuron function. Another interesting aspect of SMA is that the SMN1 protein plays a role in snRNP assembly and thus it may also be considered a trans-acting splicing disorder (see later).

**Neurological Diseases Caused by Trans-Acting Splicing Defects**

A number of neurological syndromes or diseases are caused by trans-acting splicing defects. In these disorders, the mutations in genes encoding spliceosomal components or splicing regulatory factors lead to aberrant or defective splicing events.

**Retinitis pigmentosa** A common cause of blindness is retinitis pigmentosa, which is characterized by a loss of photoreceptor cells and progressive degeneration of the retina. A fraction of patients with autosomal dominant form of retinitis pigmentosa (adRP) have mutations in genes encoding spliceosomal proteins essential for spliceosomal assembly, including PRP3, PRP8, and PRP31. Similar to SMA, in these
adRP patients, defects in the ubiquitously expressed RNA processing factors lead to specific neurological diseases. The mechanisms for the specificity of neurodegeneration in these diseases remain to be elucidated. Current models include haploinsufficiency and dominant-negative effects leading to cell-type-specific functional deficiencies.

**Myotonic dystrophy** Myotonic dystrophy (‘dystrophia myotonica’; DM) is a multisystem syndrome with prominent muscular atrophy. Two types of DM have been revealed by genetic studies: DM1 is caused by the presence of a CUG expansion in the 3’ untranslated region (UTR) of the DM protein kinase premRNA, and DM2 is caused by a CCUG expansion in intron 1 of the CNBP (ZNF9) gene. CUG/CCUG repeat expansion results in sequestration or upregulation of proteins interacting with CUG/CCUG, including neuronblind-like (MBNL) and CUG-binding protein 1 (CUG-BP1). This in turn causes a dysregulation in the splicing of a subset of pre-mRNA muscle and brain transcripts that require these regulatory factors. DM1 is also associated with changes in splicing of pre-mRNAs that encode amyloid precursor protein, NMDAR1, and tau, suggesting that aberrant splicing of these pre-mRNAs may be the basis of the central nervous system defects such as memory impairments.

**Prader–Willi Syndrome** Prader–Willi syndrome (PWS) patients often exhibit behavioral problems such as obsessive-compulsive disorder and autism. A recent report has suggested an explanation for why these patients frequently respond to treatment with serotonin reuptake inhibitors. PWS is associated with deletions of the paternally inherited copy of chromosome 15q11–q13, a maternally imprinted locus encoding multiple copies of a small nuclear RNA called H11-52 snoRNA. A stretch of 18 nucleotides in the H11-52 snoRNA is complementary to the sequence within an alternatively spliced exon (Vb) of the 5-HT2R serotonin receptor premRNA. H11-52 snoRNA binds to and antagonizes a splicing silencer, facilitating the inclusion of exon Vb within the mRNA. Changes in splicing of 5-HT2R were detected in the hippocampal RNA in PWS patients. These data suggest that altered 5-HT2R premRNA splicing caused by the loss of H11-52 snoRNA contributes to the clinical manifestations in PWS patients.

**Ataxias** Fragile X-associated tremor/ataxia syndrome (FXTAS) is a newly recognized late-onset syndrome characterized by cognitive impairment along with tremor and gait problems. FXTAS is associated with moderate expansions of 55–200 CGG repeats in the region of the fragile X mental retardation (FMR1) gene encoding the 5’ UTR. Longer expansions (>200) of CGG repeats lead to the fragile X mental retardation syndrome with numerous neurological problems, including autism, learning disabilities, anxiety disorders, and mental retardation. The FMR1 gene is regulated by alternative splicing and encodes RNA-binding proteins involved in regulation of protein synthesis at postsynaptic sites of dendrites and in the maturation of dendritic spines. The CGG trinucleotide repeat expansions in the 5’ UTR lead to transcription silencing and loss of function of FMR1. Intranuclear inclusions in neurons and astrocytes in the postmortem brains of FXTAS patients contain the RNA-binding proteins MBNL1 and hnRNP A2, suggesting that CGG expansions may affect the function of these splicing factors and that FXTAS may involve aberrant pre-mRNA splicing. Further research is needed to understand the role of MBNL1, hnRNP A2, and FMR1 in disease development.

In another form of ataxia, spinocerebellar ataxia type 2 (SCA2), there is a CAG repeat expansion in the coding region of the gene encoding ataxin-2. Ataxin-2 protein contains RNA-binding domains and interacts with the splicing regulator A2BP1 (FOX1). Disruption of the A2BP1 locus by chromosomal translocation has been reported in patients with mental retardation and epilepsy. Triplet repeat expansions in the noncoding RNAs have been found in patients with other SCA subtypes, including SCA8, SCA10, and SCA12. These observations suggest the possible involvement of pre-mRNA splicing dysfunction in various forms of ataxia. Further research is necessary to understand if there are common molecular mechanisms among numerous repeat expansion diseases, why these diseases manifest differently in patients, and whether dysregulation of alternative pre-mRNA splicing contributes to the pathogenesis of these diseases.

**Paraneoplastic neurologic disorders** In paraneoplastic neurologic disorder (PND) patients, malignancies outside of the central nervous system (CNS) induce expression of neuron-specific splicing regulatory factors (SRFs). The autoimmune response generated against these neuron-specific SRFs ultimately results in the dysfunction of neurons that normally express these proteins. Two families of neuron-specific SRFs have been identified as PND target antigens, Hu and Nova. Nova-1 and Nova-2 regulate pre-mRNA splicing of genes encoding synaptic proteins
Conclusions and Future Perspective

Overwhelming evidence supports the concept that pre-mRNA splicing and alternative splicing play an important role in mammalian gene expression and that alternative splicing is a powerful mechanism for genetic diversity. This is particularly evident in the nervous system. Alternative splicing modulates activities of the majority of genes involved in the formation and function of the nervous system. A wide range of neurologic disease is caused by genetic mutations that affect pre-mRNA splicing. Some splicing mutations act in cis to cause splicing defects in genes containing the mutations. Other mutations affect splicingomous components of splicing regulatory factors, which in turn lead to aberrant splicing or dysregulation of splicing of genes regulated by these splicing factors. Trinucleotide repeat expansion mutations can also affect pre-mRNA splicing by sequestering or altering splicing regulators that bind to the trinucleotide repeats and affecting the splicing of the genes that are normally controlled by the splicing regulators. In addition, abnormal splicing has been reported in complex diseases such as psychiatric disorders. Although the important role of pre-mRNA splicing and alternative splicing regulation in the nervous system has been established, much work needs to be done to understand the molecular mechanisms underlying the splicing regulation and the molecular pathogenesis of splicing disorders.

Numerous environmental factors influence neuronal survival and function. Very little is known about how the nuclear splicing machinery responds to various extracellular signals, although many studies have documented altered pre-mRNA splicing in response to stresses such as pH change, osmotic or temperature shock, UV exposure, and oxidative stress. For example, brain ischemia induces the translocation of serine/arginine regulatory factors from the nucleus to the cytoplasm and causes changes in alternative splicing. Further research is necessary to elucidate the molecular mechanisms by which environmental factors modulate pre-mRNA splicing and alternative splicing regulation.

The combined application of genetic, molecular, biochemical, and bioinformatic approaches will further our understanding of the role in pre-mRNA splicing and alternative splicing regulation in the nervous system. The ultimate goal is the development of strategies to correct splicing defects that cause human diseases.

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See also: Gene expression regulation: chromatin modification in the CNS (00174); Gene expression dysregulation in CNS pathophysiology (00175); Genetic influence on CNS gene expression: impact on behavior (00183); Genomic disorder and gene expression in the developing CNS (00184)

Further Reading


Non-Print Items

Abstract:
The sequencing of the human genome has revealed that the number of protein-coding genes is far fewer than initially predicted based on phenotypic diversity. This implies that posttranscriptional processing of RNA transcripts plays a more important role in generating genetic diversity than previously anticipated. One of the most powerful mechanisms contributing to transcript diversity is alternative pre-mRNA splicing. This process of generating multiple mRNAs from single pre-mRNA transcripts occurs at the highest frequency in the nervous system. This article focuses on the important role splicing plays in the nervous system and the evidence that mutations that affect splicing often lead to neurological disease.

Keywords: alternative splicing; ataxia; frontotemporal dementia and parkinsonism linked to chromosome 17; gene expression; muscular dystrophy; myotonic dystrophy; neurodegeneration; Prader–Willi syndrome; RNA; spinal muscular atrophy

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