Neuron 52, 93–101, October 5, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.neuron.2006.09.017

Splicing Regulation in Neurologic Disease

Review

Donny D. Licatalosi[1] and Robert B. Darnell[1,*]
[1] Howard Hughes Medical Institute
Laboratory of Molecular Neuro-Oncology
The Rockefeller University
1230 York Avenue
New York, New York 10021

The importance of alternative splicing in the regulation of diverse biological processes is reflected in the growing list of human diseases associated with known or suspected splicing defects. It is becoming evident that alternative splicing plays a particularly important role in neurologic disease, which is perhaps not surprising given the important role splicing plays in generating complexity and function in the brain. This review considers the evidence that defects in regulation of splicing may underlie many types of human neurologic diseases.

Thirty years have passed since the unexpected discovery that the protein-coding (exonic) sequences of genes are separated by relatively large noncoding (intronic) sequences. Pre-mRNA splicing is the process in which these long precursor (pre-) mRNAs are processed into the mature forms found in the cytoplasm via the precise joining of exonic sequences and the removal of introns that can be hundreds of kilobases long (Figure 1). Splicing is accomplished by a large macromolecular complex termed the spliceosome, which consists of numerous proteins and small RNAs (snRNPs; Rappsilber et al., 2002; Zhou et al., 2002). The spliceosome recognizes specific sequences in pre-mRNA to define intron-exon boundaries and to facilitate splicing (Figure 1). The activity of the spliceosome can be regulated by multiple splicing activator and repressor proteins that are bound to enhancer and silencer elements, respectively, in the pre-mRNA (reviewed in Black, 2003).

Alternative splicing is the process by which different combinations of exons are included in the mature mRNA, thus allowing a single gene to encode multiple protein isoforms with altered or potentially antagonistic properties. Remarkably, some genes can yield hundreds and potentially thousands of unique isoforms. For example, due to the large number of alternative exons present in the three neurexin genes, alternative splicing can generate nearly 3000 unique mRNAs (Missler and Sudhof, 1998) encoding a potentially huge array of synaptic molecules. An emerging theme in molecular neurobiology is that alternative splicing may generate cell-specific combinations of protein isoforms that define the functional properties of the cell and underlie complex processes in the nervous system such as synaptic adhesion and plasticity (Boucard et al., 2005; Ule and Darnell, 2006). Here we review evidence that splicing misregulation plays a crucial role in the development of neurologic diseases.

*Correspondence: darnelr@rockefeller.edu

Splicing in the Brain

Alternative splicing is highly abundant in brain relative to other tissues (Blencowe, 2006; Yeo et al., 2004), where it can influence neurophysiology through spatial and temporal alterations in proteins that comprise ion channels and membrane-bound receptors and are involved in neurotransmitter storage and release (Grabowski and Black, 2001; Lipscombe, 2005; Stamm et al., 2005). In the nervous system, regulated alternative splicing allows the cell to "fine-tune" its protein composition in order to respond and adapt to different stimuli. Activity-induced changes in the alternative splicing of many pre-mRNAs have been described, including those that encode clathrin light chain B, c-src, NMDA NR1, PMCA, and AChE (Daoud et al., 1999; Lipscombe, 2005; Pick et al., 2004; Strehler and Zacharias, 2001; Vallano et al., 1999; Xie and Black, 2001). Alternative splicing of *NMDAR1* pre-mRNAs represents an excellent example of how the regulated production of different protein isoforms can influence receptor properties and consequently cell physiology (Bradley et al., 2006; Ehlers et al., 1996; Mu et al., 2003; Xie and Black, 2001).

Alternative splicing patterns are dependent on the net result of multiple interactions between RNA binding proteins bound to regulatory elements in the pre-mRNAs (Black, 2003). As a result, differences in the regulation of splicing activator and repressor proteins may be able to affect the alternative splicing of different pre-mRNAs that share common regulatory elements or "splice codes" (Goren et al., 2006; Han et al., 2005; Ule and Darnell, 2006; Ule et al., 2006; Wang et al., 2004). Such regulation through combinatorial control could allow alternative splicing patterns of multiple pre-mRNAs to be altered in response to changes in the level or activity of a single splicing factor, a mechanism believed to underlie the establishment and maintenance of tissue- and developmental-stage-specific gene expression pathways (Jensen et al., 2000; Ule et al., 2003, 2005; Xu et al., 2005). Consequently, disrupting the function of a single RNA binding protein can affect many alternatively spliced transcripts, a phenomenon that is increasingly recognized as having a role in human diseases. In this review, we present examples of the different types of neurologic diseases with links to alternative splicing (Table 1). The reader is directed to other excellent recent reviews that discuss the roles of RNA binding proteins and alternative splicing in human diseases in general (Caceres and Kornblihtt, 2002; Cartegni et al., 2002; Faustino and Cooper, 2003).

Neurologic Diseases with Primary Splicing Defects: *cis*-Acting Splicing Disorders

In one set of neurologic diseases involving splicing defects, mutations within the disease-causing gene alter sequences important for the proper splicing of the resulting pre-mRNA (Figure 2), and we refer to these as *cis*-acting splicing disorders. It is estimated that at least 15% (Krawczak et al., 1992), and perhaps as many as 50% (see "The Phakomatoses," below), of point mutations that result in human disease cause *cis*-acting

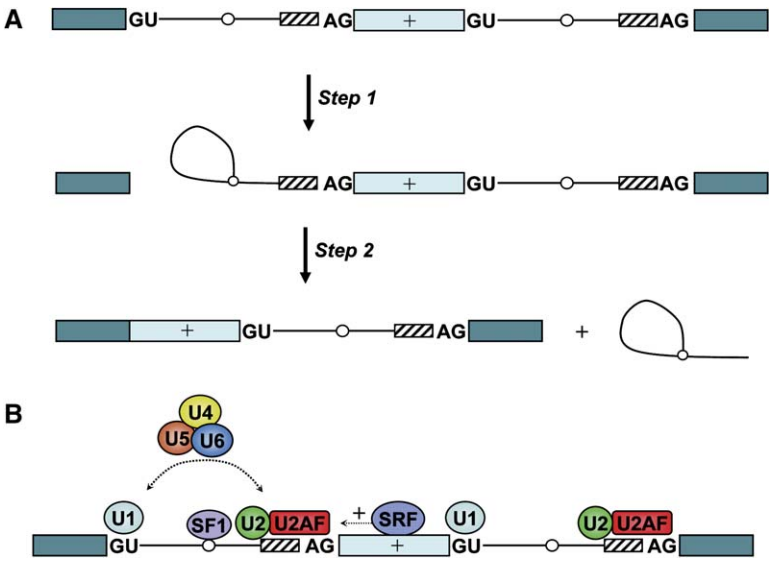

Figure 1. A Simplified Overview of Pre-mRNA Splicing

(A) Splicing of a pre-mRNA containing three exons (solid boxes) is shown as dependent on the presence of specific *cis* elements in the transcript. These elements include the GU and AG dinucleotides that define the 5′ and 3′SS, respectively; a branch point sequence (open circle); a pyrimidine-rich sequence (hatched box) located upstream of the 3′SS; and an auxiliary splicing regulatory element ("+" sign). Splicing of an intron occurs in two catalytic steps. In the first catalytic step of the splicing reaction, cleavage at the 5′SS of intron 1 leads to the formation of a branched (lariat) intermediate. In the second catalytic step, the branched intermediate is removed from the transcript by cleavage at the 3′SS, and the first two exons are ligated together.

(B) *cis* elements in pre-mRNA are recognized by components of the spliceosome. These include the snRNPs (U1, U2, U4, U5, and U6) and additional proteins, including the U2-auxilliary factor (U2AF), the branch point binding protein SF1, and in this example, an SRF that functions to promote the association of U2AF with the 3′SS of intron 1. Interactions between factors bound at the different splice sites help to define intron-exon boundaries and to initiate the removal of the first intron.

defects in pre-mRNA splicing. These mutations may weaken or strengthen splicing enhancer and silencer elements, as well as create or destroy splice sites. As a result, the splicing of constitutive and alternative exons is altered, cryptic splice sites activated, or intronic sequences retained in the mature mRNA. Specific examples of *cis*-acting mutations and their consequences in neurologic disease follow.

The Phakomatoses

Ataxia-telangiectasia and neurofibromatosis are two of a group of autosomal dominant disorders (the phakomatoses) associated with neurologic disease and a predisposition for malignancies; both disorders have been recently found to be associated in some patients with mutations altering pre-mRNA splicing. For example, a 4 base-pair deletion in intron 20 of the ataxia-telengiectasia mutated (*ATM*) gene can result in the activation of a cryptic exon and is directly responsible for ataxia-telengiectasia in an affected individual (Pagani et al., 2002). More generally, an unusually high number of ataxia-telengiectasia and neurofibromatosis type 1 patients (~50%) have mutations that affect pre-mRNA splicing (Ars et al., 2000; Teraoka et al., 1999). As emphasized by Cartegni et al. (2002), these studies highlight the importance of comparing DNA and the resulting mRNA sequences in order to determine whether silent or missense mutations might also change alternative splicing patterns. In ataxia-telengiectasia and neurofibromatosis type 1, such careful sequencing of DNA and mRNA from patients has expanded the list of disease-causing mutations due to aberrant pre-mRNA splicing. Continued efforts to define auxiliary elements that function as enhancers and suppressors of exon splicing will undoubtedly lead to an improved ability to predict which disease-causing mutations affect pre-mRNA splicing.

Frontotemporal Dementia with Parkinsonism Linked to Chromosome 17, or FTDP-17

The need to properly control the relative levels of protein isoforms generated by splicing is underscored by the disorder termed frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17). FTDP-17 is an autosomal dominant disorder in which *cis*-acting mutations result in splicing defects in the transcript encoding MAPT, the microtubule-associated protein tau that is enriched in axons of mature and growing neurons. *MAPT* gives rise to multiple transcripts that are alternatively spliced and regulated in a developmental and tissue-specific manner. The association of tau with microtubules is dependent on C-terminal repetitive microtubule binding domains encoded by exons 9 through 12. The number of repeat domains (R) encoded in the *MAPT* transcript, either 3 or 4, is developmentally regulated through the exclusion or inclusion, respectively, of exon 10, and can influence the efficiency with which tau promotes microtubule assembly (Buee et al., 2000). Perturbation of the ratio of 4R- to 3R-containing protein isoforms can lead to the formation of neurofibrillary tangles (NFTs) and neurodegeneration (Lee et al., 2001). Several mutations clustered around exon 10 which lead to increased levels of exon 10-containing isoforms in humans have been characterized. These include missense mutations that are presumed to create or disrupt a splicing enhancer or silencer, respectively (D'Souza et al., 1999; Hasegawa et al., 1999; Jiang et al., 2003), as well as mutations that disrupt the formation of an RNA stem-loop structure at the 5′ splice site (SS) of exon 10, which normally functions to restrict spliceosome assembly (Grover et al., 1999). Such structural causes of altered splicing are difficult to document and may be more widespread than currently appreciated. Finally, while the splicing of *MAPT* exon 10 has been most extensively studied for its role in the pathology in FTDP-17, altered splicing of *MAPT* exons 2, 3, and 6 have also been observed and implicated in the pathology of other diseases, including gliopathy, spinal cord degeneration, Alzheimer's disease, and myotonic dystrophy (DM; see below) (Andreadis, 2005; Glatz et al., 2006; Leroy et al., 2006; Sergeant et al., 2001).

Table 1. Neurologic Disorders with Links to Alternative Splicing

Disease	Link to alternative splicing
Ataxia-telengiectasia	Point mutations within the *ATM* gene cause aberrant splicing of *ATM* transcripts
Fascioscapulohumoral dystrophy (FSHD)	Loss of FRG1 leads to altered splicing of many pre-mRNAs
Fragile-X-associated tremor/ataxia syndrome (FXTAS)	Premutation CGG repeat expansions in the *FMR1* gene result in the sequestration of RNA-binding splicing factors
Frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17)	Point mutations within the *MAPT* gene result in altered levels of *MAPT* transcripts containing the alternatively spliced exon 10
Duschenne muscular dystrophy; Becker's muscular dystrophy	Altered splicing of *dystrophin* transcripts due to deletions and mutations in the *dystrophin* gene
Myotonic dystrophy (DM)	
DM1	CUG expansion in the 3'UTR of *DMPK* results in the misregulation of the MBNL splicing factor and consequent missplicing of MBNL target pre-mRNAs
DM2	CCUG expansion in *ZNF9* intron leading to misregulation of the CUG-BP1 splicing factor and missplicing of CUG-BP1 target pre-mRNAs
Neurofibromatosis type 1 (NF1)	Numerous mutations in the *NF1* gene, including mutations that result in aberrant splicing
Paraneoplastic neurologic disorders (PND)	
Paraneoplastic opsoclonus-myoclonus-ataxia (POMA)	Autoimmune antibodies recognize the Nova family of neuron-specific RNA-binding splicing factors; *Nova* knockout mice phenocopy POMA
Hu syndrome (PEM/SN; paraneoplastic encephalomyelitis / sensory neuronopathy)	Autoimmune antibodies recognize the Hu family of RNA-binding factors related to the *Drosophila* splicing factor ELAV
Prader Willi syndrome	Loss of a splicing regulatory snoRNA that is complementary to a splicing silencer element implicated in regulating the alternative splicing of serotonin receptor *5-HT$_{2c}$R* transcripts
Psychiatric disorders	Accumulation of aberrantly spliced transcripts in schizophrenic patients
Retinitis pigmentosa	Mutation of genes encoding U snRNP-associated proteins
Rett syndrome	Mutation of the gene encoding MeCP2, which interacts with the YB-1 RNA binding protein; mouse model of Rett syndrome shows aberrant pre-mRNA splicing
Spinal muscular atrophy	Deletion/mutation of the *SMN1* gene, and the loss of a splicing regulatory element in *SMN2* results in insufficient levels of SMN, which is involved in snRNP biogenesis
Spinocerebellar ataxias	
SCA2, SCA8, SCA10, and SCA12	Possible RNA gain of function due to triplet repeat expansions; direct and indirect interactions with RNA-binding splicing factors

See text for details and references.

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is a common autosomal recessive neurodegenerative disorder, characterized by the progressive loss of motor neurons in the anterior horns of the spinal cord (Monani, 2005). SMA results from insufficient levels of the survival of motor neuron (SMN) protein, which is encoded by two nearly identical genes, *SMN1* and *SMN2*. In most affected individuals, the disease mutation is in the *SMN1* gene, which is absent or mutated (Lefebvre et al., 1995). *SMN2*, while nearly identical to *SMN1*, is nonetheless unable to compensate for this loss due to a splicing problem; a translationally silent C to T nucleotide substitution is present in the normal *SMN2* gene that disrupts an important splicing regulatory element in exon 7 (Cartegni et al., 2006; Kashima and Manley, 2003). As a result, only ~20% of SMN2 transcripts contain exon 7 and encode a functional SMN protein, while the predominant exon 7-skipped isoform naturally produced from *SMN2* is nonfunctional. Thus, SMA is an unusual disorder in which a loss-of-function mutation in one gene (*SMN1*) unmasks a naturally occurring *cis*-acting splicing defect in a second gene (*SMN2*), leading to the insufficient production of a critical gene product.

A second link between SMA and pre-mRNA splicing has emerged from studies on the biology of the SMN protein itself, and it suggests that SMA may also turn out to be a *trans*-acting splicing disorder. SMN interacts with a complex of several gemin proteins to form a complex that is critical for the association of Sm proteins with snRNA to form snRNPs, which themselves form part of the constitutive machinery necessary for pre-mRNA splicing (Carissimi et al., 2006; Gubitz et al., 2004). SMN facilitates the assembly of spliceosomal snRNPs prior to their import into the nucleus, where splicing occurs, and can enhance pre-mRNA splicing in vitro (Pellizzoni et al., 1998). Although these observations suggest that defects in SMN production might lead to widespread alterations in pre-mRNA splicing, to date no changes in pre-mRNA splicing have been observed in mouse models of SMA. Interestingly, mutations that affect the general splicing machinery have also been described in a second neurological disorder. In autosomal dominant retinitis pigmentosa (RP), degeneration of photoreceptor cells results from mutations that affect the activity of some U snRNP-associated factors (Chakarova et al., 2002; McKie et al., 2001; Vithana et al., 2001), consistent with the suggestion made in SMA that defects in the basal splicing machinery may lead to specific neurologic disorders.

It remains unclear why a deficiency in such ubiquitously expressed proteins would lead to selective degeneration of spinal cord motor neurons or photoreceptor cells. Such proteins may have an essential function that is unique to specific cells or be required at a level in a subset of neurons not accommodated by redundant proteins (such as SMN2). One model of SMA, based on knockdown of SMN expression in animal models (Monani, 2005) and in particular rescue by coinjection of snRNPs, proposes that motor neurons have a particular sensitivity to snRNP levels (Winkler et al., 2005). A related model proposes that the cell-specific degeneration in SMA may be due to cell type-specific differences in the relative amounts of splicing silencer and enhancer

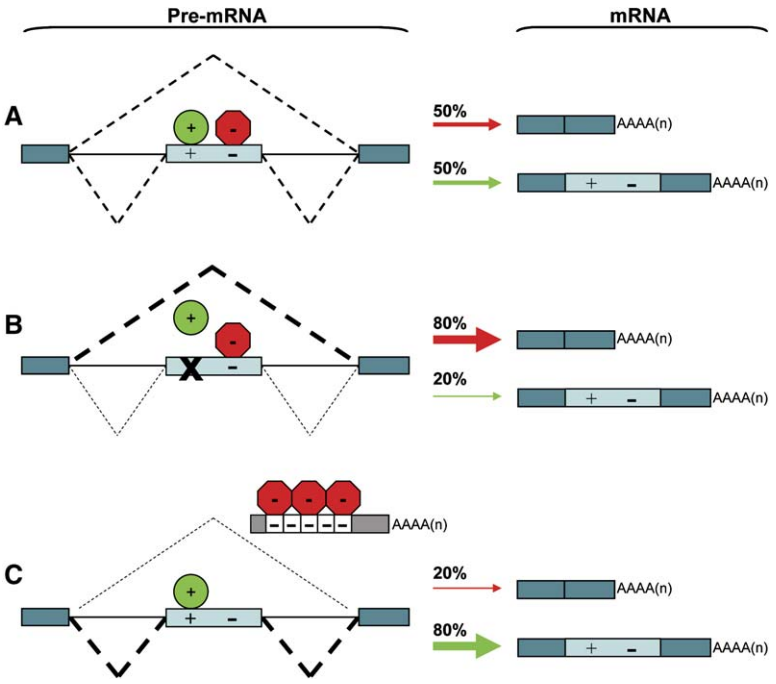

Figure 2. *cis* and *trans* Mutations Can Disrupt Levels of Alternatively Spliced mRNAs

(A) Splicing of an alternative exon (light blue) is regulated by the association of SRFs with splicing regulatory elements ("+" and "−") in the pre-mRNA. SRFs act either to promote (green circle) or repress (red stop sign) inclusion of the alternative exon in the mature mRNA. In this example, the ratio of positive and negative SRFs leads to inclusion of the alternate exon in 50% of the mature mRNA transcripts.

(B) In this example, a *cis*-acting mutation ("X") in the pre-mRNA prevents the positive-acting SRF (green) from interacting with the pre-mRNA. This results in a relative excess of negative-acting SRFs (red) binding to the pre-mRNA, leading to an increase in spliced mRNAs that have skipped the alternate exon.

(C) A mutation in one gene functions in *trans* to cause aberrant splicing of mRNA from a second gene. In this example, a repeat expansion mutation in one transcript sequesters a negative-acting SRF. This SRF is then unavailable to negatively regulate splicing of an alternative exon present in a second pre-mRNA. This leads to an increase in spliced mRNAs that contain the alternate exon.

proteins that determine the efficiency of *SMN2* exon 7 splicing (Cartegni et al., 2006). It is notable that disease severity in SMA patients (Taylor et al., 1998) and SMA mice (Hsieh-Li et al., 2000; Monani et al., 2000) can be modified by *SMN2* copy number. Finally, it remains possible that some transcripts may be more sensitive to snRNP levels, particularly in motor neurons, and that failure to properly splice such transcripts might contribute to the disease phenotype (Monani, 2005). Alternatively, other proposed functions for SMN, including a role in axonal transport (Eggert et al., 2006), may play a role in SMA.

Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is caused by mutations in the *dystrophin* gene. Mutations that induce inappropriate alternative splicing (i.e. exon skipping) can cause disease, and the mechanism has been well worked out in some cases in which mutations disrupt splicing enhancers (Ahn and Kunkel, 1993). Aberrant splicing of the *dystrophin* pre-mRNAs that preserve the open reading frame are better tolerated and may result in a milder DMD variant, Becker's muscular dystrophy (BMD). In some instances, detailed analyses of mutations have revealed splicing defects that may have otherwise been overlooked, as described for the phakomatoses. For example, a *cis*-acting nonsense mutation in the *dystrophin* gene also causes exon 31 skipping by creating an exonic splicing silencer by creating a specific binding site (UAGACA) for the hnRNP A1 protein (Disset et al., 2006). Interestingly, intronic mutations that result in aberrant splicing due to the activation of cryptic splice sites or pseudoexons have also been described (Beroud et al., 2004; Tuffery-Giraud et al., 2003).

Neurologic Diseases where RNA Binding Proteins Are Implicated: *trans*-Acting Splicing Disorders

In a second set of neurologic diseases associated with splicing defects, which we term *trans*-acting splicing disorders, aberrant splicing of pre-mRNAs occurs indirectly, from dysfunction in another gene which leads to a secondary splicing defect (Figure 2). One set of disorders are those in which splicing regulatory factors (SRFs) themselves are directly implicated in the disease process. In a second set of diseases, repeat expansion mutations are present in RNAs that are believed to act as "dominant-negative sinks" for SRFs. In these disorders, RNA binding proteins appear to be vulnerable to being bound by stretches of repeat sequences that, when amplified by disease-causing mutations, lead to amplification of SRF binding sites and SRF sequestration, and consequently to altered splicing of pre-mRNAs that would normally be regulated by those SRFs (Ranum and Cooper, 2006; Ranum and Day, 2004b).

Indirect Targeting of RNA Binding Proteins in Neurologic Diseases

Myotonic Dystrophy. Myotonic dystrophy is a multisystemic disorder representing a common form of muscular dystrophy in adults. Two types of DM have been identified: DM1, which is caused by a CUG expansion in the 3′ untranslated region (3′ UTR) of the DM protein kinase gene (*DMPK*); and DM2, which is caused by a CCUG expansion located in intron 1 of the zinc finger protein 9 (*ZNF9*) gene (Ranum and Day, 2004a). In both DM1 and DM2, misregulation of the levels of proteins involved in pre-mRNA splicing is believed to lead to the disease phenotype, which for DM1, includes cognitive impairment, personality and behavior abnormalities, abnormal white matter that is indicative of degenerative changes, and the presence of NFTs in the neocortex and subcortical nuclei (Ranum and Day, 2004b). Numerous biochemical and genetic studies with mouse models have demonstrated that the CUG and CCUG repeat expansions result in the sequestration and upregulation of the MBNL and CUG-BP1 proteins, respectively, which function to modulate the alternative splicing of transcripts that are misregulated in DM striated muscle

and brain tissues (Kanadia et al., 2006; Ranum and Cooper, 2006). Interestingly, alterations in the splicing of *amyloid precursor protein* pre-mRNA, *NMDAR1* exon 5, and *MAPT* exons 2, 6, and 10 have been reported in DM1, and are suspected to underlie various CNS abnormalities in DM1, including memory impairment (Jiang et al., 2004; Leroy et al., 2006).

Fragile-X-Associated Tremor/Ataxia Syndrome, or FXTAS. Mutations in the *FMR1* locus were originally linked to mental retardation (the fragile-X syndrome) and found to be due to long triplet repeat expansions (CGG) in the 5′ UTR, causing hypermethylation, transcriptional silencing, and loss of function. Fragile-X-associated tremor/ataxia syndrome (FXTAS) is a more recently described late-onset neurologic disorder caused by "premutation" expansions of 55–200 CGG repeats, and is associated with cognitive decline, gait difficulty, intention tremor, and white matter changes (Hagerman and Hagerman, 2004; Oostra and Willemsen, 2003). Fly (Jin and Warren, 2003) and mouse (Willemsen et al., 2003) models support the idea of an RNA gain-of-function mutation. Consistent with this model is the observation that two RNA binding proteins, hnRNP A2 and MBNL1, were among the proteins reported to be present in inclusions isolated from FXTAS brain specimens (Iwahashi et al., 2006). The presence of MBNL1 in FXTAS intranuclear inclusions suggests that similar mechanisms may underlie the pathogenesis of DM and FXTAS, although it remains to be determined whether FXTAS is also associated with altered pre-mRNA splicing.

Prader Willi Syndrome. Prader Willi syndrome (PWS) is a rare genetic disorder characterized by intellectual and behavioral disturbances, including obsessive compulsive disorder and autism, together with obesity and short stature (Veltman et al., 2005). PWS is associated with deletions on the paternally inherited copy of chromosome 15q11-13, a maternally imprinted locus harboring multiple copies of a small non-protein-coding RNA termed *HBII-52* (a small nucleolar RNA, or snoRNA). Recently, Kishore and Stamm (2006) noted complementarity (18 contiguous identical nucleotides) between *HBII-52* snoRNA and an alternative exon (Vb) of the serotonin receptor *5-HT$_{2c}$R* pre-mRNA. *HBII-52* was found to promote inclusion of exon Vb in transfected cells by binding a silencer element, thus presumably antagonizing the activity of a splicing inhibitor. Furthermore, these observations were correlated with splicing changes in *5-HT$_{2c}$R* mRNA seen in hippocampal RNA obtained from PWS patients. These results were taken to suggest that PWS may arise due to the absence of a *trans*-acting RNA which normally functions to regulate *5-HT$_{2c}$R* pre-mRNA alternative splicing, a hypothesis further correlated with the observation that patient symptoms may respond to serotonin reuptake inhibitors.

Direct Targeting of RNA Binding Proteins in Neurologic Diseases

Although numerous reports have described links between alternative splicing and neurologic disease (Table 1), in many cases the role of alternative splicing in disease is not well understood. These include diseases in which targets of the disease are thought to have a role in the regulation of alternative splicing, as well as neurologic diseases in which aberrant pre-mRNA splicing patterns have been observed. In this emerging area, the linkages between aberrant splicing and RNA binding proteins are just beginning to be understood, and the issue of direct versus indirect effects remains to be clarified.

Paraneoplastic Neurologic Disorders

An intriguing link between alternative splicing and neurologic disease is emerging from the study of paraneoplastic neurologic disorders (PNDs). In these disorders, malignancies present outside of the nervous system induce expression of what are believed to be neuron-specific SRFs. This leads to immunologic suppression of the malignancy, and, in ways that are poorly understood, immunologic breach of the blood-brain barrier and autoimmune neurologic disease, with dysfunction in those neurons normally expressing the tumor-expressed SRF. It has been proposed that PND antibody targeting of PND antigens may contribute to the specific sets of neurologic dysfunction seen in the disorders (Musunuru and Darnell, 2001; Roberts and Darnell, 2004).

Two neuron-specific families of RNA binding proteins were identified through the study of PNDs. Nova, targeted in patients with dysfunction of inhibitory motor control, is a neuron-specific protein that regulates alternative splicing in a discrete set (approximately several hundred) of pre-mRNAs encoding a biologically coherent set of synaptic proteins (Jensen et al., 2000; Ule et al., 2003, 2005). Nova RNA targets correlate with the specific neurologic defects seen in the PND patients, including several necessary for slow inhibitory postsynaptic potentials that regulate long-term potentiation (Huang et al., 2005; Ule and Darnell, 2006). Although the pathogenesis of the disorder is complex (Musunuru and Darnell, 2001; Roberts and Darnell, 2004), Nova knockout mice phenocopy the human PND (Jensen et al., 2000). A second PND antigen, Hu, is the human homolog of the *Drosophila* neuronal protein Elav (Szabo et al., 1991). Although there is no clear data relating Hu to mammalian neuronal alternative splicing, Elav has been implicated in the regulation of alternative splicing of several transcripts in *Drosophila* (Koushika et al., 2000; Soller and White, 2003).

Ataxias: a Distinct Disease Subset Associated with RNA Binding Protein Dysfunction?

Neurologic diseases associated with the mutation or dysregulation of other factors believed to be necessary for pre-mRNA splicing regulation have also been described. For example, spinocerebellar ataxia subtype 2 (SCA2) results from CAG expansion in the coding region of the gene encoding the ataxin-2 protein. Ataxin-2 contains two RNA binding modules and interacts with the known neuronal splicing regulator Fox-1/A2bp1 (Underwood et al., 2005), which has also been implicated in isolated cases of epilepsy and mental retardation (Bhalla et al., 2004). While these observations suggest that the activity of splicing factors may be altered in SCA2, it is not known whether splicing is affected in SCA2 patients.

A number of other defects related to RNA regulation have been observed in patients with ataxic disorders. For example, triplet repeat expansions have been observed in noncoding RNAs associated with spinocerebellar ataxias (SCAs) 8, 10, and 12 (Ranum and Cooper,

2006). These observations raise the question of how specific subsets of neurologic symptoms arise in patients with related mutations. For example, patients with SCA8 and Huntington's Disease-Like 2 (HDL2) both harbor noncoding CUG expansions, as do patients with DM, but rather than presenting with muscle defects, they present with signs of either cerebellar ataxia (Koob et al., 1999) or movement abnormalities, dementia, and psychiatric problems (Holmes et al., 2001), respectively. It is not clear whether different phenotypes relate to differences in the cell types expressing each of these CUG repeat-containing genes, or to other causes. Differences in adjacent sequences, such as CTA tracts adjacent to the CTG expansion in SCA8, interruptions within the repeats themselves, antisense transcripts, or other variables, may contribute to phenotypic differences (Martins et al., 2005; Moseley et al., 2006; Ranum and Cooper, 2006).

Neurologic Disorders Targeting Other Nuclear Proteins

As alternative splicing becomes more closely scrutinized in neurologic disease, splicing defects are beginning to be described in disorders where mutations are present in proteins that were not previously known to participate in pre-mRNA splicing. For example, in fascioscapulohumeral muscular dystrophy (FSHD), an autosomal dominant neuromuscular disorder, deletion of a transcriptional silencer leads to overexpression of several genes, including FRG1. While there has been little biochemical analysis of FRG1 function, the protein is nuclear, copurifies (along with RNA binding proteins and transcription factors) with spliceosomal complexes (Rappsilber et al., 2002), and is present in nuclear speckles (in addition to the nucleolus and Cajal bodies) in tissue culture cells (van Koningsbruggen et al., 2004). This localization has also been described for the nuclear poly(A) binding protein PABPN1 protein (Bao et al., 2002), which interestingly, accumulates in the nucleus due to triplet repeat expansions in the neuromuscular disorder oculopharyngeal muscular dystrophy (Brais et al., 1998). In Rett syndrome (RTT), a developmental disorder characterized by motor dysfunction and autistic features, patients harbor mutations in the *MeCP2* gene that encodes a DNA methylase believed to affect transcription, but which is also able to bind to the RNA binding protein YB1 (Young et al., 2005). The links between FRG1 and RNA binding led to analysis of alternative splicing patterns of candidate genes in FSHD and, more globally, to a genome-wide analysis of alternative splicing in RTT, leading in both cases to identification of misspliced transcripts that may play roles in the disorders (Gabellini et al., 2006; Young et al., 2005). Such splicing defects indicate that the disease-causing protein may have a previously unsuspected function as an SRF, or that alternative splicing may by influenced indirectly by these proteins.

Challenges for the Future

Although significant progress has been made in identifying links between alternative splicing and neurologic disease, in many cases our comprehension of the significance of these links and the molecular basis for these diseases is far from complete. Splicing abnormalities have been noted in complex polygenic disorders, including schizophrenia and other psychiatric disorders (Black and Grabowski, 2003; Clinton et al., 2003). However, the causes of these splicing defects and their contributions to disease pathogenesis have not been determined.

More generally, there remains a large body of neurologic disorders without a known pathophysiology. Given that alternative splicing contributes to the regulation of virtually all aspects of biology, from transcription and translation to neuronal excitation and inhibition, at the current time virtually every aspect of neurologic disease could be considered as having a potential component of alternative splicing-mediated pathophysiology. In order for splicing to be able to be linked to disease pathophysiology, it will first be necessary to identify candidate alternate exons that are correlated with disease, a process that will be greatly aided by the development of high throughput technologies such as splicing arrays. Second, it will be necessary to generate animal models in which such splicing changes recapitulate a disease process. For a complete pathophysiologic understanding of such correlations, it will be necessary to understand the function of proteins at the exonic level and correlate splicing changes with physiologic changes.

The application of genetic, bioinformatic, and biochemical approaches should continue to enhance our understanding of the role of alternative splicing in neurobiology and neurologic disease. For example, a recent study employing an unbiased screen to define the "ataxia-ome," a protein-protein interaction network of proteins that are involved in human cerebellar ataxias, found that many ataxia-causing proteins interact directly or indirectly with one another and with a common set of proteins, consistent with the fact that many neurodegenerative disorders share similar phenotypes (Lim et al., 2006). Remarkably, proteins involved in RNA binding and splicing (including Fox-1/A2bp1) were found to interact with many ataxia-causing proteins, suggesting that alternative splicing defects may contribute to the pathogenicity of various ataxias. Additional support for this model comes from studies demonstrating that alternative splicing factors can function as modifiers of disease severity (Nissim-Rafinia and Kerem, 2005). Similar attempts to identify proteins that interact with splicing factors, as well as their downstream pre-mRNA targets, will better enable us to understand and perhaps predict the functional consequences of compromising these factors in disease.

Together, these efforts will provide the foundation for the development of therapeutic strategies aimed at correcting splicing defects that are present in human diseases in an attempt to improve human health. Exciting steps have been taken in recent years toward the development of therapeutic strategies to correct the aberrant splicing programs that underlie disease. In contrast to conventional gene therapy approaches involving the introduction and expression of large genomic or cDNA fragments, many efforts have focused on the targeting and repair of endogenous pre-mRNA to allow the generation of a preferred spliced isoform. One scheme involves *trans*-splicing of an endogenous pre-mRNA to an exogenous transcript in order to create a chimeric molecule. Two different *trans*-splicing methods have been developed which differ primarily in the mechanism

by which the two disparate RNAs are ligated together. One approach requires the activity of the spliceosome to join the two RNAs, whereas the other requires the activity of a ribozyme that is encoded within the exogenous RNA itself (Puttaraju et al., 1999; Sullenger and Cech, 1994). A second strategy to repair aberrant splicing patterns is through the introduction of chimeric molecules consisting of an RNA complimentary to a specific pre-mRNA sequence and a second domain that can either directly affect pre-mRNA splicing or recruit endogenous factors that can do so (Cartegni and Krainer, 2003; Skordis et al., 2003). Although it remains to be determined whether these approaches will provide a useful therapeutic tool to combat neurologic disease in humans, considerable progress has been made in demonstrating that efforts to reprogram pre-mRNA splicing patterns in cell-culture systems and mouse models of disease can succeed.

Acknowledgments

We thank Kevin O'Donovan, Graeme Couture, and Jernej Ule for critical review of the manuscript. This work was supported by the NIH (R01 NS34389 and NS40955 to R.B.D.) and the Howard Hughes Medical Institute. R.B.D. is an Investigator of the Howard Hughes Medical Institute.

References

Ahn, A.H., and Kunkel, L.M. (1993). The structural and functional diversity of dystrophin. Nat. Genet. *3*, 283–291.

Andreadis, A. (2005). Tau gene alternative splicing: expression patterns, regulation and modulation of function in normal brain and neurodegenerative diseases. Biochim. Biophys. Acta. *1739*, 91–103.

Ars, E., Serra, E., Garcia, J., Kruyer, H., Gaona, A., Lazaro, C., and Estivill, X. (2000). Mutations affecting mRNA splicing are the most common molecular defects in patients with neurofibromatosis type 1. Hum. Mol. Genet. *9*, 237–247.

Bao, Y.P., Cook, L.J., O'Donovan, D., Uyama, E., and Rubinsztein, D.C. (2002). Mammalian, yeast, bacterial, and chemical chaperones reduce aggregate formation and death in a cell model of oculopharyngeal muscular dystrophy. J. Biol. Chem. *277*, 12263–12269.

Beroud, C., Carrie, A., Beldjord, C., Deburgrave, N., Llense, S., Carelle, N., Peccate, C., Cuisset, J.M., Pandit, F., Carre-Pigeon, F., et al. (2004). Dystrophinopathy caused by mid-intronic substitutions activating cryptic exons in the DMD gene. Neuromuscul. Disord. *14*, 10–18.

Bhalla, K., Phillips, H.A., Crawford, J., McKenzie, O.L., Mulley, J.C., Eyre, H., Gardner, A.E., Kremmidiotis, G., and Callen, D.F. (2004). The de novo chromosome 16 translocations of two patients with abnormal phenotypes (mental retardation and epilepsy) disrupt the A2BP1 gene. J. Hum. Genet. *49*, 308–311.

Black, D.L. (2003). Mechanisms of alternative pre-messenger RNA splicing. Annu. Rev. Biochem. *72*, 291–336.

Black, D.L., and Grabowski, P.J. (2003). Alternative pre-mRNA splicing and neuronal function. Prog. Mol. Subcell. Biol. *31*, 187–216.

Blencowe, B.J. (2006). Alternative splicing: new insights from global analyses. Cell *126*, 37–47.

Boucard, A.A., Chubykin, A.A., Comoletti, D., Taylor, P., and Sudhof, T.C. (2005). A splice code for trans-synaptic cell adhesion mediated by binding of neuroligin 1 to alpha- and beta-neurexins. Neuron *48*, 229–236.

Bradley, J., Carter, S.R., Rao, V.R., Wang, J., and Finkbeiner, S. (2006). Splice variants of the NR1 subunit differentially induce NMDA receptor-dependent gene expression. J. Neurosci. *26*, 1065–1076.

Brais, B., Bouchard, J.P., Xie, Y.G., Rochefort, D.L., Chretien, N., Tome, F.M., Lafreniere, R.G., Rommens, J.M., Uyama, E., Nohira, O., et al. (1998). Short GCG expansions in the PABP2 gene cause oculopharyngeal muscular dystrophy. Nat. Genet. *18*, 164–167.

Buee, L., Bussiere, T., Buee-Scherrer, V., Delacourte, A., and Hof, P.R. (2000). Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res. Brain Res. Rev. *33*, 95–130.

Caceres, J.F., and Kornblihtt, A.R. (2002). Alternative splicing: multiple control mechanisms and involvement in human disease. Trends Genet. *18*, 186–193.

Carissimi, C., Saieva, L., Baccon, J., Chiarella, P., Maiolica, A., Sawyer, A., Rappsilber, J., and Pellizzoni, L. (2006). Gemin8 is a novel component of the survival motor neuron complex and functions in small nuclear ribonucleoprotein assembly. J. Biol. Chem. *281*, 8126–8134.

Cartegni, L., and Krainer, A.R. (2003). Correction of disease-associated exon skipping by synthetic exon-specific activators. Nat. Struct. Biol. *10*, 120–125.

Cartegni, L., Chew, S.L., and Krainer, A.R. (2002). Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nat. Rev. Genet. *3*, 285–298.

Cartegni, L., Hastings, M.L., Calarco, J.A., de Stanchina, E., and Krainer, A.R. (2006). Determinants of exon 7 splicing in the spinal muscular atrophy genes, SMN1 and SMN2. Am. J. Hum. Genet. *78*, 63–77.

Chakarova, C.F., Hims, M.M., Bolz, H., Abu-Safieh, L., Patel, R.J., Papaioannou, M.G., Inglehearn, C.F., Keen, T.J., Willis, C., Moore, A.T., et al. (2002). Mutations in HPRP3, a third member of pre-mRNA splicing factor genes, implicated in autosomal dominant retinitis pigmentosa. Hum. Mol. Genet. *11*, 87–92.

Clinton, S.M., Haroutunian, V., Davis, K.L., and Meador-Woodruff, J.H. (2003). Altered transcript expression of NMDA receptor-associated postsynaptic proteins in the thalamus of subjects with schizophrenia. Am. J. Psychiatry *160*, 1100–1109.

D'Souza, I., Poorkaj, P., Hong, M., Nochlin, D., Lee, V.M., Bird, T.D., and Schellenberg, G.D. (1999). Missense and silent tau gene mutations cause frontotemporal dementia with parkinsonism-chromosome 17 type, by affecting multiple alternative RNA splicing regulatory elements. Proc. Natl. Acad. Sci. USA *96*, 5598–5603.

Daoud, R., Da Penha Berzaghi, M., Siedler, F., Hubener, M., and Stamm, S. (1999). Activity-dependent regulation of alternative splicing patterns in the rat brain. Eur. J. Neurosci. *11*, 788–802.

Disset, A., Bourgeois, C.F., Benmalek, N., Claustres, M., Stevenin, J., and Tuffery-Giraud, S. (2006). An exon skipping-associated nonsense mutation in the dystrophin gene uncovers a complex interplay between multiple antagonistic splicing elements. Hum. Mol. Genet. *15*, 999–1013.

Eggert, C., Chari, A., Laggerbauer, B., and Fischer, U. (2006). Spinal muscular atrophy: the RNP connection. Trends Mol. Med. *12*, 113–121.

Ehlers, M.D., Zhang, S., Bernhadt, J.P., and Huganir, R.L. (1996). Inactivation of NMDA receptors by direct interaction of calmodulin with the NR1 subunit. Cell *84*, 745–755.

Faustino, N.A., and Cooper, T.A. (2003). Pre-mRNA splicing and human disease. Genes Dev. *17*, 419–437.

Gabellini, D., D'Antona, G., Moggio, M., Prelle, A., Zecca, C., Adami, R., Angeletti, B., Ciscato, P., Pellegrino, M.A., Bottinelli, R., et al. (2006). Facioscapulohumeral muscular dystrophy in mice overexpressing FRG1. Nature *439*, 973–977.

Glatz, D.C., Rujescu, D., Tang, Y., Berendt, F.J., Hartmann, A.M., Faltraco, F., Rosenberg, C., Hulette, C., Jellinger, K., Hampel, H., et al. (2006). The alternative splicing of tau exon 10 and its regulatory proteins CLK2 and TRA2-BETA1 changes in sporadic Alzheimer's disease. J. Neurochem. *96*, 635–644.

Goren, A., Ram, O., Amit, M., Keren, H., Lev-Maor, G., Vig, I., Pupko, T., and Ast, G. (2006). Comparative analysis identifies exonic splicing regulatory sequences–The complex definition of enhancers and silencers. Mol. Cell *22*, 769–781.

Grabowski, P.J., and Black, D.L. (2001). Alternative RNA splicing in the nervous system. Prog. Neurobiol. *65*, 289–308.

Grover, A., Houlden, H., Baker, M., Adamson, J., Lewis, J., Prihar, G., Pickering-Brown, S., Duff, K., and Hutton, M. (1999). 5′ splice site

mutations in tau associated with the inherited dementia FTDP-17 affect a stem-loop structure that regulates alternative splicing of exon 10. J. Biol. Chem. *274*, 15134–15143.

Gubitz, A.K., Feng, W., and Dreyfuss, G. (2004). The SMN complex. Exp. Cell Res. *296*, 51–56.

Hagerman, P.J., and Hagerman, R.J. (2004). The fragile-X premutation: a maturing perspective. Am. J. Hum. Genet. *74*, 805–816.

Han, K., Yeo, G., An, P., Burge, C.B., and Grabowski, P.J. (2005). A combinatorial code for splicing silencing: UAGG and GGGG motifs. PLoS Biol. *3*, e158. 10.1371/journal.pbio.0030158.

Hasegawa, M., Smith, M.J., Iijima, M., Tabira, T., and Goedert, M. (1999). FTDP-17 mutations N279K and S305N in tau produce increased splicing of exon 10. FEBS Lett. *443*, 93–96.

Holmes, S.E., O'Hearn, E., Rosenblatt, A., Callahan, C., Hwang, H.S., Ingersoll-Ashworth, R.G., Fleisher, A., Stevanin, G., Brice, A., Potter, N.T., et al. (2001). A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. Nat. Genet. *29*, 377–378.

Hsieh-Li, H.M., Chang, J.G., Jong, Y.J., Wu, M.H., Wang, N.M., Tsai, C.H., and Li, H. (2000). A mouse model for spinal muscular atrophy. Nat. Genet. *24*, 66–70.

Huang, C.S., Shi, S.H., Ule, J., Ruggiu, M., Barker, L.A., Darnell, R.B., Jan, Y.N., and Jan, L.Y. (2005). Common molecular pathways mediate long-term potentiation of synaptic excitation and slow synaptic inhibition. Cell *123*, 105–118.

Iwahashi, C.K., Yasui, D.H., An, H.J., Greco, C.M., Tassone, F., Nannen, K., Babineau, B., Lebrilla, C.B., Hagerman, R.J., and Hagerman, P.J. (2006). Protein composition of the intranuclear inclusions of FXTAS. Brain *129*, 256–271.

Jensen, K.B., Dredge, B.K., Stefani, G., Zhong, R., Buckanovich, R.J., Okano, H.J., Yang, Y.Y., and Darnell, R.B. (2000). Nova-1 regulates neuron-specific alternative splicing and is essential for neuronal viability. Neuron *25*, 359–371.

Jiang, H., Mankodi, A., Swanson, M.S., Moxley, R.T., and Thornton, C.A. (2004). Myotonic dystrophy type 1 is associated with nuclear foci of mutant RNA, sequestration of muscleblind proteins and deregulated alternative splicing in neurons. Hum. Mol. Genet. *13*, 3079–3088.

Jiang, Z., Tang, H., Havlioglu, N., Zhang, X., Stamm, S., Yan, R., and Wu, J.Y. (2003). Mutations in tau gene exon 10 associated with FTDP-17 alter the activity of an exonic splicing enhancer to interact with Tra2 beta. J. Biol. Chem. *278*, 18997–19007.

Jin, P., and Warren, S.T. (2003). New insights into fragile X syndrome: from molecules to neurobehaviors. Trends Biochem. Sci. *28*, 152–158.

Kanadia, R.N., Shin, J., Yuan, Y., Beattie, S.G., Wheeler, T.M., Thornton, C.A., and Swanson, M.S. (2006). Reversal of RNA missplicing and myotonia after muscleblind overexpression in a mouse poly(CUG) model for myotonic dystrophy. Proc. Natl. Acad. Sci. USA *103*, 11748–11753.

Kashima, T., and Manley, J.L. (2003). A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. Nat. Genet. *34*, 460–463.

Kishore, S., and Stamm, S. (2006). The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. Science *311*, 230–232.

Koob, M.D., Moseley, M.L., Schut, L.J., Benzow, K.A., Bird, T.D., Day, J.W., and Ranum, L.P. (1999). An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). Nat. Genet. *21*, 379–384.

Koushika, S.P., Soller, M., and White, K. (2000). The neuron-enriched splicing pattern of Drosophila erect wing is dependent on the presence of ELAV protein. Mol. Cell. Biol. *20*, 1836–1845.

Krawczak, M., Reiss, J., and Cooper, D.N. (1992). The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. Hum. Genet. *90*, 41–54.

Lee, V.M., Goedert, M., and Trojanowski, J.Q. (2001). Neurodegenerative tauopathies. Annu. Rev. Neurosci. *24*, 1121–1159.

Lefebvre, S., Burglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M., et al. (1995). Identification and characterization of a spinal muscular atrophy-determining gene. Cell *80*, 155–165.

Leroy, O., Wang, J., Maurage, C.A., Parent, M., Cooper, T., Buee, L., Sergeant, N., Andreadis, A., and Caillet-Boudin, M.L. (2006). Brain-specific change in alternative splicing of Tau exon 6 in myotonic dystrophy type 1. Biochim. Biophys. Acta *1762*, 460–467.

Lim, J., Hao, T., Shaw, C., Patel, A.J., Szabo, G., Rual, J.F., Fisk, C.J., Li, N., Smolyar, A., Hill, D.E., et al. (2006). A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. Cell *125*, 801–814.

Lipscombe, D. (2005). Neuronal proteins custom designed by alternative splicing. Curr. Opin. Neurobiol. *15*, 358–363.

Martins, S., Seixas, A.I., Magalhaes, P., Coutinho, P., Sequeiros, J., and Silveira, I. (2005). Haplotype diversity and somatic instability in normal and expanded SCA8 alleles. Am. J. Med. Genet. B. Neuropsychiatr. Genet. *139*, 109–114.

McKie, A.B., McHale, J.C., Keen, T.J., Tarttelin, E.E., Goliath, R., van Lith-Verhoeven, J.J., Greenberg, J., Ramesar, R.S., Hoyng, C.B., Cremers, F.P., et al. (2001). Mutations in the pre-mRNA splicing factor gene PRPC8 in autosomal dominant retinitis pigmentosa (RP13). Hum. Mol. Genet. *10*, 1555–1562.

Missler, M., and Sudhof, T.C. (1998). Neurexins: three genes and 1001 products. Trends Genet. *14*, 20–26.

Monani, U.R. (2005). Spinal muscular atrophy: a deficiency in a ubiquitous protein; a motor neuron-specific disease. Neuron *48*, 885–896.

Monani, U.R., Sendtner, M., Coovert, D.D., Parsons, D.W., Andreassi, C., Le, T.T., Jablonka, S., Schrank, B., Rossol, W., Prior, T.W., et al. (2000). The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn(−/−) mice and results in a mouse with spinal muscular atrophy. Hum. Mol. Genet. *9*, 333–339.

Moseley, M.L., Zu, T., Ikeda, Y., Gao, W., Mosemiller, A.K., Daughters, R.S., Chen, G., Weatherspoon, M.R., Clark, H.B., Ebner, T.J., et al. (2006). Bidirectional expression of CUG and CAG expansion transcripts and intranuclear polyglutamine inclusions in spinocerebellar ataxia type 8. Nat. Genet. *38*, 758–769.

Mu, Y., Otsuka, T., Horton, A.C., Scott, D.B., and Ehlers, M.D. (2003). Activity-dependent mRNA splicing controls ER export and synaptic delivery of NMDA receptors. Neuron *40*, 581–594.

Musunuru, K., and Darnell, R.B. (2001). Paraneoplastic neurologic disease antigens: RNA-binding proteins and signaling proteins in neuronal degeneration. Annu. Rev. Neurosci. *24*, 239–262.

Nissim-Rafinia, M., and Kerem, B. (2005). The splicing machinery is a genetic modifier of disease severity. Trends Genet. *21*, 480–483.

Oostra, B.A., and Willemsen, R. (2003). A fragile balance: FMR1 expression levels. Hum. Mol. Genet. *12*(Spec No 2), R249–R257.

Pagani, F., Buratti, E., Stuani, C., Bendix, R., Dork, T., and Baralle, F.E. (2002). A new type of mutation causes a splicing defect in ATM. Nat. Genet. *30*, 426–429.

Pellizzoni, L., Kataoka, N., Charroux, B., and Dreyfuss, G. (1998). A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. Cell *95*, 615–624.

Pick, M., Flores-Flores, C., and Soreq, H. (2004). From brain to blood: alternative splicing evidence for the cholinergic basis of Mammalian stress responses. Ann. N Y Acad. Sci. *1018*, 85–98.

Puttaraju, M., Jamison, S.F., Mansfield, S.G., Garcia-Blanco, M.A., and Mitchell, L.G. (1999). Spliceosome-mediated RNA trans-splicing as a tool for gene therapy. Nat. Biotechnol. *17*, 246–252.

Ranum, L.P., and Cooper, T.A. (2006). RNA-mediated neuromuscular disorders. Annu. Rev. Neurosci. *29*, 259–277. Published online April 20, 2006. 10.1146/annurev.neuro.29.051605.113014.

Ranum, L.P., and Day, J.W. (2004a). Myotonic dystrophy: RNA pathogenesis comes into focus. Am. J. Hum. Genet. *74*, 793–804.

Ranum, L.P., and Day, J.W. (2004b). Pathogenic RNA repeats: an expanding role in genetic disease. Trends Genet. *20*, 506–512.

Rappsilber, J., Ryder, U., Lamond, A.I., and Mann, M. (2002). Large-scale proteomic analysis of the human spliceosome. Genome Res. *12*, 1231–1245.

Roberts, W.K., and Darnell, R.B. (2004). Neuroimmunology of the paraneoplastic neurological degenerations. Curr. Opin. Immunol. *16*, 616–622.

Sergeant, N., Sablonniere, B., Schraen-Maschke, S., Ghestem, A., Maurage, C.A., Wattez, A., Vermersch, P., and Delacourte, A. (2001). Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1. Hum. Mol. Genet. *10*, 2143–2155.

Skordis, L.A., Dunckley, M.G., Yue, B., Eperon, I.C., and Muntoni, F. (2003). Bifunctional antisense oligonucleotides provide a trans-acting splicing enhancer that stimulates SMN2 gene expression in patient fibroblasts. Proc. Natl. Acad. Sci. USA *100*, 4114–4119.

Soller, M., and White, K. (2003). ELAV inhibits 3′-end processing to promote neural splicing of ewg pre-mRNA. Genes Dev. *17*, 2526–2538.

Stamm, S., Ben-Ari, S., Rafalska, I., Tang, Y., Zhang, Z., Toiber, D., Thanaraj, T.A., and Soreq, H. (2005). Function of alternative splicing. Gene *344*, 1–20.

Strehler, E.E., and Zacharias, D.A. (2001). Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. Physiol. Rev. *81*, 21–50.

Sullenger, B.A., and Cech, T.R. (1994). Ribozyme-mediated repair of defective mRNA by targeted, trans-splicing. Nature *371*, 619–622.

Szabo, A., Dalmau, J., Manley, G., Rosenfeld, M., Wong, E., Henson, J., Posner, J.B., and Furneaux, H.M. (1991). HuD, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to Elav and Sex-lethal. Cell *67*, 325–333.

Taylor, J.E., Thomas, N.H., Lewis, C.M., Abbs, S.J., Rodrigues, N.R., Davies, K.E., and Mathew, C.G. (1998). Correlation of SMNt and SMNc gene copy number with age of onset and survival in spinal muscular atrophy. Eur. J. Hum. Genet. *6*, 467–474.

Teraoka, S.N., Telatar, M., Becker-Catania, S., Liang, T., Onengut, S., Tolun, A., Chessa, L., Sanal, O., Bernatowska, E., Gatti, R.A., and Concannon, P. (1999). Splicing defects in the ataxia-telangiectasia gene, ATM: underlying mutations and consequences. Am. J. Hum. Genet. *64*, 1617–1631.

Tuffery-Giraud, S., Saquet, C., Chambert, S., and Claustres, M. (2003). Pseudoexon activation in the DMD gene as a novel mechanism for Becker muscular dystrophy. Hum. Mutat. *21*, 608–614.

Ule, J., and Darnell, R.B. (2006). RNA binding proteins and the regulation of neuronal synaptic plasticity. Curr. Opin. Neurobiol. *16*, 102–110.

Ule, J., Jensen, K.B., Ruggiu, M., Mele, A., Ule, A., and Darnell, R.B. (2003). CLIP identifies Nova-regulated RNA networks in the brain. Science *302*, 1212–1215.

Ule, J., Ule, A., Spencer, J., Williams, A., Hu, J.S., Cline, M., Wang, H., Clark, T., Fraser, C., Ruggiu, M., et al. (2005). Nova regulates brain-specific splicing to shape the synapse. Nat. Genet. *37*, 844–852.

Ule, J., Stefani, G., Mele, A., Ruggiu, M., Wang, X., Taneri, B., Gaasterland, T., Blencowe, B.J., and Darnell, R.B. (2006). An RNA map predicting Nova-dependent splicing regulation. Nature, in press.

Underwood, J.G., Boutz, P.L., Dougherty, J.D., Stoilov, P., and Black, D.L. (2005). Homologues of the Caenorhabditis elegans Fox-1 protein are neuronal splicing regulators in mammals. Mol. Cell. Biol. *25*, 10005–10016.

Vallano, M.L., Beaman-Hall, C.M., and Benmansour, S. (1999). Ca2+ and pH modulate alternative splicing of exon 5 in NMDA receptor subunit 1. Neuroreport *10*, 3659–3664.

van Koningsbruggen, S., Dirks, R.W., Mommaas, A.M., Onderwater, J.J., Deidda, G., Padberg, G.W., Frants, R.R., and van der Maarel, S.M. (2004). FRG1P is localised in the nucleolus, Cajal bodies, and speckles. J. Med. Genet. *41*, e46. 10.1136/jmg 2003.012781.

Veltman, M.W., Craig, E.E., and Bolton, P.F. (2005). Autism spectrum disorders in Prader-Willi and Angelman syndromes: a systematic review. Psychiatr. Genet. *15*, 243–254.

Vithana, E.N., Abu-Safieh, L., Allen, M.J., Carey, A., Papaioannou, M., Chakarova, C., Al-Maghtheh, M., Ebenezer, N.D., Willis, C., Moore, A.T., et al. (2001). A human homolog of yeast pre-mRNA splicing gene, PRP31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (RP11). Mol. Cell *8*, 375–381.

Wang, Z., Rolish, M.E., Yeo, G., Tung, V., Mawson, M., and Burge, C.B. (2004). Systematic identification and analysis of exonic splicing silencers. Cell *119*, 831–845.

Willemsen, R., Hoogeveen-Westerveld, M., Reis, S., Holstege, J., Severijnen, L.A., Nieuwenhuizen, I.M., Schrier, M., van Unen, L., Tassone, F., Hoogeveen, A.T., et al. (2003). The FMR1 CGG repeat mouse displays ubiquitin-positive intranuclear neuronal inclusions; implications for the cerebellar tremor/ataxia syndrome. Hum. Mol. Genet. *12*, 949–959.

Winkler, C., Eggert, C., Gradl, D., Meister, G., Giegerich, M., Wedlich, D., Laggerbauer, B., and Fischer, U. (2005). Reduced U snRNP assembly causes motor axon degeneration in an animal model for spinal muscular atrophy. Genes Dev. *19*, 2320–2330.

Xie, J., and Black, D.L. (2001). A CaMK IV responsive RNA element mediates depolarization-induced alternative splicing of ion channels. Nature *410*, 936–939.

Xu, X., Yang, D., Ding, J.H., Wang, W., Chu, P.H., Dalton, N.D., Wang, H.Y., Bermingham, J.R., Jr., Ye, Z., Liu, F., et al. (2005). ASF/SF2-regulated CaMKIIdelta alternative splicing temporally reprograms excitation-contraction coupling in cardiac muscle. Cell *120*, 59–72.

Yeo, G., Holste, D., Kreiman, G., and Burge, C.B. (2004). Variation in alternative splicing across human tissues. Genome Biol. *5*, R74. Published online September 13, 2004. 10.1186/gb-2004-5-10-r74.

Young, J.I., Hong, E.P., Castle, J.C., Crespo-Barreto, J., Bowman, A.B., Rose, M.F., Kang, D., Richman, R., Johnson, J.M., Berget, S., and Zoghbi, H.Y. (2005). Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. Proc. Natl. Acad. Sci. USA *102*, 17551–17558.

Zhou, Z., Licklider, L.J., Gygi, S.P., and Reed, R. (2002). Comprehensive proteomic analysis of the human spliceosome. Nature *419*, 182–185.