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Case Report

# Knight in Splicing Armor: Alternative Splicing as a Neuroprotective Mechanism

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**Abstract**

By adjusting gene expression in response to environmental changes, cells can optimize fitness as needed. Alternative splicing is one of the most important post-transcriptional regulation steps, broadly involved in diverse physiological and pathological conditions. Here, we present 5 cases of alternative splicing conferring increased neuroprotection through diverse mechanisms. These examples highlight the enormous power of alternative splicing in maintaining viability of neurons. From pre-mRNA secondary structure alterations to exon skipping and alternative splice sites, we describe how various mechanisms can be utilized in a functionally significant manner. While these instances focus on endogenous splicing control, it highlights the therapeutic potential of modifying these genes. With the urgency for neurodegenerative disease intervention rising, these targets represent excellent targets to focus on.

**Keywords**

NMNAT; CD-33; RAGE; XBP-1; MGF; neurodegenerative diseases

## 1. Introduction

In most cases, newly transcribed precursor mRNA (pre-mRNA) will be spliced to form mature mRNA during or post transcription. In this process, the spliceosome, a complex of small nuclear RNAs and associated proteins (snRNPs), forms on splice sites in pre-mRNA to remove the introns (non-coding regions) and join together exons (coding regions). Under different conditions splicing of a pre-mRNA can occur with different combinations of splice sites, a process known as alternative splicing. Alternative splicing allows for the expression of different possible mRNA variants from a single pre-mRNA. By mixing and matching different exons, cells can diversify the protein amino acid sequences and its functions to better adapt to the intrinsic or external micro-environmental change [1, 2]. Alternative splicing plays important roles in cellular differentiation and stress response in both physiological and pathological conditions. Alternative splicing has been a subject for disease association and for therapeutics. About 88% of human genes are alternatively spliced [3], it has been reported that 22% of disease causing mutations are splicing sensitive [4], and 25% of disease-causing exonic mutations induce exon skipping [5].

Neurodegenerative diseases are a class of diseases characterized by neuronal death and/or dysfunction [6-9], such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and ALS. So far, therapies for most of these diseases are limited. With an aging population, these diseases are predicted to have a large socioeconomic impact in the near future [10]. Developing therapies for these diseases is paramount. In past decades, efforts to explore the mechanisms of these diseases were mainly focused on mutated proteins. Recently, RNA dysregulation in these conditions was discovered to be an important contributor to pathogenesis. Mechanistic understanding of dysregulation of RNA processing may reveal novel targets and provide new insights for neuroprotective therapy.

Here, we present several examples of splice variants conferring differential neuroprotection. In this review, we describe the role of splicing in modulating the neuroprotective capabilities of 5 genes- Nicotinamide Mononucleotide Adenylyltransferase (NMNAT), Cluster of Differentiation 33 (CD33), Receptor for Advanced Glycation End-products (RAGE), X-Box Binding Protein-1 (XBP-1), and Mechano-Growth Factor (MGF). All of these genes have been demonstrated to protect against neurodegenerative diseases in a robust, splicing-dependent manner. NMNAT is a conserved enzyme whose neuroprotection has been demonstrated in a variety of disease models in many species. We describe how *Drosophila Nmnat* pre-mRNA splicing is determined by a microRNA disrupting its secondary structure in order to upregulate the more neuroprotective NMNAT variant. A CD33 variant which skips an exon has been linked to reduced risk of Late-Onset Alzheimer's disease. The soluble isoform of RAGE is produced via an alternative 3' splice site, and results in decreased Amyloid beta (Aβ) uptake in Alzheimer's. XBP-1 (X Box binding protein 1) pre-mRNA is normally localized to the endoplasmic reticulum. During stress conditions, it is spliced and translated to its active form, a transcription factor which can activate pro-neuroprotective targets. MGF is a variant of Insulin-like Growth factor which results from a frame shift coupled to the inclusion of an exon. This variant has demonstrated protection in many models for neurodegenerative diseases, such as Amyotrophic lateral sclerosis (ALS), ischemia, and MPP+ (1-methyl-4-phenylpyridinium) treatment. With there already being reports of developing

therapeutics to influence splice variants [11, 12], these genes present excellent opportunities to combat neurodegenerative diseases.

## 2. Mutually Exclusive Last Exons-NMNAT

Nicotinamide mononucleotide adenylyltransferase (NMNAT) is a housekeeping enzyme that is ubiquitous across the biosphere. It is the sole enzyme responsible for the synthesis of nicotinamide adenine dinucleotide ($NAD^+$) from Nicotinamide mononucleotide (NMN) in all living organisms [13]. Its highly conserved and essential nature makes it a central player in many processes throughout the biosphere.

In addition to its enzymatic role, NMNAT proteins are essential for neuronal maintenance and protection against neurodegeneration [12-24]. Specifically in models of proteinopathies, NMNAT has been found to function as a chaperone [14], and protect against neurodegeneration through direct interaction with disease-associated proteins, including Ataxin-1, Tau [15, 16] and Huntingtin [17]. NMNAT was able to protect neurons even when enzyme deactivated through mutation, showing the importance of its chaperone functions in neuroprotection [18]. NMNAT has also been shown to confer robust resistance against various stressors across many species, including hypoxia [19, 20], heat [19], oxidative stress [21, 22], disease states [14-16, 23], and aging [24]. Indeed, NMNAT proteins can serve as a general protector to improve cellular viability.

*Drosophila melanogaster* has a single *Nmnat* gene [25] which is alternatively spliced into two distinct mRNA variants, RA and RB, giving rise to protein isoforms $NMNAT^{PC}$ and $NMNAT^{PD}$, respectively [23].While NMNAT has an alternate distal start codon which can translate to two additional protein isoforms, $NMNAT^{PA}$ and $NMNAT^{PB}$, the proximal start site is strongly preferred. Isoforms $NMNAT^{PC}$ and $NMNAT^{PD}$ share similar enzymatic properties but have distinct subcellular localizations and divergent neuroprotective functions; isoform $NMNAT^{PD}$ is cytosolic and is a better neuroprotector than the RA-translated nuclear $NMNAT^{PC}$ isoform [23]. $NMNAT^{PD}$ has been shown to protect against spinocerebellar ataxia-1, while $NMNAT^{PC}$ exacerbated it [23].

The divergent function of $NMNAT^{PC}$ and $NMNAT^{PD}$ protein isoforms posts an intriguing question regarding the regulation of isoform-specific expression. *Drosophila* NMNAT pre-mRNA has 7 exons [23] (Figure 1). The first 4 exons are commonly spliced to the two RNA variants, RA and RB. While NMNAT pre-mRNA is slightly preferentially spliced to variant RA during basal conditions, it has been shown that the splicing of NMNAT pre-mRNA is shifted to variant RB in response to stress. A heat shock response element has been identified in the promoter of *Drosophila Nmnat*, allowing transcriptional upregulation of NMNAT pre-mRNA during stress conditions by transcription factor Heat Shock Factor (HSF) [19]. This is significant as it allows for the upregulation of a stress resistance factor in response to stress. However, this only accounts for the upregulation of NMNAT pre-mRNA, and doesn't explain the variant specific stress response. While NMNAT pre-mRNA transcription is upregulated in response to stress, only NMNAT splice variant RB is increased in stress conditions. Thus, a post-transcriptional "switch" is activated to promote the splicing of NMNAT pre-mRNA to RB and to serve as an effective homeostasis strategy to increase the production of the more protective protein $NMNAT^{PD}$ isoform under stress [23].

Key sequences regulating splicing are present in the $4^{th}$ intron and $5^{th}$ exon, termed Box 1 (Cyan in Figure 1) and Box 2 (Blue), respectively [26]. When these two complementary sequences bind, they form a stem-loop structure. This brings the 3' splice site of exon 6 closer to exon 4, allowing

for the splicing to RA (On bottom, with green exons 6 and 7). When the stem-loop doesn't form, the 4th exon is preferentially spliced into the 3' SS of the 5th exon (Light red), producing RB. We recently identified a direct role of microRNA miR-1002 in regulating alternative splicing [27]. Specifically, miR-1002 binds to a sequence in the pre-mRNA of NMNAT which overlaps with Box 2 and prevents the formation of the stem-loop from Box 1 and Box 2, and promotes splicing towards the RB variant. Importantly, both NMNAT pre-mRNA and miR-1002 transcription is upregulated under stress. This increases the production of the more neuroprotective NMNAT$^{PD}$ protein isoform and confers stress resistance [27]. Such microRNA-mediated regulation of alternative splicing increases the repertoire of transcriptional regulation and allow a rapid response to stress.

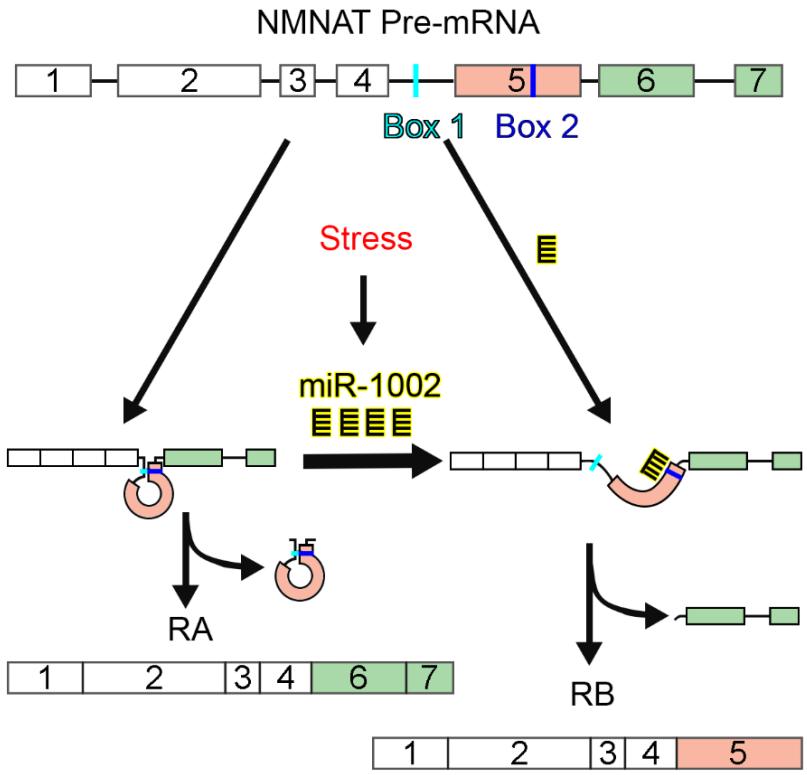


**Figure 1** Stress can induce splicing-mediated neuroprotection for NMNAT. NMNAT pre-mRNA can be alternatively spliced to the RA or RB variants. This is mediated by the presence or absence of a pre-mRNA stem-loop structure which facilitates the exclusion of the RB-exclusive 5th exon. In stress conditions, miR-1002 binds to complementary sequences in the pre-mRNA and prevents the formation of the stem-loop structure. This pushes alternative splicing towards RB, which translates to the more neuroprotective PD protein isoform.

It remains to be seen how prevalent this mechanism is. While this is the only known example of a microRNA directly regulating mRNA splicing, there is ample opportunity for therapeutic development. It has already been shown that dsRNAs can be utilized to correct the splicing of a disease-causing transcript, SMN2 [11]. Furthermore, the first FDA approved drug for Spinal Muscular Atrophy (SMA) is Antisense Oligonucleotides which target SMN2 splicing [28-30]. Using small oligonucleotides to correct aberrant splicing is an attractive avenue for further research.

## 3. Skipping a LOAD: CD33 and Exon Skipping

CD33, or Siglec-3, is a cell surface receptor that is part of the Siglecs family of type I transmembrane immunoglobulin-like lectins that bind sialic acids [31]. Primarily expressed by monocytes, hematopoietic cells, and microglia, it is an immunomodulator which can bind sialic acid via an extracellular N-terminal V-set immunoglobulin domain [32-34]. CD33 pre-mRNA has 7 exons, of which the 2nd exon encodes for the extracellular IG V-set domain [35] (Figure 2).

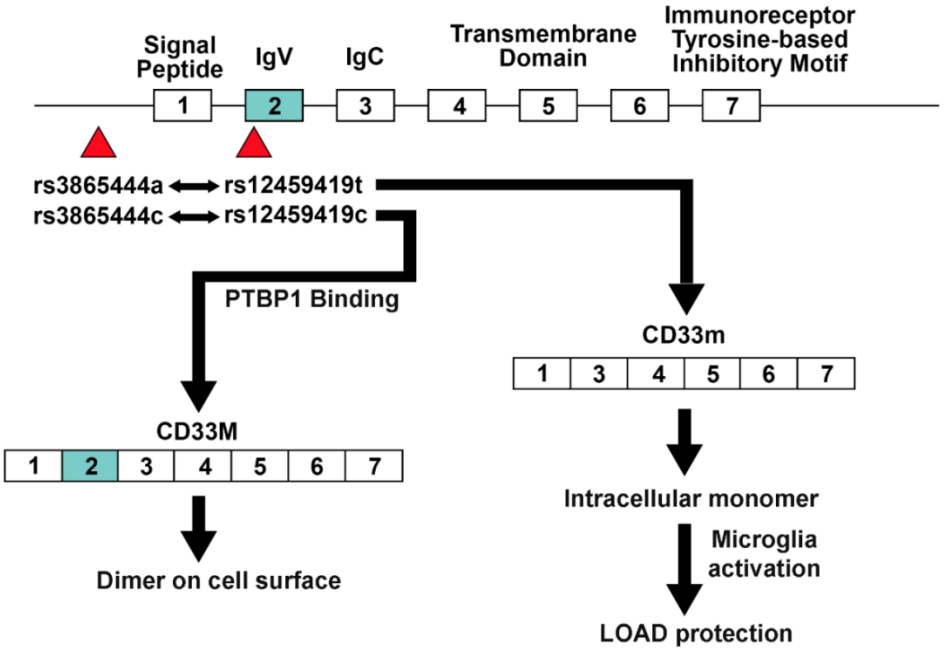


**Figure 2** Two SNPs result in differential splicing of CD33. CD33's seven exons can be spliced to the full CD33M with all seven exons, whose protein forms a dimer on the cell surface. When the second exon is spliced out, the resulting CD33m protein remains a monomer and protects against LOAD. Two SNPs in the 2nd exon (rs12459419t and rs12459419c) affect the splicing of CD33 pre-mRNA differently, with the latter allowing PTBP1 binding to include the 2nd exon. These SNPs were discovered when they were found to be in linkage disequilibrium with two SNPs in the promoter region, with rs12459419t being associated with rs3865444a and rs12459419c with rs3865444c. The original GWAS study linked rs3865444a with lower rates of LOAD compared to rs3865444c.

CD33's role in Late-onset Alzheimer's disease (LOAD) first came to prominence as a result of 2 GWAS studies [36, 37]. These studies identified a SNP in the proximal promoter of CD33, rs3865444, as a locus associated with LOAD. While rs3865444C is associated with a higher risk of LOAD, rs3865444A is protective. Later, it was discovered that rs3865444 is in complete linkage disequilibrium with rs12459419, another SNP in the 2nd exon of CD33 [38]. Further investigation lead to the discovery that this SNP affects the splicing of CD33, which in turn affects its neuroprotective efficacy [35, 38-40]

The SNP rs12459419C in the 4th base of exon 2, which is linked to rs3865444C, promotes exon 2 inclusion and splicing of the CD33M variant. rs12459419T, which is linked to rs3865444A and is

associated with LOAD protection, promotes exon 2 exclusion and splicing of the CD33m variant [41]. The exclusion of the 2nd exon is likely facilitated by the inhibition of Polypyrimidine tract binding protein 1 (PTBP1) binding due to the SNP [42].

The exclusion of the 2nd exon and its encoded IG-V domain results in an intracellular isoform of CD33 [43]. This is likely due to the loss of a disulfide bond involving the V-set domain, which is important for CD33 dimer formation. Lack of this CD33 dimer leads to an inability to mobilize to the cell surface and creates an intracellular pool of CD33m. This intracellular pool facilitates the activation of TREM2 in microglia, which in turn activates microglia for Aβ clearance [44].

The increased amount of CD33 on the cell surface is associated with AD pathology [34]. In fact, deletion of CD33 in mice has been proven to be protective [44]. The minor allele CD33m has been shown to inhibit the uptake of Aβ by microglia [33].

CD33 has been considered as a target for therapies, including CRISPR/cas9 deletion of the 2nd exon [45], antibody-drug conjugate gemtuzumab ozogamicin [46], and CD33/CD3-bispecific BiTE antibody construct [47]. The CRISPR/cas9 deletion showed efficient excision of the second exon and engraftment of knockout hematopoetic stem cells into mice, and the latter two antibody-based drugs showed efficacy against acute myeloid leukemia. These results demonstrate that CD33 modulation is feasible, and a potential pathway to neuroprotection.

## 4. Warrior's RAGE: Alternative 3' Splice Site of RAGE

Receptor for Advanced Glycation End-products (RAGE) is a multi-ligand receptor that is a part of the immunoglobulin superfamily and is implicated in Aβ uptake in Alzheimer's disease [48]. RAGE is expressed in many cell types including macrophages, muscle cells, Purkinje cells, astrocytes, and hippocampal neurons [49-52]. Its protein expression has been reported to be increased in the brains and microglia of patients with AD [49-51]. RAGE can activate APP cleavage via activation of Nuclear factor of activated T cells-1 (NFAT1) [53] or through the p38-MAPK and GSK3β pathway [54]. RAGE is also implicated in inflammation via microglia-mediated stress response [55]. RAGE has been shown to mediate the expression of proinflammatory cytokines at the [51], and its expression in microglia increases production of IL-1β and TNF-α in AD-model mice [56]. Treatment of a mouse model of AD with RAGE-specific inhibitor FPS-ZM1 prevented the influx of Aβ into the brain [57]. Consistently, overexpression of RAGE exacerbated cognitive decline in AD-model mice [58]. In addition, inhibition of RAGE in microglia prevented oxygen/glucose deprivation-induced synaptic dysfunction [59].

RAGE pre-mRNA has 11 exons, giving rise to a possible 13 variants through alternative splicing [60] (Figure 3). Of particular interest is endogenous secretory RAGE (esRAGE or RAGE_v1) and membrane-bound RAGE (mRAGE). esRAGE is a shorter, secretary form of RAGE, formed through the exclusion of exon 10 and inclusion of a portion of intron 9, mediated by an alternative 3' splice site in the 9th intron, which results in an exclusion of the transmembrane domain [61]. esRAGE acts as a decoy receptor for mRAGE, competitively binding to ligands and attenuating cellular toxicity. This soluble form of RAGE has been shown to be reduced in the plasma of patients with AD, or ischemic stroke patients with dementia, schizophrenia, and MCI (Mild cognitive impairment) [62-68]. esRAGE has been shown to be protective relative to mRAGE in Alzheimer's disease. This protection has been attributed to decreased uptake of glycated Aβ and tau at the BBB [69, 70].

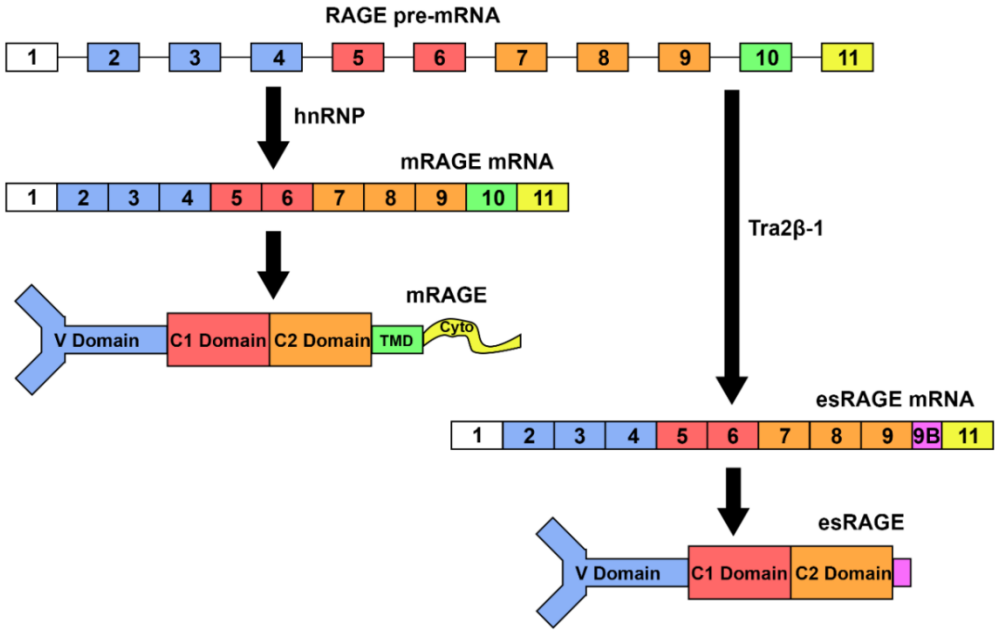


**Figure 3** A retained intron removed the transmembrane domain of RAGE, producing the soluble esRAGE. RAGE pre-mRNA contains 11 exons, with the 10$^{th}$ exon encoding a TMD. hnRNP binding to the pre-mRNA promotes splicing towards the inclusion of all 11 exons in the mature transcript. This produces the membrane bound mRAGE due to the presence of the TMD. Tra2β-1 binding to the pre-mRNA induces the retention of a part of intron 9 and the skipping of exon 10. The resulting protein, esRAGE, lacks a TMD and is neuroprotective.

esRAGE/mRAGE splicing is mediated by Tra2β-1 and hnRNP [71]. G-rich elements in exon 9B are bound by hnRNP H/A1, promoting the splicing of the upstream 5' splice site, and production of mRAGE [71]. On the other hand, Tra2β-1 upregulates the production of esRAGE [72]. Both Tra2β-1 and hnRNP A1 dysfunction has been reported for Alzheimer's disease patients [73, 74].

Disease modifying drugs directed at multiple sclerosis have been shown to increase esRAGE serum levels in MS patients [75]. Pioglitazone has been shown to increase circulating esRAGe in type 2 diabetic patients [76], while insulin has been shown to increase esRAGE *in vitro* [77]. Further work with these therapeutics may prove to be beneficial in the fight against neurodegenerative diseases.

## 5. Splicing itself as Signal-XBP-1

X-Box Binding protein-1 (XBP-1) is a leucine zipper transcription factor involved in the Unfolded Protein Response (UPR) stress response. XBP-1 exists in 2 forms, XBP1u (unspliced) and XBP1s (spliced). In contrast to other transcripts described in this review, XBP-1 is activated through splicing itself (Figure 4). XBP1 pre-mRNA is localized to the ER in basal conditions. In response to various stresses, XBP-1 is spliced to mRNA and translated into active form XBP-1s. XBP-1s translocates to the nucleus to activate many stress response genes [78-82]. Almost all XBP-1 activation pathways converge onto IRE1α. When various signals reach the ER, Binding immunoglobulin protein (BiP) dissociates from IRE1α [83, 84]. IRE1α then dimerizes and

autophosphorylates to activate itself [85-89]. This results in IRE1α unconventionally splicing the ER-localized XBP-1 transcript [90, 91]. The spliced transcript is then translated before translocation of the active XBP-1 protein to the nucleus.

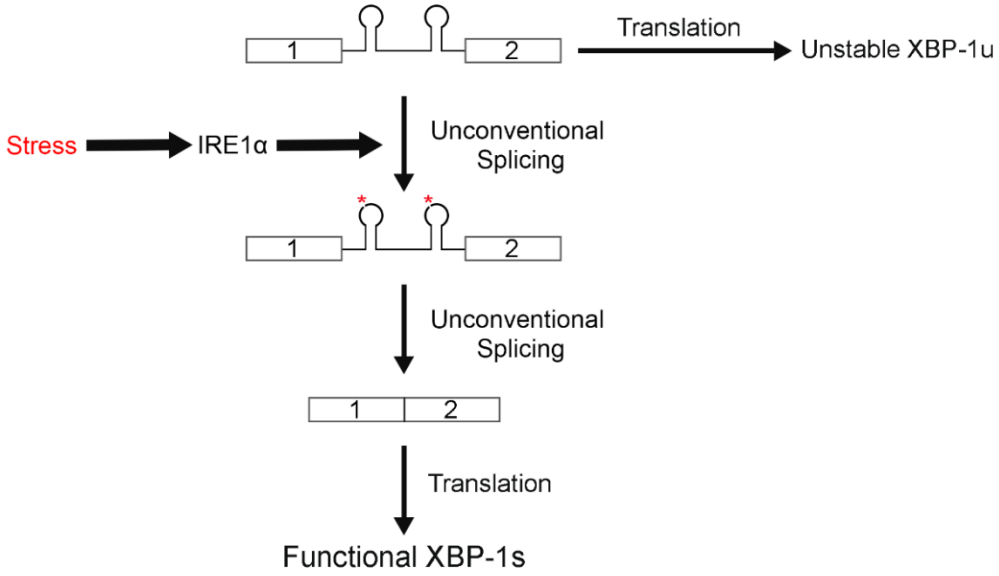


**Figure 4** Stress induces the IRE1α-mediated splicing of XBP-1 mRNA. Unspliced XBP-1 mRNA is localized to the ER during basal conditions. IRE1α is activated by stress, and induces the unconventional splicing of XBP-1 mRNA to its fully processed form. This leads to the translation of XBP-1 to its active protein, which is a transcription factor that translocated to the nucleus and activates many neuroprotective genes. When unspliced XBP-1 is translated, the resulting protein is quickly degraded.

Under normal conditions, XBP-1 transcript is unspliced, and the translated product from unspliced transcript is rapidly degraded [92]. Thus, only the fully spliced form can produce functional protein. A removal of a 26-nucleotide intron from XBP1u by IRE1α is required to induce a frameshift that will produce the functional XBP1s spliced variant [78]. This intron removal is not carried out through conventional spliceosome-mediated splicing, but through unconventional splicing [83, 84]. Specifically, IRE1α cleaves XBP1u at 2 specific positions at 2 separate stem-loop structures. This removes the intron before phosphorylation of the 5' end of the 5$^{th}$ exon, then ligation forms the final XBP1s product. It is important to note that the unconventional splicing of XBP-1 has also been observed to occur in the nucleus in an ER-stress-independent manner [93].

XBP-1 translocation to the nucleus has many roles in gene activation. XBP-1 can regulate memory formation through Brain-derived neurotrophic factor (BDNF) and kalirin-7 [95, 96]. It has also been found to be protective in oxygen/glucose deprivation [97], α-syn-induced neurodegeneration [98], 6-hydroxydopamine (6-OHDA) [99], MPP+ and MPTP induced Parkinson's models [100], mutant Huntingtin's inclusions [101], stroke [102], and in the Retinal Ganglion Cells of Experimental autoimmune encephalomyelitis (EAE) mouse Multiple Sclerosis model [103]. It is likely that many of XBP-1's protective qualities are due to its decreasing proteotoxic load by activating autophagy and lysosomes [104]. Furthermore, XBP-1 is likely involved in contextual memory formation [95, 96, 105] and axonal guidance [106],

However, XBP-1 is double-edged sword. There is evidence suggesting that multiple myeloma is sensitized by knockdown of XBP-1 [107]. Similarly, XBP-1 knockdown sensitized glioma cells to Reactive Oxygen Species (ROS) induced death [108]. Contradictory to other reports, ablation of XBP-1 decreased mutant Huntingtin aggregation [109], most likely due to the increase of another transcription factor, FoxO1. Mice with mutated Superoxide dismutase 1 (SOD-1) to model ALS had decreased toxicity when XBP-1 was deleted in the nervous system [110].

Due to its central role in UPR, XBP-1 has many triggers (Table 1). While many hurdles still remain, this provides hope that its splicing can be regulated in a medically feasible manner with further study.

**Table 1** XBP-1 has been reported to be regulated by many factors. Reported effectors of XBP-1 shown with its overall effect on XBP-1 activation.

| Trigger | Effect | Species/Model | Citation |
|---|---|---|---|
| **IRE1α inhibitor 4μ8C** | Inhibition | HFL1 cells | [111] |
| **ATF6** | Enhance | HeLa Cells | [78] |
| **BCL-2** | Enhance | Mouse Embryonic Fibroblast cells | [112] |
| **Single-Prolonged stress** | Enhance | Wistar rats | [113] |
| **Aminoglycosides** | Enhances | HEK cells | [114] |
| **Mutant Human TDP43** | Depletes XBP1s | Transgenic Rats | [115] |
| **MKC-3946** | Inhibits | Multiple Myeloma cells | [107] |
| **eIF2a phosphorylation** | Stabilizes mRNA | MEF cells | [116] |
| **BDNF** | Increased splicing | Mouse primary neuron | [117] |
| **Zika Virus infection** | Increased | AG6 mice | [118] |
| **Immobilization stress** | Increased | C57BL/6 Cr Slc mice | [119] |
| **Heat stress** | Increased | Primary horse muscle | [120] |
| **miR-326** | downregulation | MPTP-injected mice | [121] |
| **CDK5** | Nuclear translocation and activation through phosphorylation | Primary rat cortical neurons | [122] |

## 6. MGF - Exon Inclusion + Frameshift

Mechano-Growth Factor (MGF) is a splice variant of Insulin-like Growth Factor-1 (IGF-1). It is so named due to its upregulation in muscle during mechanical loading [123]. IGF-1 pre-mRNA has 6 exons and can produce 3 splice variants in humans and 2 in rodents (Figure 5). During IGF-1 splicing, a 49 bp insertion of the 5$^{th}$ exon (52 bp in rodents) causes a frame shift, resulting in MGF [124]. The splicing of IGF-1 to MGF is mediated by a purine-rich splicing enhancer in exon 5 [125]. SF2/ASF binds to this 18-nucleotide sequence in the pre-mRNA and increases the inclusion of the 5$^{th}$ exon. A series of deletion mutants identified this sequence as necessary for SF2/ASF binding to the transcript *in vitro*, and increased the splicing inclusion of exon 5 by almost 6 fold. What mediated the splicing of the alternative 3' splice site of the 5$^{th}$ exon to the 6$^{th}$ exon is yet unknown.

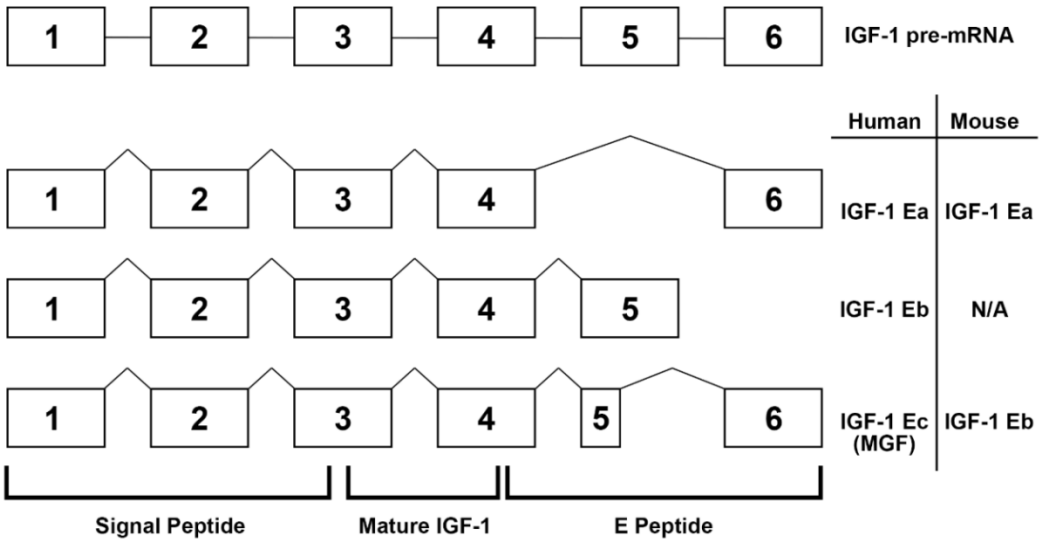


**Figure 5** IGF-1 pre-mRNA produces three splice variants in humans and two variants in mouse. The 6$^{th}$ exons in the pre-mRNA can be spliced differentially to produce proteins with different E peptides, while the mature IGF-1 peptide and single peptide remain the same. IGF-1Ea is produced from the skipping of the 5$^{th}$ exon. Skipping of the 6$^{th}$ exon results in human IGF-1Eb. An alternate 3' splice site in the 5th exon produces the human IGF-1Ec or mouse IGF-1Eb variant. The resulting E peptide is also known as MGF.

It should be noted here that the nomenclature regarding IGF-1 and MGF has been a topic of debate [126]. Humans have 3 IGF-1 splice variants- IGF-1Ea, IGF-1Eb, IGF-1Ec. Rodents have 2 IGF-1 splice variants- IGF-1Ea and IGF-1Eb [126, 127]. Human IGF-1Ec mRNA is referred to as MGF and is homologous to rodent IGF-1Eb. All variants most likely use 2 alternative leader sequences in the 1$^{st}$ exon [128]. The first 2 exons are mutually exclusive, with variants containing exon 1 are termed class 1 transcripts and those containing exon 2 are termed class 2 transcripts. When translated all IGF-1 isoforms are cleaved into 3 products: a signal peptide, the mature IGF-1 peptide, and an E peptide. The differences in the splice variants are due to the sequence encoding E peptide, the alternatively spliced 5$^{th}$ and 6$^{th}$ exons. When Human IGF-1Ec/Rodent IGF-1Eb is translated, its resulting E peptide is referred to as MGF as well.

Both the full-length MGF isoform and the E peptide have been shown to provide protection. MGF protects neurons after facial nerve avulsion in rats significantly better than the IGF-IEa [129]. This is most likely due to the MGF E-peptide activating a different set of receptors than the other E peptide variants. For example, it has been shown that MGF works through a different receptor than the other IGF-1 isoforms and doesn't activate Akt [130-134]. The MGF C-terminal peptide has been shown to induce heme oxygenase-1 (HO-1) [135]. Injection of MGF into the hindlimbs of SOD1$^{G93A}$ mice, a model for ALS, improved muscle strength and slowed neurodegeneration [136]. Injection of the C-terminal peptide of MGF prevented neurodegeneration in the hippocampus after ischemia in gerbils [137]. MGF has been shown to promote neurogenesis as well [138]. Overexpression of MGF in mice starting at various timepoints induced increased growth in the dentate gyrus and subventricular zone, up to 3 months after birth. The C-terminal peptide has been shown to protect SH-SY5Y cells from 6-hydroxydopamine (6-OHDA), 1-methyl-4-

phenylpyridinium (MPP+), or rotenone treatment [135]. It has also been shown to protect rats from 6-OHDA-lesion induced damage [135].

MGF is expressed in neurons during development [139]. In addition to mechanical stress [123, 140] and mechanical damage [130, 141], MGF expression is induced by hypoxia [139]. Growth hormone (GH) has been shown to increase MGF expression in C2C12 cells [142], rat muscles [143], mouse muscle [144], and elderly humans [145]. GH mostly works through the JAK/Stat pathway to induce GH expression. GH induces the activity of stat5b to bind to a sequence in the 2$^{nd}$ exon of IGF-1, termed HS7 [146-148]. Cyclic stretching upregulates the expression of SRSF1, which in turn regulates the expression of MGF in osteoblasts [149]. Other reported inducers of MGF expression are cAMP, Phorbol ester (PMA), and PGE2 [150].

Due to its attractiveness as a therapeutic target, compelling *in vivo* groundwork has been laid for MGF modulation. The MGF E peptide can promote the healing of rat tendons [151] and protect rat brains against traumatic injury [152]. Simulated weightlessness was able to increase MGF mRNA in rat hippocampus [153], and magnesium alloy may provide a convenient delivery vehicle for MGF [154]. Indeed, MGF has an exciting future and is poised to be a major player in neuroprotection in the years to come.

## 7. Remarks

Neurons are terminally differentiated cells. Regulation of gene expression is critical to maintaining neuronal function against stress. Post-transcriptional steps, including alternative splicing, mRNA stability and localization, translation, and protein modification, stability and localization are emerging as essential targets for regulating neuronal stress response. Among them, the molecular mechanism of alternative splicing is least studied. A number of diversified cis- and trans-elements mediating alternative splicing in the nervous system have been identified. While the identity of the cis-elements is still elusive, most of the trans-elements are RNA binding proteins with the exception of the most recent identification of a microRNA as a trans-element. It will be exciting to uncover the details of how these cis- and trans-elements cooperate to promote or inhibit alternative splicing site selection, and similarly for the characterization of the induction and the initiation of the assembly of the alternative splicing complex. The outcome of these studies will provide new targets for increasing aging and neurodegeneration disease therapy.

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## Author Contributions

Conceptualization: J.P. and R.G.Z.
Funding acquisition: R.G.Z.
Manuscript drafting and editing: J.P., X.T., and R.G.Z.

## Competing Interests

The authors have declared that no competing interests exist.

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