Published in final edited form as:
Biochemistry. 2012 July 17; 51(28): 5541–5556. doi:10.1021/bi3007065.

Tailoring of Membrane Proteins by Alternative Splicing of Pre-mRNA†

Kathleen F. Mittendorf[‡,§], **Catherine L. Deatherage**[‡,§], **Melanie D. Ohi**[§,#], and **Charles R. Sanders**[‡,§,*]

[‡]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232

[§]Center for Structural Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

[#]Department of Cellular and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

Abstract

Alternative splicing (ASfootnote_1) of RNA is a key mechanism for diversification of the eukaryotic proteome. In this process, different mRNA transcripts can be produced through altered excision/inclusion of exons during processing of the pre-mRNA molecule. Since its discovery, AS has been shown to play roles in protein structure, function, and localization. Dysregulation of this process can result in disease phenotypes. Moreover, AS pathways are promising therapeutic targets for a number of diseases. Integral membrane proteins (MPs) represent a class of proteins that may be particularly amenable to regulation by alternative splicing due to the distinctive topological restraints associated with their folding, structure, trafficking, and function. Here, we review the impact of AS on MP form and function, and the roles of AS in MP-related disorders such as Alzheimer's disease.

Introduction to Splicing of Pre-mRNA

In 1941, Beadle and Tatum provided data that led to the "one gene, one protein" paradigm (1). This paradigm persisted until researchers began to explore the human genome in depth, at which point it was discovered that the number of protein-encoding genes is much lower than originally predicted—leading to the "one gene, many proteins" hypothesis. Seminal work with adenovirus (2, 3) first led to the notion that multiple transcripts can arise from the same precursor RNA molecule. In 1978 a new mechanism, now known as alternative splicing (AS), was proposed (4) to generate proteomic diversity from a single gene in

†This work was supported by US NIH grants U54 GM94608, PO1 GM080512, and RO1 NS0508815 (to CRS) and by 1DP2OD004483 to MDO. KFM was supported by an NSF Graduate Research Fellowship (Grant DGE0909667).

[1]Abbreviations: AA, alternative acceptor sites; Aβ, amyloid beta; AD, alternative donor sites; AICD, amyloid precursor protein intracellular domain; AlzD, Alzheimer's disease; APH-1, anterior phalanx defective-1 protein; APP, amyloid precursor protein; AS, alternative splicing; BACE1, β-site APP cleavage enzyme 1; CA, carbonic anhydrase; ClC1, muscle-specific chloride channel; CNS, central nervous system; CTF, presenilin C-terminal fragment; D_2, dopamine receptor 2; DM1, myotonic dystrophy type 1; DMPK, myotonic dystrophy protein kinase; DNA, deoxyribonucleic acid; ER, endoplasmic reticulum; ES, exon skipping; GLT1, glutamate transporter 1; GlyR, glycine receptor; GPCR, G protein-coupled receptor; 5-HT_4, serotonin (5-hydroxytryptamine) receptor; IR, intron retention; InsR, insulin receptor; KPI, Kunitz protease inhibitor domain; mRNA, messenger ribonucleic acid; ME, mutually exclusive exon splicing; MP, integral membrane protein; mRNA, messenger ribonucleic acid; MNBL, muscleblind protein; NMD, nonsense-mediated decay; NMDA, N-methyl-D-aspartate; NTC, nineteen complex; NTF, presenilin N-terminal fragment; PEN-2, presenilin ehancer 2 protein; PKC, protein kinase C; PLP, proteolipid protein; PMP22, peripheral myelin protein 22; PS, presenilin; Rh, Rh blood group antigen protein; RNA, ribonucleic acid; RyR1, ryanodine receptor; SDC-4, syndecan receptor 4; SERCA, sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase; snRNA, small nuclear ribonucleic acid; TM, transmembrane; TRP, transient receptor potential; V2R, vasopressin V2 receptor.

*To whom correspondence should be addressed. chuck.sanders@vanderbilt.edu; phone, 615-936-3656; fax, 615-936-2211.

eukaryotes, and experimental confirmation quickly followed (5). During the maturation of mRNA in eukaryotes, RNA is spliced to remove regions of pre-mRNA referred to as introns (6–11). The remaining segments, referred to as exons, are linked and can act in both protein-coding and non-coding functions. Differential splicing, in which different exons or regions of exons are included or excluded in construction of the final coding mRNA, provides the basis for allowing a relatively small number of genes to encode for a large and diverse number of proteins. In addition to providing diversity to the proteome, AS may also regulate translation through the introduction of premature stop codons (via several possible mechanisms) that promote nonsense-mediated decay (NMD) of the alternatively spliced transcript (see (12–16)).

While the removal of introns is common to all eukaryotic life forms, the percentage of genes that undergo splicing varies by species. Generally, the abundance of AS events is proportional to organismal complexity, with AS being most prevalent in more complex organisms such as vertebrates (17). In higher eukaryotes, AS is ubiquitous, with at least 90% of human gene transcripts subjected to this process (18). Additionally, typical exon length decreases with increasing organism complexity (19), with 80% of human exons being less than 200 base pairs in length (20), which means that many exons encode only short segments of the protein product. In addition to differences among species, there is also diversity of AS within an organism. The number of potential AS products varies by gene and the expressed isoform(s) of any given protein can vary by cell type and even state of differentiation (21).

There are several types of AS that can take place within a given transcript, including exon skipping (ES), alternative donor sites (AD), alternative acceptor sites (AA), intron retention (IR), and the rare complex event known as mutually exclusive (ME) exon splicing. Other rare complex events, such as the skipping of multiple exons in a row, also occur (22). In a recent analysis of the human AS landscape, it was demonstrated that by far the most common AS events are the four “simple” modes of AS: ES, AD, AA, and IR, respectively, with ES accounting for approximately 45% of AS events (23) (see Figure 1). However, more complex types of AS, such as the skipping of two exons in a row (ca. 6–12% of total AS events), skipping of three exons in a row (ca. 2–3% of total AS events, and ME (ca. 3–5% of total AS events), do account for roughly 30% of total AS outcomes, making complex modes of AS important contributors to genomic diversity. ME exons often encode the same structural domain of the protein with slight variations (24), giving this mode of AS important roles in tuning protein structure and functions.

Splicing occurs by two consecutive transesterification reactions (25) that are catalyzed by the spliceosome, a ~3 MDa macromolecular machine composed of four of snRNAs (small nuclear RNAs) and proteins (see Figure 2). In a popular model based on *in vitro* studies, this apparatus is postulated to assemble in a step-wise and dynamic fashion (26, 27). In the process of spliceosome assembly, several intermediary complexes form (see Figure 2, (6–11) and (28)). The spliceosome recognizes three primary splicing signals: the 5' and 3' splice sites and the branch site. In the first transesterification reaction, the 2'-OH of a conserved adenosine nucleotide within the intervening intron region performs a nucleophilic attack, resulting in the release of the 5' exon containing a free 3'-OH group and the formation of the lariat intermediate structure. In the second step, the 3'-OH of the 5' exon serves as the nucleophile at the 3' splice site, producing the ligated exons and releasing the 2'–5' branched lariat intron. Spliceosome assembly begins with the recognition of the 5' splice site and branch point sequences of the pre-mRNA by the U1 snRNP and the U2 snRNP respectively. After binding of the U4/U6.U5 tri-snRNP, the U4/U6 snRNA duplex is replaced by a U2/U6 snRNA duplex. Furthermore, the U1 snRNA base pairing at the 5' splice site is disrupted and exchanged for base pairing between the 5' splice site and the U6 snRNA. The

subsequent addition of another complex, the NTC (Nineteen complex) and the release of the U1 and U4 snRNPs marks the transition from an inactive to an active spliceosome composed of the NTC, and the U5 and U2/U6 snRNPs. 5' splice site cleavage and lariat formation, followed by 3' splice site cleavage and exon ligation, occurs within the activated spliceosome. After completion of the second transesterification step and ligation of the two exons, the post-spliceosomal complex is dismantled and the lariat intron is released (see Figure 2). In each step of spliceosome assembly, catalysis, and disassembly, there are a number of RNA helicases and other enzymes that are needed to aid and regulate the many RNA:RNA, RNA:protein, and protein:protein interactions (9).

Most introns contain conventional consensus sequences that delineate the primary splice sites (8–10, 29). The 5' splice site typically begins with a conserved GU, which is flanked by three residues on the exon side and seven residues on the intron side that are generally similar among 5' splice sites. The 3' splice site usually follows the trinucleotide CAG or UAG (or, more rarely, AAG). The first nucleotide of the adjacent exon is also partially conserved. In addition to these consensus sequences, a tract of pyrimidine-rich bases upstream of the 3' splice site also contributes to recognition by the spliceosome. The final primary determinant of splice site recognition is the branch site consensus sequence, typically UACUAAC, where the final A serves as the nucleophilic branch site nucleotide and forms the lariat structure depicted in Figure 2. While this sequence is the most preferred by the splicing machinery, variation does occur around the branch site in certain cases. There is also another, less common, class of introns present in higher eukaryotes that utilizes both distinct consensus sequences (|ATATCCTT at the 5' splice site, CCTTRACCY at the branch site, and YAC| at the 3' splice site, where the vertical line indicates the actual site of the splicing event) and distinct spliceosomal components (the U11, U12, U4atac, and U6atac snRNPs, which are analogous to the U1, U2, U4, and U6 snRNPs, respectively). This minor spliceosome form proceeds through a step-wise reaction pathway identical to that depicted in Figure 2 and accounts for less than 1% of introns in human cells.

In addition to the primary determinants discussed above, there are other sequence signals outside of the conventional splice sites that regulate splicing. For instance, splicing enhancers and suppressors either promote or reduce the incidence of splicing at a particular site (30, 31). The relative strengths of multiple splice sites in the same sequence vicinity can also play roles in determining which site the spliceosome recognizes. RNA secondary structure also often appears to play a role in splice site selection (32–34). In another surprising mechanism, histone modifications present on the nucleosomes binding to exonic regions of DNA have also been directly linked to splicing outcomes (35). Together, these extra determinants outside the canonical splice site sequences provide additional ways for the spliceosome to distinguish the “correct” splice site from similar, but inappropriate, sites found elsewhere in the gene. Although these extra determinants complicate the “splicing code”, making it difficult to untangle which transcripts form from various genes, they serve as an important mechanism for “fine-tuning” splice site selection, providing the flexibility to precisely regulate which sequences are spliced or retained

With the currently available understanding about splice site consensus sequences and other signals that affect splicing, one might expect there to be robust computational approaches that would accurately predict exon/intron boundaries. Indeed this is the goal of several online splice site locator and gene structure prediction tools (36–39); however, the additional signals that regulate splicing discussed above often make these predictions complicated and occasionally inaccurate. A recent study (40) has taken further steps to incorporate regulatory information in the prediction algorithms allowing the prediction of tissue-specific alternative splicing. That study utilized information on experimentally-determined splice variants from four types of major tissue in mice, as well as information regarding the potential “splicing

code". This code includes sequences: (1) shown to recruit regulatory proteins, (2) that are enriched around splice sites, (3) containing RNA secondary structure, and (4) that contain spatial relationships between exons, introns, and other sequence motifs (40, 41). As a result of that study, an online tool (Website for Alternative Splicing Prediction, or WASP) was developed for *de novo* gene structure prediction as well as *de novo* prediction of the splice variants present in four major tissues (http://genes.toronto.edu/wasp/). Hopefully, the continued updates of WASP to reflect our improving and growing knowledge of the splicing code will eventually lead to an accurate tool for predicting gene structure and cell- and tissue-specific profiles of transcript variant expression. Finally, it should be noted that in addition to the computational algorithms for *de novo* prediction of gene structure and alternative splicing events, there are several databases that compile known splice variants and tissue expression profiles of genes (36), including a database (TMSPLICE) that specifically documents alternative splicing events in MPs within the mouse genome (42).

Alternative splicing provides an important mechanism for higher eukaryotes to both increase genetic diversity and regulate protein activity. Splicing variation affects the structure, function, and expression levels of protein products. Exploring alterations in physiology caused by AS is important to better understand the ramifications of misregulated AS and, ultimately, the role of AS in disease processes (43–46). This review will focus on how AS impacts membrane proteins (MP).

RNA Splicing and Membrane Proteins (MP)

MPs play key roles in numerous cellular functions including cell signaling, transport, energy transduction, as well as in the organization and stabilization of cellular structure. MPs differ from soluble proteins in terms of native environment, folding topology, and organization of domains and subunits. It is well established that RNA splicing can have a wide range of effects on soluble protein expression, localization, folding, structure, and function (47). There are also many examples of the role of AS in regulating MPs. However, there is currently no published survey of how AS impacts membrane proteins in ways that reflect the unique properties of this class of proteins. Here, we present a number of examples to illustrate how RNA splicing can alter MP expression, distribution, folding, topology, structure, and function. Care has been taken to highlight examples that represent a cross-function of MP types, including channels, transporters, receptors, structural proteins, and enzymes. The wide range of effects exerted by AS on MPs suggest that AS acts as a little-recognized global regulator of MP structure and function in higher organisms and thus has important implications for human disease.

AS Can Alter MP Topology or Produce Soluble Proteins

The number and arrangement of transmembrane (TM) segments in a MP defines the orientation of a protein and is often closely integrated with function and stability. Alternative or aberrant splicing can disrupt TM segments when splice sites are found within a TM-encoding region of the transcript. Likewise, it is possible that removal of an entire TM segment via splicing can significantly alter the topology of a MP or even eliminate membrane anchoring altogether. In this section, we review placement of splice junction locations within MP gene structure and examine examples where splicing significantly alters MP topology. We also briefly discuss the impact of these AS events on MP function.

Intron Junctions Tend Not to be Localized Within TM-Encoding Segments—

The aberrant introduction of splice site junctions within TM segments would likely lead to serious disruption of the encoded membrane protein structure. Indeed, computational studies assessing the likelihood of an intronic division within a TM region have found that that the probability of a TM not being divided by an intron was slightly higher than the expected

probability for a random 22-mer amino acid sequence (42). This difference was most pronounced in single-pass MPs, where TM regions were encoded on single exons 84.6% of the time, in comparison with the random sequence expectation of 58.5%. Thus, there seems to be evolutionary pressure for MPs, especially single pass MPs, to keep introns out of regions coding TMs within pre-mRNA.

AS Can Produce MPs with Altered Topology—Rh blood group antigens are important in blood-typing for blood transfusion and organ transplantation. These antigens are typically predicted to have 12 TM segments (48). The RhCcEe proteins represent an example of MPs whose topologies are altered due to AS (49). The characterization of Rh cDNA clones led to the conclusion that at least four different transcripts are produced from the RhCcEe gene by AS (Figure 3). Exon removal in one of the isoforms (RhVIII) results in the removal of residues 163–313, which form five TM segments. As a result, three of the helices are predicted to cross the membrane in reverse orientation relative to the original full-length transcript. Inversion of TM segment topology also occurs in isoform RhVI, which, as a result of exon removal, is missing residues 163–267, which form three TM segments. The reversal alters the residues that are surface-exposed and may alter antigenicity (see Figure 3A). Additionally, RhVI has another deletion (corresponding to residues 359–384) due to exon removal. This deletion causes a frameshift and results in a different C-terminal sequence with a premature stop codon. The Rh4 isoform is missing amino acid residues 314 to 358 as a result of exon removal; this exon deletion event induces a frameshift resulting in a different C-terminal sequence and a premature stop codon at the same location as in RhVI. Interestingly, the new C-terminal sequence generated by the frameshift in RhVI and Rh4 encodes the same 14 amino acids, indicating that this sequence is likely important, although its function is not yet known (see Figure 3).

Another example of altered TM topology is found in the V2 vasopressin receptor, a G-protein coupled receptor (GPCR). Its two isoforms, V2a and V2b, were found to have different topological states and different levels of stability (50) (Figure 3B). V2a is a stably expressed seven-TM segment (7-TM) protein found at the plasma membrane. Conversely, V2b differs in sequence from V2a in the C-terminal region after the sixth TM segment. This altered C-terminal tail results from the use of an alternate 3' splice site 76 base pairs downstream of the canonical 3' splice site, which results in a frameshift. The V2b protein isoform was experimentally shown to significantly populate two different topologies: a 7-TM topology similar to canonical GPCRs with the C-terminus located intracellularly, and a 6-TM topology with the C-terminus oriented to the extracellular matrix (Figure 3B). The V2b receptor also differs in that it is localized to the ER and Golgi apparatus rather than trafficking to the plasma membrane. Retention of the protein in the ER and Golgi could be due either to the splicing-based deletion of C-terminal forwards-trafficking motifs or may reflect sequestration of the truncated protein by MP folding quality control.

A third protein whose TM topology is affected by alternative splicing is the glycine receptor (51), which assembles into heteropentameric ($\alpha_2\beta_3$) ion-conducting pores and acts to mediate neurotransmissions in the central nervous system. It was found that the β subunit of this receptor was alternatively spliced to remove exon 7 (βΔ7). The long isoform of the β subunit including exon 7 possesses a predicted four-TM topology while the βΔ7 variant possesses a predicted two-TM segment topology due to the removal of TM segment 1, the first intracellular loop and a portion of TM segment 2 (see Figure 3C). Interestingly, the extracellular domain is kept intact, the protein is stably expressed in the cerebral cortex and can hetero-oligomerize with the glycine receptor α-subunits or with the glycine receptor-anchoring protein gephyrin. Because the TM segment 2 (which is shortened in βΔ7, where it is instead predicted to form the tenth beta strand in the large extracellular domain) is part of the ion conduction pathway, the functional properties of glycine receptors containing βΔ7

may be different. Indeed, experiments showed that the $\alpha_1\beta\Delta7$ complexes did not have resistance to the channel blocking agent picrotoxin while $\alpha_1\beta$ complexes display this property (51), suggesting that the pore is formed only by the α-subunits.

AS Can Alter the Signal Peptide—Signal peptides are important for membrane targeting and determination of protein subcellular localization and are often altered as a result of AS. In a computational analysis of the mouse transcriptome, 40% of transcriptional units (sets of transcripts derived from the same gene) were shown to have signal peptide variation, with the majority of transcript variation being the consequence of AS (52). These alterations are important for the targeting soluble proteins to various subcellular compartments (lumen of endoplasmic reticulum vs. cytosol, for example). Signal peptides also play an important role for targeting and integration of proteins into membrane, where they help determine membrane protein topology. The same study investigated a high-confidence set of 782 transcriptional units, where the presence of alternative transcription initiation start and termination sites in transcripts were independently confirmed, with variations in membrane organization. While this set most likely excludes most examples of AS based on the selection criteria, it is important to note that the AS may accomplish a similar task in cells based on alternative initial exons. In the same set of transcripts, there were 41 instances of signal peptide removal that resulted in a switch from Type I (C-terminal cytosolic) TM topology to Type II (N-terminal) TM topology in single-pass TM proteins. It is likely that use of alternative initial exons, which make up 58% of the mechanisms of variation among the set of transcriptional units with signal peptide variation investigated in this study, could result in a similar total inversion of topology. It seems feasible that AS could also result in complete inversion of topology of multi-span membrane proteins. There are MPs of this class that are known to populate opposite topologies (53), although splicing has not so far been established as the driving mechanism.

Signal peptide removal by AS has been documented in the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptor (54). The gene for this GPCR can be transcribed from two different transcription start sites. Use of a novel promoter in kidney results in the generation of three alternatively spliced transcripts (type I-type III), of which the signal peptide is replaced with a hydrophilic sequence in the type III splice variant. This splice variant, which was shown to retain reactivity, was expressed only minimally on the cell surface, with the majority being localized to intracellular compartments, likely as a result of replacement of the signal peptide. The type III receptor could be reactive to an intracellular form of PTHrP or may simply represent the outcome of a downregulatory mechanism.

AS Can Result in Production of Soluble Proteins from MP-Encoding Genes—In addition to altered TM topology, alternative splicing can eliminate TM domains from transcripts that would otherwise encode MPs. This results in soluble protein isoforms that may have vastly different roles in (or out) of the cell. A survey (55) of a portion of the human MP transcriptome revealed that nearly 40% of the 464 surveyed alternatively spliced single-pass MP genes have isoforms that completely lack a TM domain (Figure 4A). For example, the alternatively spliced soluble isoform of the syndecan-4 receptor (SCD-4) is very similar to the ectodomain of the canonical isoform. Syndecan receptors normally function as co-receptors by concentrating and presenting a variety of ligands (e.g. extracellular matrix proteins, growth factors, cytokines, and cell adhesion molecules) to other cell surface receptors, or they act to internalize ligand, sequestering them away from other receptors, thereby modulating signaling. The ectodomain of SCD-4 can be proteolytically cleaved, an event that is regulated by multiple signaling pathways, and this solubilized domain can still bind ligand, affecting signaling by competing with the membrane-bound SCD-4 counterpart (56). It is possible that the AS soluble isoform may

play a similar ligand-sequestration role as that played by the ectodomain generated by proteolytic cleavage.

Another example of production of a soluble isoform from a MP transcript is cadherin-7, a cell-adhesion TM glycoprotein. In developing chickens, it was found that a soluble form of cadherin-7 was expressed (57) and that this isoform can interact with the corresponding domain in the TM variant of this same protein, resulting in inhibition of cell-cell adhesion by interfering with homomeric interactions of the TM form.

AS Can Alter MP Function

Channels—Ions cannot cross the hydrophobic lipid bilayer via diffusion. Cells therefore express numerous types of channels, both to regulate cytosolic ion content and, in the case of specialized cells such as neurons and muscle cells, for signaling purposes. Ion channel activity can be regulated by AS. For example, transient receptor potential (TRP) potassium channels are heavily regulated by AS (58). The functional impact can range from altered conductance to changes in selectivity or activation. More drastic changes include the generation of dominant negative isoforms that prevent native channel function through heteromultimerization with the canonical isoforms. Consider the cold/menthol sensitive TRPM8 channel. Bidaux et al (59) have described the activity of two newly discovered short isoforms of TRPM8 (dubbed short TRPM8α and short TRPM8β). These two short isoforms contain only the cytosolic N-terminal domain and are thus are not capable of forming functional channels, but bind to and regulate canonical long-form TRPM8 channel tetramers (Figure 4B). Specifically, these variants stabilize the closed form of the channel, reducing channel activity and sensitivity to cold.

The TRPM3 channel is alternatively spliced to produce different sequences within the presumed pore domain, generating different cation selectivities. For instance, TRPM3α1 and TRPM3α2 are both outwardly rectifying cation channel isoforms, meaning they allow higher current flux out of the cell than in, whereas other TRPM3 variants are inwardly rectifying. TRPM3α2 is highly permeable to divalent cations while TRPM3α1 favors monovalent conductance. Additionally, monovalent cations block currents through TRPM3α2 but not TRPM3α1. These TRPM3 variants clearly demonstrate a role for AS in regulating channel ion permeability, selectivity, and regulation (60).

Transporters—Transporters facilitate the passage or flip-flop of solutes across the membrane, often in an energy-consuming manner against the TM concentration gradient (active transport). Transporter activity is typically tightly regulated, with AS being one mechanism of regulation.

An important family of regulated transporters are the P-type ATPases. This family includes the plasma membrane calcium pump, which is present in all eukaryotic cells and helps to maintain appropriate Ca^{2+} levels within the cytosol by moving calcium ions from the cytosol to the extracellular matrix. The calcium pump is alternatively spliced such that its calmodulin-binding regulatory domain is altered to reduce its net positive charge, resulting in a lower calmodulin affinity (61). Reduced affinity of the ATPase for calmodulin results in reduced transporter affinity for cytosolic Ca^{2+} ions.

Another example is the glutamate transporter family. After release of neurotransmitter into the synaptic cleft, transporters are responsible for transmitter reuptake by the cell and regulation of the volume of these vital molecules at the synapse. Levels of glutamate, the major excitatory neurotransmitter in the brain, are regulated by glutamate transporters, the most prominent of which is GLT1 (EEAT2 in humans). The three alternatively spliced isoforms of this protein (GLT1a, GLT1b, GLT1c) differ in their C-termini (62), with both

GLT1b and GLT1c containing a PDZ-recognition motif. GLT1a lacks this sequence (Figure 4C). Inclusion of a PDZ-binding sequence likely has functional consequences for the different GLT1 isoforms, since they may then recruit PDZ domain-containing proteins that modulate transporter function, trafficking, or associated signaling. GLT1a and GLT1b have similar expression profiles, and are primarily expressed in the brain, while GLT1c is expressed in the retina.

Receptors—G protein-coupled receptors (GPCRs) are an essential subset of MP, the target of almost 40% of the drugs on the market today (63) and represent an interesting example in terms of AS. Although many GPCR-encoding genes do not contain introns, some GPCR genes do (64–66). Besides the V2R and PTHrP receptors discussed earlier, classic examples include the D_2 dopamine receptor and the rat type I pituitary adenylate cyclase-activating peptide receptor (PAC_1 receptor). The D_2 receptor is present in one of two isoforms in the central nervous system (CNS): short (D_{2S}) or long (D_{2L}). The D_{2S} receptor lacks 29 residues within the third intracellular loop, likely resulting in different signaling properties due to the role of the intracellular loops in interaction with its cognate G-protein (64). The PAC_1 receptor is expressed in five different isoforms, all with variations in the third intracellular loop resulting in different signaling properties. The alternative forms of this loop differentially regulate activation of the downstream effectors adenylate cyclase and phospholipase C (64), again, most likely by altering interactions and specificity between the receptor and heterotrimeric G proteins.

In the case of the cannabinoid receptor hCB1, two splice variants have been identified (hCB1a and hCB1b) in addition to the canonical receptor. These variants have altered amino termini; hCB1a has an altered N-terminal sequence and hCB1b is missing the first 33 N-terminal amino acid residues. In both cases AS occurs through use of different splice site donor/acceptor sites. hCB1a and hCB1b display altered pharmacological properties in relation to the canonical hCB1 receptor (Figure 4E). Their affinity for the native ligand anandamide is dramatically reduced and their affinity for 2-arachidonoylglycerol is somewhat decreased. Additionally, it was found that instead of acting as an agonist, as it does in the hCB1, 2-arachidonoylglycerol functioned as an inverse agonist when interacting with hCB1a and hCB1b (67). Because the N-termini are not in the ligand-binding site, this example points to a function of AS in allosteric regulation of receptors.

Enzymes—TM enzymes are also affected by AS. Carbonic anhydrase XII (CA XII) is an enzyme whose alternative splicing is associated with cancer (68). There are two alternative transcripts of CA XII, the longer of which encodes 11 additional amino acid residues immediately preceding the predicted TM domain. Both transcripts are found in healthy tissue, but the longer isoform normally dominates. However, in astrocytomas expression of the shorter isoform is greatly increased and the longer isoform was rarely detected. Why the shorter isoform is more prevalent in cancer cells is not well understood; although, it is likely that the shorter isoform of CA XII may have difficulty in forming its normal quaternary structure. This is because a portion (GXXX) of the GXXXG motif, important for homodimerization of the enzyme, is missing due to alternative splicing (Figure 4D). Thus quaternary structure disruption likely contributes to catalytic dysfunction. For additional examples of enzymes affected by AS, see the Alzheimer's disease section.

Structural Proteins—The proteolipid protein (PLP) is the most abundant protein of central nervous system (CNS) myelin and is thought to maintain structural integrity of myelin and support myelin compaction, although it may have additional roles in signaling (69). PLP is a tetraspan MP with an intracellular "principal loop" connecting TM helices II and III. DM-20 is a splice variant of PLP and lacks a large portion (34 amino acids) of the principal loop (70), including two palmitoylation sites (71) (Figure 4F). It is likely that this

region allows for differential regulation of the functions of PLP and DM-20, altering their trafficking, structure, and/or signaling functions. It is interesting to note that AS-based elimination of two palmitoylation sites may also reduce the propensity of DM-20 to interact with cholesterol-rich membrane domains often referred to as “lipid rafts” or other specialized membrane domains in myelin membranes.

Regulation of Protein-Protein Interactions—Differences in alternatively spliced isoforms can impact the ability of the protein product to interact with partners. As discussed above, the GLT1 transporter isoforms differ in their possession of a PDZ-binding motif, a motif likely used to bind protein partners containing PDZ-binding domains. Another example is the N-methyl-D-asparate (NMDA) receptor. Yotiao, an A-kinase anchoring protein, scaffolds to the NR1A splice variant of the NR1 subunit of the NMDA receptor but not to the NR1C splice variant (72). This interaction is modulated by either the presence or absence of a subdomain encoded by an alternatively spliced C1 exon cassette that is included in NR1A but not the NR1C splice isoform. Additional research suggests that Yotiao may mediate a ternary complex between itself, the NMDA receptor and cAMP-dependent protein kinase II. This interaction is postulated to provide a mechanism for cAMP to modulate NMDA-receptor signaling (73). Similarly, calmodulin interacts avidly with the receptor through binding of NR1A to the region encoded by the very same C1 exon cassette, thereby inhibiting NMDA receptor function (74, 75).

Tissue-Specific AS

Through tissue-specific isoform expression, AS can alter the function of the protein product of a gene to better suit the requirements of its environment. For example, TMEM16A is thought to function as a calcium-dependent chloride channel (CaCC) with eight proposed TM segments. Recent studies of the patterns of alternative splicing that produce distinct isoforms of this protein show that differential exclusion/inclusion of exons 6b, 13, and 15 in the mRNA transcript occurs in different organs (76). The liver, placenta, prostate, thyroid, and trachea tend toward inclusion of exon 6b and exclusion of exon 15. However, in the brain the mRNA is most often missing exon 6b but contains exon 15. Exon 13 skipping was only found to occur only in brain and skeletal muscle. These changes in mRNA structure are reflected in the protein structure with functional consequences. For instance, skipping of exon 6b results in a deletion of a 22-amino acid intracellular segment in the N-terminus of TMEM16A, leading to a much higher affinity of TMEM16A for intracellular Ca^{2+} ions. Deletion of exon 13 results in removal of four amino acids in the intracellular loop between TM segments 2 and 3, reducing the voltage-dependence of the Cl^{-} currents.

AS can also involve non-coding regions of the mRNA. For instance, peripheral myelin protein 22 (PMP22), a protein involved in the inherited peripheral neuropathy Charcot-Marie-Tooth Disease, is spliced in a tissue-specific manner. The first exon in the 5' untranslated region (UTR) is alternatively spliced to include exon 1a or exon 1b (77). Exon 1a dominates in myelinating Schwann cells, while exon 1b is common to non-neural tissues expressing PMP22. Evidence suggests that expression of these two alternative transcripts is actually regulated by two different upstream promoters in the DNA, with the resultant pre-mRNA containing either exon 1a or 1b. The resultant proteins are identical, and it appears that the different promoters and the different 5' exons act to differentially regulate PMP22 expression in a tissue-specific manner at both transcriptional and translational stages.

Impact of AS on Trafficking and Subcellular Distribution

Trafficking sequence motifs play key roles in directing the trafficking and subcellular distribution of proteins within the cell. AS can result in the removal or retention of these signals to directly affect protein trafficking and organelle sub-localization. For instance,

there are two sets of *N*-ethylmaleimide- sensitive factor attachment protein (SNAP) receptors (SNAREs). v-SNAREs are single-pass MPs embedded in the membranes of vesicles, while t-SNAREs are embedded in the membranes of the target organelle. Together these SNAREs work to fuse vesicles to their target destination, releasing the vesicular contents. Importantly, there are two isoforms of the well-characterized v-SNARE vesicle-associated MP-1 (VAMP-1), VAMP-1A and VAMP-1B, that differ in the sequence at their C-termini, presumably through use of an alternative 3' exon. The VAMP-1B isoform is targeted to the mitochondria via conferral of a positive charge to the C-terminus and shortening of the hydrophobic membrane-embedded portion of the protein by four amino acid residues, while the VAMP-1A isoform is localized to the plasma membrane and the endosomes (78). Consequently, AS affects not only the trafficking of these important proteins, but also likely affects the destination of the vesicular contents of VAMP-directed vesicles (to the mitochondrial matrix for VAMP-1B and to extracellular matrix for VAMP-1A). In another example, the lysosome-associated MP (LAMP) 2, a major component of the lysosomal membrane, has three splice variants (LAMP-2a, -2b, and -2c) that are trafficked either to lysosomal compartments (LAMP2c) or to the cell surface (LAMP-2a and -2b). These differences are dictated by variation of their C-terminal targeting sequence (79), which is governed by alternative splicing of the last exon in the transcript, presumably through a mutually exclusive splicing event of the 3' exon.

Impact of AS on Folding

MP folding is still poorly understood in comparison to soluble protein folding and the evidence for the impact of alternative splicing on MP stability, folding pathways, and interactions with chaperones is currently sparse. Some AS events do result in the retention of specific variants (but not others) in the ER, which may indicate that AS sometimes results in changes in protein structure that are perceived by the protein folding quality control as signatures of misfolding. For instance, the V2b variant of the vasopressin receptor (50) is retained in the ER, as are two alterative isoforms of the TM aspartyl protease β-site APP cleavage enzyme 1 (BACE1), BACE1 I-476 and I-457, which are also far less active than the canonical BACE1 isoform I-501, (80). It has also been specifically suggested that V2b might represent a destabilized form of the V2 vasopressin repressor (50), which is not surprising considering that the V2b isoform has an altered topology (see Figure 3). To what extent these apparent AS-encoded “misfolding” events are important for normal cell biology (and are therefore evolutionarily selected for) remains to be seen. In any case, as the technology for examining MP folding and misfolding becomes more accessible, care should be taken to investigate the possible impact of AS on MP trafficking and folding efficiency.

AS of MPs and Disease

Alternative splicing of soluble proteins has long been documented as a contributing factor to disease (43–46). Here, we review two disorders in which aberrant RNA splicing has a pathological impact on MPs.

Myotonic Dystrophy Type 1

Myotonic Dystrophy Type 1 (DM1) is an inherited multisystem progressive disorder affecting 1 in 8000 people. DM1 is characterized by myotonia, heart defects, cataracts, gastrointestinal defects, insulin resistance, muscle wasting, and neuropsychiatric disorders (81, 82). DM1 appears to be triggered by expansion of a CUG repeat in the 3' untranslated region (3'-UTR) of the mytonic dystrophy protein kinase (DMPK) gene transcript (82). The number of CUG repeats correlates with disease severity (83). The CUG-expanded RNA accumulates in nuclear foci (84) and likely has a toxic gain of function mediated by hyperphosphorylation. This leads to stabilization of CUG-repeat binding proteins, such as

CUG binding protein (CUG-BP) (85, 86), a member of the CELF family of splicing regulators, and sequestration of muscleblind (MNBL) (84, 87). Sequestration of MNBL and stabilization of CUG-BP aberrantly alters AS of their downstream target transcripts (see Figure 5). As such, DM1 can be classified as an indirect spliceopathy since, while no splice sites are altered, AS of multiple proteins is dysregulated. While both soluble and MPs are adversely affected, below we review the AS of MPs affected in this disease.

Insulin Receptor—Aberrant AS of the insulin receptor (InsR) results in the insulin resistance phenotype observed in DM1 patients. The insulin receptor functions as a heterotetramer with two α- and two β-subunits. In DM1, there is a switch from the normal production in skeletal muscle of the IR-B splice variant of the α subunit, which includes a 12 residue C-terminus, to production of the IR-A variant lacking this terminus (88, 89). Omission of the C-terminus results in reduced responsiveness of the InsR to insulin and reduced kinase activity (and hence, signaling) for the IR-A isoform that is expressed in DM1 (90, 91).

Muscle-Specific Chloride Channel—One of the most common symptoms of DM-1 is myotonia, a delay in the relaxation of muscle after contraction. Hyperexcitability is likely caused by loss of function of chloride channels. It is thought that this is due to aberrant alternative splicing of the muscle-specific chloride channel (ClC-1), which results in decreased levels of the channel (92). Three aberrant splice variants of ClC-1 were found in the skeletal muscle of DM-1 patients. All three of these disease-generated isoforms contain premature stop codons introduced by the splicing changes and the transcripts are likely degraded by nonsense-mediated decay. This results in the loss of functional translated ClC-1 in skeletal muscle and thus a decrease in chloride conductance across the membrane, causing myotonia (93).

Ryanodine Receptor and Sarcoplasmic Endoplasmic Reticulum Ca^{2+} ATPase —Perhaps the most debilitating symptom of DM-1 is muscle wasting and weakness. It has been shown in other disorders that this symptom can result from changes to calcium homeostasis in muscle cells that increase intracellular Ca^{2+} levels (94). Two proteins essential to maintaining calcium homeostasis are the ryanodine receptor (RyR1) and the sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase (SERCA). The RyR1 is responsible for the release of Ca^{2+} in storage in the sarcoplasmic reticulum during muscle contraction and SERCA transports Ca^{2+} back across the ER membrane for storage. Research has shown that the transcripts for both of these proteins undergo AS in DM-1 patients (95).

RyR1 has 2 variants seen in DM1: the neonatal form of RyR1 lacks exon 70 (residues 3481–3485), which corresponds to five residues found in the receptor modulatory region, (96) and the adult form RyR1 includes exon 70. Adult DM-1 patients have been found to have elevated levels of the neonatal RyR1 isoform in their skeletal muscle. This form of RyR1 has lower channel activity than the adult isoform, which would result in increased depolarization-dependent Ca^{2+} release (95).

Two SERCA genes, SERCA1 and SERCA2 are also implicated in DM-1. SERCA1 has two splice variants: SERCA1a is the adult version of the protein and contains exon 22, which encodes 7 amino acids at the C-terminus. The neonatal form, SERCA1b lacks exon 22, and its C-terminus has an eight-residue, highly-charged C-terminal tail. In DM-1, a switch occurs, where the major isoform produced is the neonatal form (SERCA1b) as opposed to the adult form. It was also found that SERCA2 has a novel variant (SERCA2d) with lowered expression in DM-1. SERCA2d contains intron 19, which results in the addition of 27 amino acids, a frameshift, and a premature stop codon in exon 20. The aberrantly expressed

SERCA isoforms both have an altered C-terminus, but how this impacts overall pump function is not known (95).

$Ca_V1.1$ L-type Calcium Channel—The voltage-dependent $Ca_V1.1$ calcium channel plays a central and important role in excitation/contraction coupling in skeletal muscle (97). This channel also undergoes aberrant splicing that is associated with DM-1. AS of $Ca_V1.1$ in DM patients results in omission of exon 29 (ΔE29), removing a portion of the extracellular loop near the voltage sensor. As a consequence, the ΔE29 isoform exhibits altered calcium channel gating, which increases Ca^{2+} influx. These defects result in Ca^{2+} overload, likely contributing to muscle weakness and wasting.

Alzheimer's Disease

Alzheimer's Disease (AlzD) is a progressive neurodegenerative disorder affecting over 5 million people in the United States alone (98, 99). AlzD can arise sporadically or, in rare cases, can be directly inherited (familial AlzD). While the etiology of the disease remains the subject of intense scrutiny, the amyloidogenic pathway appears to play a central role (100). Amyloid plaques containing the amyloid-β (Aβ) peptide build up in the brains of AlzD patients and are the histological hallmark of the disease. It is thought, however, that it is soluble Aβ oligomers or aggregates that confer neurotoxicity and are at least partially responsible for AlzD progression (101). Aβ production occurs through the successive release of the ectodomain of the amyloid precursor protein (APP) by β-secretase followed by γ-secretase cleavage of the remaining 99-residue TM C-terminus to release the Aβ peptides. In a competing (and benign) pathway of APP processing initiated by α-secretase, a non-amyloidogenic peptide known as p3 is generated (102). The β- and γ-secretase enzymes are TM proteins, with the β-secretase being the aspartyl protease BACE1 (103–106). The γ-secretase complex is composed of four TM subunits: presenilin, nicastrin, APH-1, and presenilin enhancer 2 (PEN2) (107). As discussed below, the β-secretase and components of the γ-secretase complex are subject to AS, and this AS may regulate their activities (see Figure 6). Additionally, there is evidence for the involvement of aberrant AS in the progression of AlzD. Finally, APP is itself affected by AS. Here we review instances of AS of MPs in AlzD and discuss the potential impact on disease progression and therapeutics.

Amyloid Precursor Protein—Three major splice isoforms of APP have been documented: APP695 (lacking exons 7 and 8; the predominant neuronal isoform), APP751 (lacking exon 8 and expressed abundantly in non-neuronal CNS tissue and peripheral tissue), and APP770 (containing both exons 7 and 8 and expressed predominantly in peripheral tissues and at only low levels in the CNS) (108, 109). Exon 7 encodes a Kunitz protease inhibitor (KPI) domain in the ectodomain of APP that is possibly involved in blocking the α-secretase-initiated non-amyloidogenic APP processing pathway, since inclusion of exon 7 promotes production of Aβ and a decreased production of the p3 peptide (110). However, it should be noted that this domain is a serine-protease inhibitor domain, so would be unlikely to block the metalloproteinase α-secretase directly. Exon 8 encodes a region of the protein between the KPI domain and a heparin-binding domain. All three APP variants are subject to proteolytic processing along the amyloidogenic and non-amyloidogenic pathways described above. There is much inconsistency in the literature regarding differential expression of these isoforms in non-AlzD versus AlzD brains. Because it is difficult to compare relative values obtained from relative quantification-PCR among different studies, definitive *in vivo* data showing that differential AS of APP predisposes a patient to AlzD is still lacking.

BACE1—BACE1 is a single span MP with its catalytic subunit located in the ectoplasm. BACE1 is a prominent target in the as-of-yet unsuccessful search for a drug to lower amyloid-β production. A recent series of publications (111–114) has described the various isoforms of BACE1 produced through AS. The longest isoform, I-501 (named for the number of amino acids), is both the most active and most commonly produced. However, AS events can also generate a series of shorter isoforms, including I-476, I-455, I-432, and I-127. The I-127 mRNA transcript contains a premature stop codon and is degraded by NMD; in addition, the small amount of translated I-127 protein isoform is subject to degradation by the proteasome (113). The BACE1 I-476, I-457, I-455, and I-432 all exhibit dramatically reduced activity relative to I-501, such that promotion of AS to generate the shorter isoforms results in decreased Aβ secretion (114). Accordingly, promotion of AS of the BACE1 pre-mRNA may be a viable therapeutic strategy for reducing Aβ production. Recent experiments in the laboratory of Michael Wolfe have shown that I-501 production is activated through the binding of heterogeneous nuclear ribonucleoprotein H (hnRNP H) to a G-rich element in the third exon of BACE1 pre-mRNA (115). Because the 5' splice site responsible for I-501 production is thought to be weaker than the upstream 5' splice site (115) leading to the I-457 and I-453 isoforms, targeting the activation of I-501 production by hnRNP H is a reasonable therapeutic strategy in the search for a way to reduce BACE1 activity, in this case by reducing production of the most active isoform of the enzyme.

Presenilin (PS)—PS is the aspartyl protease in the heterotetrameric γ-secretase complex responsible for intramembrane cleavage of a large number of different single span MP substrates (116–118), including the 99-residue TM C-terminus (C99) of the APP that is released by BACE1 cleavage. Cleavage of C99 by γ-secretase releases the Aβ peptides (see Figure 6A). After assembly into the γ-secretase, cleavage of auto-inhibited presenilin within the intracellular loop located between TM segments 6 and 7 generates active γ-secretase. The now active presenilin is comprised of a heterodimer of its N-terminal fragment (NTF) and a loop-containing C-terminal fragment (CTF). The NTF and CTF each contribute an aspartic acid residue necessary for catalysis (102, 119–121). Prior to its cleavage, the loop that is clipped to generate active presenilin is thought to extend into the active site to block catalysis.

Inherited mutations of presenilin-1 or presenilin-2 (PS-1 and PS-2) are one of the known causes of familial (early onset) AlzD. Some of these mutations appear to impact pre-mRNA splicing. Additionally, aberrant splicing of the presenilin transcript has been shown to occur in the more common sporadic AlzD.

Presenilin-1—It has been shown that presenilin-1 (PS1) is affected by multiple splicing events, and that some heritable PS-1 mutations modulate splicing. Here, we will discuss three instances where splicing may play a role in disease progression.

First, in human tissues, two isoforms of PS1 are expressed that differ only by the inclusion or exclusion of four amino acids, VRSQ, in the N-terminal domain (122). The VRSQ motif, which is conserved among humans, rats, and mice, provides a putative phosphorylation consensus site for protein kinase C (PKC) at a downstream threonine (122). This phosphorylation event may play a role in regulating the trafficking of PS1. There is a relative decrease of the longer isoform in some cases of sporadic (123) and familial AlzD (124), suggesting a link between altered trafficking of PS1 and AlzD etiology.

Aberrant splicing of PS1 can also be induced by mutation. For instance, a mutation within intron 4 was identified in several patients with early-onset AlzD (125, 126), which results in production of three aberrant species of PS1. Two of these contain full or partial deletions of exon 4 and result in truncation of the protein product; the third encodes insertion of a single

threonine residue between the canonical residues 113 and 114 (126). Only the threonine-insertion species was detected in brain homogenates, suggesting the AlzD phenotype most likely results from the insertion species. This is supported by observation that transfection of the Thr-insertion form of the enzyme into HEK-293 cells resulted in increased $A\beta_{42}$ secretion (126).

Another interesting aberrant splicing event occurs when a mutation destroys a splice acceptor site at exon 9 (127). This causes the deletion of exon 9, which encodes part of the autoinhibitory loop including the site of activating cleavage of presenilin within the auto-inhibitory loop (128). Because disruption of this loop is required to activate presenilin activity (128, 129) and the elimination of the autocleavage site results in constitutively active presenilin (116), it was originally thought that its elimination via AS is solely responsible for the associated AlzD phenotype. However, it was later demonstrated that the single point mutation (S290C) that also results from this splicing event may contribute to AlzD pathogenesis by promoting increased $A\beta_{42}$ production (Figure 6B) (130).

Presenilin-2—For presenilin-2 (PS2), an aberrant splicing event in sporadic AlzD, resulting in exon 5 skipping (PS2V) and protein truncation, has been extensively characterized (131–135). The removal of exon 5 causes a frameshift in exon 6 and introduces a premature stop codon. The resulting PS2V mRNA encodes only the amino terminus containing one predicted TM segment and an additional five C-terminal residues (131, 132). The PS2V protein is found in the hippocampus and cerebral cortex of patients with sporadic AlzD, accumulates in inclusion bodies that impair the unfolded protein response, and results in increased Aβ production through an undefined mechanism (132). It is possible that it may exert its effect through associations with other γ-secretase proteins or through disruption of the unfolded protein response, preventing disposal of Aβ.

Recently, a novel splice variant of PS2, termed PS2β, was described to be a γ-secretase inhibitor (136). Its expression is therefore predicted to be beneficial in terms of reducing the risk for AlzD. This splice variant encodes the entire NTF plus the autoinhibitory hydrophilic loop domain that typically resides between TM segments 6 and 7. However, the exons encoding the entire CTF are not included in this isoform, such that this splice variant is catalytically inactive. PS2β was found to reduce the interaction between APH-1 and nicastrin and to inhibit Aβ secretion (136). It seems then, that PS2β may inhibit γ-secretase activity by preventing assembly of the holoenzyme. Perhaps this isoform may represent an endogenous means of repressing γ-secretase activity (Figure 6B).

Anterior Pharynx Defective-1—Another γ-secretase subunit, anterior pharynx defective-1 (APH-1) is a seven-TM spanning protein encoded by two homologous human genes, either APH-1a or APH-1b (137). APH-1 is known to be responsible for assembly of an initial subcomplex with nicastrin, and together these two proteins recruit the presenilin and PEN2 (138–142). The APH-1a transcript can be alternatively spliced to produce one of two isoforms, APH-1aS and APH-1aL, which differ only at their C-termini (137). These splice variants have been shown to reside in distinct, active γ-secretase complexes (143). Additionally, a novel (third) splice variant, APH-1bΔ4, has been identified that lacks the entire fourth exon, meaning it also lacks the entire fourth TM segment (144), resulting in predicted inversion of topology for the three C-terminal TM segments. This omitted TM segment also contains a GXXXG motif that is thought to be critical for assembly of the γ-secretase multisubunit complex (145) (Figure 6C). Endogenous expression of this variant form of the protein is very low, and the protein is destabilized (144). Whether alternative or aberrant splicing of APH-1 plays any role in promoting or preventing AlzD has yet to be determined.

Nicastrin—Nicastrin is a highly glycosylated type I MP that participates, along with APH-1, in the assembly of the γ-secretase complex. Two independent genome surveys (146, 147) have found evidence for association of sporadic AlzD with the region of chromosome 1 on which the nicastrin gene resides.

In 2005, a novel skipped splice variant of nicastrin was reported to be expressed in rat tissues and a human neuroblastoma cell line (148). This variant, which encodes a truncated 62-residue protein due to exon 3 removal resulting in a premature stop-codon, was shown to be preferentially made in the central nervous system and is subject to degradation by NMD. It is possible that NMD plays a specific role in the healthy CNS to down-regulate expression of active γ-secretase, thereby reducing Aβ production.

Soon thereafter, a second novel nicastrin splice variant was identified, this time lacking the entirety of exon 16 (149). As a result, this splice variant is missing 71 residues preceding the N-terminal region of the TM domain, which was shown to be required for interaction with the γ-secretase complex (150).

5-HT_4 Receptor—The serotonin (5-hydroxytryptamine) receptor 5-HT_4, a GPCR, has been implicated in the regulation of APP processing and receptor agonists have been shown to increase the production of the soluble APP ectodomain released by α-secretase cleavage of full length APP to initiate the non-amyloidogenic pathway (151–153). Alternatively spliced isoforms of 5-HT_4 have intracellular C-terminal domains of different lengths: a short isoform, 5-$HT_{4(d)}$, which contains only two amino acids after the C-terminal splice site, located after the region encoding TM segment 7 and preceding the intracellular C-terminal tail, and a larger isoform that contains 20 amino acids after the C-terminal splice site, 5-HT_4. These isoforms were compared with respect to the promotion of non-amyloidogenic APP processing (154). It was found that not only does activation of the short 5-$HT_{4(d)}$ isoform by agonist promote generation of non-amyloidogenic soluble-APPα (sAPPα, the ectodomain released upon α-secretase cleavage), but also that, unlike the longer isoform, stimulation of the short isoform reduces Aβ production. It is possible then, that 5-HT_4 receptor isoforms may differentially regulate APP processing. Presumably, this may come from signal transduction differences related to the differing C-terminal tail lengths, possibly involving signaling through arrestins, which become activated when they bind to the multiply-phosphorylated C-termini of activated GPCRs. Such arrestin-based signaling would be eliminated in splice variants that removed all or key parts of the C-terminal domain of the 5-HT_4 receptor. Further insight into how 5-HT_4 receptors influence APP processing and their relationship with the secretases is needed to shed light on this data.

Concluding Remarks

Alternative splicing of pre-mRNA can impact membrane protein trafficking, structure, function, and ultimately, contribute to disease. The examples highlighted in this review represent only a sample from what is already a very large pool of literature on this subject. Given that studies of RNA splicing remain in a rapid state of development, these examples represent only modest beginnings. There are undoubtedly new discoveries waiting to be made. It is already clear that splicing can alter almost every aspect of a MP's structure, topology, function, trafficking, and interactions. When studying MPs from eukaryotes, the possibility of different isoforms with varying properties should not be overlooked.

Acknowledgments

We thank Nicholas Reiter for his helpful comments and suggestions and Wade Van Horn for his help with figures.

References

1. Beadle GW, Tatum EL. Genetic Control of Biochemical Reactions in Neurospora. P Natl Acad Sci USA. 1941; 27:499–506.
2. Chow LT, Roberts JM, Lewis JB, Broker TR. A map of cytoplasmic RNA transcripts from lytic adenovirus type 2, determined by electron microscopy of RNA:DNA hybrids. Cell. 1977; 11:819–836. [PubMed: 890740]
3. Berget SM, Moore C, Sharp PA. Spliced segments at the 5' terminus of adenovirus 2 late mRNA. P Natl Acad Sci USA. 1977; 74:3171–3175.
4. Gilbert W. Why genes in pieces? Nature. 1978; 271:501. [PubMed: 622185]
5. Medford RM, Nguyen HT, Destree AT, Summers E, Nadal-Ginard B. A novel mechanism of alternative RNA splicing for the developmentally regulated generation of troponin T isoforms from a single gene. Cell. 1984; 38:409–421. [PubMed: 6205765]
6. Matlin AJ, Moore MJ. Spliceosome assembly and composition. Adv Exp Med Biol. 2007; 623:14–35. [PubMed: 18380338]
7. Grainger, RJ.; Beggs, JD. eLS. John Wiley & Sons, Ltd.; 2007. Spliceosomal Machinery.
8. Will CL, Luhrmann R. Spliceosome structure and function. Cold Spring Harb Perspect Biol. 2011; 3
9. Wahl MC, Will CL, Luhrmann R. The spliceosome: design principles of a dynamic RNP machine. Cell. 2009; 136:701–718. [PubMed: 19239890]
10. Mount, SM. eLS. John Wiley & Sons, Ltd.; 2001. Messenger RNA Splicing Signals.
11. Lu, Y-S.; Tarn, W-Y. eLS. John Wiley & Sons, Ltd.; 2010. Splicing of Pre-mRNA.
12. Chang YF, Imam JS, Wilkinson MF. The nonsense-mediated decay RNA surveillance pathway. Annu Rev Biochem. 2007; 76:51–74. [PubMed: 17352659]
13. Baker KE, Parker R. Nonsense-mediated mRNA decay: terminating erroneous gene expression. Curr Opin Cell Biol. 2004; 16:293–299. [PubMed: 15145354]
14. Brogna S, Wen J. Nonsense-mediated mRNA decay (NMD) mechanisms. Nat Struct Mol Biol. 2009; 16:107–113. [PubMed: 19190664]
15. Maquat LE. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. Nat Rev Mol Cell Biol. 2004; 5:89–99. [PubMed: 15040442]
16. Mendell, JT.; Dietz, HC. eLS. John Wiley & Sons, Ltd.; 2006. Nonsense-mediated mRNA Decay.
17. Keren H, Lev-Maor G, Ast G. Alternative splicing and evolution: diversification, exon definition and function. Nat Rev Genet. 2010; 11:345–355. [PubMed: 20376054]
18. Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. Nature. 2008; 456:470–476. [PubMed: 18978772]
19. Koralewski TE, Krutovsky KV. Evolution of exon-intron structure and alternative splicing. PLoS One. 2011; 6:e18055. [PubMed: 21464961]
20. Sakharkar MK, Chow VT, Kangueane P. Distributions of exons and introns in the human genome. In Silico Biol. 2004; 4:387–393. [PubMed: 15217358]
21. Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing. Nature. 2010; 463:457–463. [PubMed: 20110989]
22. Breitbart RE, Andreadis A, Nadal-Ginard B. Alternative splicing: a ubiquitous mechanism for the generation of multiple protein isoforms from single genes. Annu Rev Biochem. 1987; 56:467–495. [PubMed: 3304142]
23. Sammeth M, Foissac S, Guigo R. A general definition and nomenclature for alternative splicing events. PLoS Comput Biol. 2008; 4:e1000147. [PubMed: 18688268]
24. Pillmann H, Hatje K, Odronitz F, Hammesfahr B, Kollmar M. Predicting mutually exclusive spliced exons based on exon length, splice site and reading frame conservation, and exon sequence homology. BMC Bioinformatics. 2011; 12:270. [PubMed: 21718515]
25. Moore MJ, Sharp PA. Evidence for two active sites in the spliceosome provided by stereochemistry of pre-mRNA splicing. Nature. 1993; 365:364–368. [PubMed: 8397340]

26. Gornemann J, Kotovic KM, Hujer K, Neugebauer KM. Cotranscriptional spliceosome assembly occurs in a stepwise fashion and requires the cap binding complex. Mol Cell. 2005; 19:53–63. [PubMed: 15989964]
27. Tardiff DF, Rosbash M. Arrested yeast splicing complexes indicate stepwise snRNP recruitment during in vivo spliceosome assembly. Rna. 2006; 12:968–979. [PubMed: 16618970]
28. Hoskins AA, Friedman LJ, Gallagher SS, Crawford DJ, Anderson EG, Wombacher R, Ramirez N, Cornish VW, Gelles J, Moore MJ. Ordered and dynamic assembly of single spliceosomes. Science. 2011; 331:1289–1295. [PubMed: 21393538]
29. Padgett, RA.; Burge, CB. eLS. John Wiley & Sons, Ltd.; 2005. Splice Sites.
30. Smith CW, Valcarcel J. Alternative pre-mRNA splicing: the logic of combinatorial control. Trends Biochem Sci. 2000; 25:381–388. [PubMed: 10916158]
31. Wang Z, Burge CB. Splicing regulation: from a parts list of regulatory elements to an integrated splicing code. Rna. 2008; 14:802–813. [PubMed: 18369186]
32. Buratti E, Baralle FE. Influence of RNA secondary structure on the pre-mRNA splicing process. Mol Cell Biol. 2004; 24:10505–10514. [PubMed: 15572659]
33. Shepard PJ, Hertel KJ. Conserved RNA secondary structures promote alternative splicing. Rna. 2008; 14:1463–1469. [PubMed: 18579871]
34. Yang Y, Zhan L, Zhang W, Sun F, Wang W, Tian N, Bi J, Wang H, Shi D, Jiang Y, Zhang Y, Jin Y. RNA secondary structure in mutually exclusive splicing. Nat Struct Mol Biol. 2011; 18:159–168. [PubMed: 21217700]
35. Luco RF, Pan Q, Tominaga K, Blencowe BJ, Pereira-Smith OM, Misteli T. Regulation of alternative splicing by histone modifications. Science. 2010; 327:996–1000. [PubMed: 20133523]
36. Holste D, Ohler U. Strategies for identifying RNA splicing regulatory motifs and predicting alternative splicing events. PLoS Comput Biol. 2008; 4:e21. [PubMed: 18225947]
37. Thanaraj TA, Robinson AJ. Prediction of exact boundaries of exons. Brief Bioinform. 2000; 1:343–356. [PubMed: 11465052]
38. Jones SJ. Prediction of genomic functional elements. Annu Rev Genomics Hum Genet. 2006; 7:315–338. [PubMed: 16824019]
39. Thanaraj TA, Stamm S. Prediction and statistical analysis of alternatively spliced exons. Prog Mol Subcell Biol. 2003; 31:1–31. [PubMed: 12494761]
40. Barash Y, Calarco JA, Gao W, Pan Q, Wang X, Shai O, Blencowe BJ, Frey BJ. Deciphering the splicing code. Nature. 2010; 465:53–59. [PubMed: 20445623]
41. Tejedor JR, Valcarcel J. Gene regulation: Breaking the second genetic code. Nature. 2010; 465:45–46. [PubMed: 20445621]
42. Cline MS, Shigeta R, Wheeler RL, Siani-Rose MA, Kulp D, Loraine AE. The effects of alternative splicing on transmembrane proteins in the mouse genome. Pac Symp Biocomput. 2004:17–28. [PubMed: 14992489]
43. Garcia-Blanco MA, Baraniak AP, Lasda EL. Alternative splicing in disease and therapy. Nat Biotechnol. 2004; 22:535–546. [PubMed: 15122293]
44. Tazi J, Bakkour N, Stamm S. Alternative splicing and disease. Biochim Biophys Acta. 2009; 1792:14–26. [PubMed: 18992329]
45. Faustino NA, Cooper TA. Pre-mRNA splicing and human disease. Gene Dev. 2003; 17:419–437. [PubMed: 12600935]
46. Wang GS, Cooper TA. Splicing in disease: disruption of the splicing code and the decoding machinery. Nat Rev Genet. 2007; 8:749–761. [PubMed: 17726481]
47. Stamm S, Ben-Ari S, Rafalska I, Tang Y, Zhang Z, Toiber D, Thanaraj TA, Soreq H. Function of alternative splicing. Gene. 2005; 344:1–20. [PubMed: 15656968]
48. Avent ND, Reid ME. The Rh blood group system: a review. Blood. 2000; 95:375–387. [PubMed: 10627438]
49. Le Van Kim C, Cherif-Zahar B, Raynal V, Mouro I, Lopez M, Cartron JP, Colin Y. Multiple Rh messenger RNA isoforms are produced by alternative splicing. Blood. 1992; 80:1074–1078. [PubMed: 1379850]

50. Gonzalez A, Borquez M, Trigo CA, Brenet M, Sarmiento JM, Figueroa CD, Navarro J, Gonzalez CB. The splice variant of the V2 vasopressin receptor adopts alternative topologies. Biochemistry-Us. 2011; 50:4981–4986.
51. Oertel J, Villmann C, Kettenmann H, Kirchhoff F, Becker CM. A novel glycine receptor beta subunit splice variant predicts an unorthodox transmembrane topology. Assembly into heteromeric receptor complexes. The Journal of biological chemistry. 2007; 282:2798–2807. [PubMed: 17145751]
52. Davis MJ, Hanson KA, Clark F, Fink JL, Zhang F, Kasukawa T, Kai C, Kawai J, Carninci P, Hayashizaki Y, Teasdale RD. Differential Use of Signal Peptides and Membrane Domains is a Common Occurence in the Protein Output of Transcriptional Units. PLoS Genetics. 2006; 2:0554–0563.
53. von Heijne G. Membrane-protein topology. Nat Rev Mol Cell Biol. 2006; 7:909–918. [PubMed: 17139331]
54. Joun H, Lanske B, Karperien M, Qian F, Defize L, Abou-Samra A. Tissue-specific transcription start sites and alternative splicing of the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptor gene: a new PTH/PTHrP receptor splice variant that lacks the signal peptide. Endocrinology. 1997; 138:1742–1749. [PubMed: 9075739]
55. Xing Y, Xu Q, Lee C. Widespread production of novel soluble protein isoforms by alternative splicing removal of transmembrane anchoring domains. Febs Lett. 2003; 555:572–578. [PubMed: 14675776]
56. Fitzgerald ML, Wang Z, Park PW, Murphy G, Bernfield M. Shedding of syndecan-1 and -4 ectodomains is regulated by multiple signaling pathways and mediated by a TIMP-3-sensitive metalloproteinase. The Journal of cell biology. 2000; 148:811–824. [PubMed: 10684261]
57. Kawano R, Matsuo N, Tanaka H, Nasu M, Yoshioka H, Shirabe K. Identification and characterization of a soluble cadherin-7 isoform produced by alternative splicing. The Journal of biological chemistry. 2002; 277:47679–47685. [PubMed: 12364338]
58. Vazquez E, Valverde MA. A review of TRP channels splicing. Semin Cell Dev Biol. 2006; 17:607–617. [PubMed: 17174125]
59. Bidaux G, Beck B, Zholos A, Gordienko D, Lemonnier L, Flourakis M, Roudbaraki M, Borowiec AS, Fernandez J, Delcourt P, Lepage G, Shuba Y, Skryma R, Prevarskaya N. Regulation of activity of transient receptor potential melastatin 8 (TRPM8) channel by its short isoforms. The Journal of biological chemistry. 2012; 287:2948–2962. [PubMed: 22128173]
60. Oberwinkler J, Lis A, Giehl KM, Flockerzi V, Philipp SE. Alternative splicing switches the divalent cation selectivity of TRPM3 channels. The Journal of biological chemistry. 2005; 280:22540–22548. [PubMed: 15824111]
61. Enyedi A, Verma AK, Heim R, Adamo HP, Filoteo AG, Strehler EE, Penniston JT. The Ca2+ affinity of the plasma membrane Ca2+ pump is controlled by alternative splicing. The Journal of biological chemistry. 1994; 269:41–43. [PubMed: 8276828]
62. Lauriat TL, McInnes LA. EAAT2 regulation and splicing: relevance to psychiatric and neurological disorders. Mol Psychiatry. 2007; 12:1065–1078. [PubMed: 17684493]
63. Filmore D. It's a GPCR World. Modern Drug Discovery. 2004:24–28.
64. Markovic D, Challiss RA. Alternative splicing of G protein-coupled receptors: physiology and pathophysiology. Cell Mol Life Sci. 2009; 66:3337–3352. [PubMed: 19629391]
65. Kilpatrick GJ, Dautzenberg FM, Martin GR, Eglen RM. 7TM receptors: the splicing on the cake. Trends Pharmacol Sci. 1999; 20:294–301. [PubMed: 10390648]
66. Minneman KP. Splice variants of G protein-coupled receptors. Mol Interv. 2001; 1:108–116. [PubMed: 14993330]
67. Ryberg E, Vu HK, Larsson N, Groblewski T, Hjorth S, Elebring T, Sjogren S, Greasley PJ. Identification and characterisation of a novel splice variant of the human CB1 receptor. Febs Lett. 2005; 579:259–264. [PubMed: 15620723]
68. Haapasalo J, Hilvo M, Nordfors K, Haapasalo H, Parkkila S, Hyrskyluoto A, Rantala I, Waheed A, Sly WS, Pastorekova S, Pastorek J, Parkkila AK. Identification of an alternatively spliced isoform of carbonic anhydrase XII in diffusely infiltrating astrocytic gliomas. Neuro Oncol. 2008; 10:131–138. [PubMed: 18322268]

69. Gudz TI, Schneider TE, Haas TA, Macklin WB. Myelin proteolipid protein forms a complex with integrins and may participate in integrin receptor signaling in oligodendrocytes. J Neurosci. 2002; 22:7398–7407. [PubMed: 12196561]
70. Nave KA, Lai C, Bloom FE, Milner RJ. Splice site selection in the proteolipid protein (PLP) gene transcript and primary structure of the DM-20 protein of central nervous system myelin. P Natl Acad Sci USA. 1987; 84:5665–5669.
71. Weimbs T, Stoffel W. Proteolipid protein (PLP) of CNS myelin: positions of free, disulfide-bonded, and fatty acid thioester-linked cysteine residues and implications for the membrane topology of PLP. Biochemistry-Us. 1992; 31:12289–12296.
72. Lin JW, Wyszynski M, Madhavan R, Sealock R, Kim JU, Sheng M. Yotiao, a novel protein of neuromuscular junction and brain that interacts with specific splice variants of NMDA receptor subunit NR1. J Neurosci. 1998; 18:2017–2027. [PubMed: 9482789]
73. Feliciello A, Cardone L, Garbi C, Ginsberg MD, Varrone S, Rubin CS, Avvedimento EV, Gottesman ME. Yotiao protein, a ligand for the NMDA receptor, binds and targets cAMP-dependent protein kinase II(1). Febs Lett. 1999; 464:174–178. [PubMed: 10618500]
74. Ehlers MD, Mammen AL, Lau LF, Huganir RL. Synaptic targeting of glutamate receptors. Curr Opin Cell Biol. 1996; 8:484–489. [PubMed: 8791455]
75. Ehlers MD, Zhang S, Bernhadt JP, Huganir RL. Inactivation of NMDA receptors by direct interaction of calmodulin with the NR1 subunit. Cell. 1996; 84:745–755. [PubMed: 8625412]
76. Ferrera L, Caputo A, Ubby I, Bussani E, Zegarra-Moran O, Ravazzolo R, Pagani F, Galietta LJ. Regulation of TMEM16A chloride channel properties by alternative splicing. The Journal of biological chemistry. 2009; 284:33360–33368. [PubMed: 19819874]
77. Suter U, Snipes GJ, Schoener-Scott R, Welcher AA, Pareek S, Lupski JR, Murphy RA, Shooter EM, Patel PI. Regulation of tissue-specific expression of alternative peripheral myelin protein-22 (PMP22) gene transcripts by two promoters. The Journal of biological chemistry. 1994; 269:25795–25808. [PubMed: 7929285]
78. Isenmann S, Khew-Goodall Y, Gamble J, Vadas M, Wattenberg BW. A splice-isoform of vesicle-associated membrane protein-1 (VAMP-1) contains a mitochondrial targeting signal. Mol Biol Cell. 1998; 9:1649–1660. [PubMed: 9658161]
79. Gough NR, Fambrough DM. Different steady state subcellular distributions of the three splice variants of lysosome-associated membrane protein LAMP-2 are determined largely by the COOH-terminal amino acid residue. The Journal of cell biology. 1997; 137:1161–1169. [PubMed: 9166415]
80. Ehehalt R, Michel B, De Pietri Tonelli D, Zacchetti D, Simons K, Keller P. Splice variants of the beta-site APP-cleaving enzyme BACE1 in human brain and pancreas. Biochem Biophys Res Commun. 2002; 293:30–37. [PubMed: 12054559]
81. Bird, TD. Myotonic Dystrophy Type 1. 1999 Sep 17 [Updated 2011 Feb 8]. In: Pagon, RA.; Bird, TD.; Dolan, CR., et al., editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1165/
82. Mahadevan M, Tsilfidis C, Sabourin L, Shutler G, Amemiya C, Jansen G, Neville C, Narang M, Barcelo J, O'Hoy K, et al. Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. Science. 1992; 255:1253–1255. [PubMed: 1546325]
83. Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, Fenton I, Shaw DJ, Harper PS. Size of the unstable CTG repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. Am J Hum Genet. 1993; 52:1164–1174. [PubMed: 8503448]
84. Jiang H, Mankodi A, Swanson MS, Moxley RT, Thornton CA. 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. 2004; 13:3079–3088. [PubMed: 15496431]
85. Timchenko LT, Miller JW, Timchenko NA, DeVore DR, Datar KV, Lin L, Roberts R, Caskey CT, Swanson MS. Identification of a (CUG)n triplet repeat RNA-binding protein and its expression in myotonic dystrophy. Nucleic Acids Res. 1996; 24:4407–4414. [PubMed: 8948631]

86. Kuyumcu-Martinez NM, Wang GS, Cooper TA. Increased steady-state levels of CUGBP1 in myotonic dystrophy 1 are due to PKC-mediated hyperphosphorylation. Mol Cell. 2007; 28:68–78. [PubMed: 17936705]

87. Miller JW, Urbinati CR, Teng-Umnuay P, Stenberg MG, Byrne BJ, Thornton CA, Swanson MS. Recruitment of human muscleblind proteins to (CUG)(n) expansions associated with myotonic dystrophy. The EMBO journal. 2000; 19:4439–4448. [PubMed: 10970838]

88. Moller DE, Yokota A, Caro JF, Flier JS. Tissue-specific expression of two alternatively spliced insulin receptor mRNAs in man. Mol Endocrinol. 1989; 3:1263–1269. [PubMed: 2779582]

89. Seino S, Bell GI. Alternative splicing of human insulin receptor messenger RNA. Biochem Biophys Res Commun. 1989; 159:312–316. [PubMed: 2538124]

90. Knudsen L, De Meyts P, Kiselyov VV. Insight into the molecular basis for the kinetic differences between the two insulin receptor isoforms. The Biochemical journal. 2011; 440:397–403. [PubMed: 21838706]

91. Savkur RS, Philips AV, Cooper TA. Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. Nat Genet. 2001; 29:40–47. [PubMed: 11528389]

92. Charlet BN, Savkur RS, Singh G, Philips AV, Grice EA, Cooper TA. Loss of the muscle-specific chloride channel in type 1 myotonic dystrophy due to misregulated alternative splicing. Mol Cell. 2002; 10:45–53. [PubMed: 12150906]

93. Wheeler TM, Lueck JD, Swanson MS, Dirksen RT, Thornton CA. Correction of CIC-1 splicing eliminates chloride channelopathy and myotonia in mouse models of myotonic dystrophy. J Clin Invest. 2007; 117:3952–3957. [PubMed: 18008009]

94. Lyfenko AD, Goonasekera SA, Dirksen RT. Dynamic alterations in myoplasmic Ca2+ in malignant hyperthermia and central core disease. Biochem Biophys Res Commun. 2004; 322:1256–1266. [PubMed: 15336973]

95. Kimura T, Nakamori M, Lueck JD, Pouliquin P, Aoike F, Fujimura H, Dirksen RT, Takahashi MP, Dulhunty AF, Sakoda S. Altered mRNA splicing of the skeletal muscle ryanodine receptor and sarcoplasmic/endoplasmic reticulum Ca2+-ATPase in myotonic dystrophy type 1. Hum Mol Genet. 2005; 14:2189–2200. [PubMed: 15972723]

96. Futatsugi A, Kuwajima G, Mikoshiba K. Tissue-specific and developmentally regulated alternative splicing in mouse skeletal muscle ryanodine receptor mRNA. The Biochemical journal. 1995; 305(Pt 2):373–378. [PubMed: 7832748]

97. Tang ZZ, Yarotskyy V, Wei L, Sobczak K, Nakamori M, Eichinger K, Moxley RT, Dirksen RT, Thornton CA. Muscle weakness in myotonic dystrophy associated with misregulated splicing and altered gating of Ca(V)1.1 calcium channel. Hum Mol Genet. 2012; 21:1312–1324. [PubMed: 22140091]

98. Thies W, Bleiler L. 2011 Alzheimer's disease facts and figures. Alzheimers Dement. 2011; 7:208–244. [PubMed: 21414557]

99. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet. 2006; 368:387–403. [PubMed: 16876668]

100. Karran E, Mercken M, De Strooper B. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov. 2011; 10:698–712. [PubMed: 21852788]

101. Walsh DM, Selkoe DJ. A beta oligomers - a decade of discovery. J Neurochem. 2007; 101:1172–1184. [PubMed: 17286590]

102. Prox J, Rittger A, Saftig P. Physiological functions of the amyloid precursor protein secretases ADAM10, BACE1, and Presenilin. Exp Brain Res. 2012; 217:331–341. [PubMed: 22120156]

103. Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaari SM, Wang S, Walker D, Zhao J, McConlogue L, John V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature. 1999; 402:537–540. [PubMed: 10591214]

104. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL,

Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science. 1999; 286:735–741. [PubMed: 10531052]

105. Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR, Stratman NC, Mathews WR, Buhl AE, Carter DB, Tomasselli AG, Parodi LA, Heinrikson RL, Gurney ME. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. Nature. 1999; 402:533–537. [PubMed: 10591213]
106. Lin X, Koelsch G, Wu S, Downs D, Dashti A, Tang J. Human aspartic protease memapsin 2 cleaves the beta-secretase site of beta-amyloid precursor protein. P Natl Acad Sci USA. 2000; 97:1456–1460.
107. Kimberly WT, LaVoie MJ, Ostaszewski BL, Ye W, Wolfe MS, Selkoe DJ. Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. P Natl Acad Sci USA. 2003; 100:6382–6387.
108. Yoshikai S, Sasaki H, Doh-ura K, Furuya H, Sakaki Y. Genomic organization of the human amyloid beta-protein precursor gene. Gene. 1990; 87:257–263. [PubMed: 2110105]
109. Ling Y, Morgan K, Kalsheker N. Amyloid precursor protein (APP) and the biology of proteolytic processing: relevance to Alzheimer's disease. Int J Biochem Cell Biol. 2003; 35:1505–1535. [PubMed: 12824062]
110. Ho L, Fukuchi K, Younkin SG. The alternatively spliced Kunitz protease inhibitor domain alters amyloid beta protein precursor processing and amyloid beta protein production in cultured cells. The Journal of biological chemistry. 1996; 271:30929–30934. [PubMed: 8940079]
111. Tanahashi H, Tabira T. Three novel alternatively spliced isoforms of the human beta-site amyloid precursor protein cleaving enzyme (BACE) and their effect on amyloid betapeptide production. Neurosci Lett. 2001; 307:9–12. [PubMed: 11516562]
112. Bodendorf U, Fischer F, Bodian D, Multhaup G, Paganetti P. A splice variant of beta-secretase deficient in the amyloidogenic processing of the amyloid precursor protein. The Journal of biological chemistry. 2001; 276:12019–12023. [PubMed: 11152683]
113. Tanahashi H, Tabira T. A novel beta-site amyloid precursor protein cleaving enzyme (BACE) isoform regulated by nonsense-mediated mRNA decay and proteasome-dependent degradation. Neurosci Lett. 2007; 428:103–108. [PubMed: 17961921]
114. Mowrer KR, Wolfe MS. Promotion of BACE1 mRNA alternative splicing reduces amyloid beta-peptide production. The Journal of biological chemistry. 2008; 283:18694–18701. [PubMed: 18468996]
115. Fisette JF, Montagna DR, Mihailescu MR, Wolfe MS. A G-Rich Element Forms a G-Quadruplex and Regulates Bace1 mRNA Alternative Splicing. J Neurochem. 2012; 121:763–773. [PubMed: 22303960]
116. Ahn K, Shelton CC, Tian Y, Zhang X, Gilchrist ML, Sisodia SS, Li YM. Activation and intrinsic gamma-secretase activity of presenilin 1. P Natl Acad Sci USA. 2010; 107:21435–21440.
117. Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature. 1999; 398:513–517. [PubMed: 10206644]
118. Figueroa DJ, Morris JA, Ma L, Kandpal G, Chen E, Li YM, Austin CP. Presenilin-dependent gamma-secretase activity modulates neurite outgrowth. Neurobiol Dis. 2002; 9:49–60. [PubMed: 11848684]
119. Tolia A, De Strooper B. Structure and function of gamma-secretase. Semin Cell Dev Biol. 2009; 20:211–218. [PubMed: 19007897]
120. Zhang H, Ma Q, Zhang YW, Xu H. Proteolytic processing of Alzheimer's beta-amyloid precursor protein. J Neurochem. 2012; 120(Suppl 1):9–21. [PubMed: 22122372]
121. De Strooper B, Iwatsubo T, Wolfe MS. Presenilins and gamma-Secretase: Structure, Function, and Role in Alzheimer Disease. Cold Spring Harb Perspect Med. 2012; 2:a006304. [PubMed: 22315713]
122. Scheper W, Zwart R, Baas F. Alternative splicing in the N-terminus of Alzheimer's presenilin 1. Neurogenetics. 2004; 5:223–227. [PubMed: 15480879]

123. Isoe-Wada K, Urakami K, Wakutani Y, Adachi Y, Arai H, Sasaki H, Nakashima K. Alteration in brain presenilin-1 mRNA expression in sporadic Alzheimer's disease. Eur J Neurol. 1999; 6:163–167. [PubMed: 10053228]

124. Barton AJ, Crook BW, Karran EH, Brown F, Dewar D, Mann DM, Pearson RC, Graham DI, Hardy J, Hutton M, Duff K, Goate AM, Clark RF, Roberts GW. Alteration in brain presenilin 1 mRNA expression in early onset familial Alzheimer's disease. Neurodegeneration. 1996; 5:213–218. [PubMed: 8910899]

125. Tysoe C, Whittaker J, Xuereb J, Cairns NJ, Cruts M, Van Broeckhoven C, Wilcock G, Rubinsztein DC. A presenilin-1 truncating mutation is present in two cases with autopsy-confirmed early-onset Alzheimer disease. Am J Hum Genet. 1998; 62:70–76. [PubMed: 9443865]

126. De Jonghe C, Cruts M, Rogaeva EA, Tysoe C, Singleton A, Vanderstichele H, Meschino W, Dermaut B, Vanderhoeven I, Backhovens H, Vanmechelen E, Morris CM, Hardy J, Rubinsztein DC, St George-Hyslop PH, Van Broeckhoven C. Aberrant splicing in the presenilin-1 intron 4 mutation causes presenile Alzheimer's disease by increased Abeta42 secretion. Hum Mol Genet. 1999; 8:1529–1540. [PubMed: 10401002]

127. Perez-Tur J, Froelich S, Prihar G, Crook R, Baker M, Duff K, Wragg M, Busfield F, Lendon C, Clark RF, et al. A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene. Neuroreport. 1995; 7:297–301. [PubMed: 8742474]

128. Thinakaran G, Borchelt DR, Lee MK, Slunt HH, Spitzer L, Kim G, Ratovitsky T, Davenport F, Nordstedt C, Seeger M, Hardy J, Levey AI, Gandy SE, Jenkins NA, Copeland NG, Price DL, Sisodia SS. Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. Neuron. 1996; 17:181–190. [PubMed: 8755489]

129. Podlisny MB, Citron M, Amarante P, Sherrington R, Xia W, Zhang J, Diehl T, Levesque G, Fraser P, Haass C, Koo EH, Seubert P, St George-Hyslop P, Teplow DB, Selkoe DJ. Presenilin proteins undergo heterogeneous endoproteolysis between Thr291 and Ala299 and occur as stable N- and C-terminal fragments in normal and Alzheimer brain tissue. Neurobiol Dis. 1997; 3:325–337. [PubMed: 9173929]

130. Steiner H, Romig H, Grim MG, Philipp U, Pesold B, Citron M, Baumeister R, Haass C. The biological and pathological function of the presenilin-1 Deltaexon 9 mutation is independent of its defect to undergo proteolytic processing. The Journal of biological chemistry. 1999; 274:7615–7618. [PubMed: 10075646]

131. Sato N, Hori O, Yamaguchi A, Lambert JC, Chartier-Harlin MC, Robinson PA, Delacourte A, Schmidt AM, Furuyama T, Imaizumi K, Tohyama M, Takagi T. A novel presenilin-2 splice variant in human Alzheimer's disease brain tissue. J Neurochem. 1999; 72:2498–2505. [PubMed: 10349860]

132. Sato N, Imaizumi K, Manabe T, Taniguchi M, Hitomi J, Katayama T, Yoneda T, Morihara T, Yasuda Y, Takagi T, Kudo T, Tsuda T, Itoyama Y, Makifuchi T, Fraser PE, St George-Hyslop P, Tohyama M. Increased production of beta-amyloid and vulnerability to endoplasmic reticulum stress by an aberrant spliced form of presenilin 2. The Journal of biological chemistry. 2001; 276:2108–2114. [PubMed: 11031265]

133. Manabe T, Katayama T, Sato N, Gomi F, Hitomi J, Yanagita T, Kudo T, Honda A, Mori Y, Matsuzaki S, Imaizumi K, Mayeda A, Tohyama M. Induced HMGA1a expression causes aberrant splicing of Presenilin-2 pre-mRNA in sporadic Alzheimer's disease. Cell Death Differ. 2003; 10:698–708. [PubMed: 12761578]

134. Manabe T, Ohe K, Katayama T, Matsuzaki S, Yanagita T, Okuda H, Bando Y, Imaizumi K, Reeves R, Tohyama M, Mayeda A. HMGA1a: sequence-specific RNA-binding factor causing sporadic Alzheimer's disease-linked exon skipping of presenilin-2 pre-mRNA. Genes Cells. 2007; 12:1179–1191. [PubMed: 17903177]

135. Ohe K, Mayeda A. HMGA1a trapping of U1 snRNP at an authentic 5' splice site induces aberrant exon skipping in sporadic Alzheimer's disease. Mol Cell Biol. 2010; 30:2220–2228. [PubMed: 20194618]

136. Suzuki Y, Ohta K, Itoh M, Sakoh-Sumitomo Y, Mitsuda T, Ueda M, Hayakawa-Yano Y, Li S, Hida Y, Inuzuka T, Jung YK, Nakagawa T. An alternative spliced mouse presenilin-2 mRNA

encodes a novel gamma-secretase inhibitor. Febs Lett. 2009; 583:1403–1408. [PubMed: 19376115]

137. Lee SF, Shah S, Li H, Yu C, Han W, Yu G. Mammalian APH-1 interacts with presenilin and nicastrin and is required for intramembrane proteolysis of amyloid-beta precursor protein and Notch. The Journal of biological chemistry. 2002; 277:45013–45019. [PubMed: 12297508]
138. Hu Y, Fortini ME. Different cofactor activities in gamma-secretase assembly: evidence for a nicastrin-Aph-1 subcomplex. The Journal of cell biology. 2003; 161:685–690. [PubMed: 12771124]
139. LaVoie MJ, Fraering PC, Ostaszewski BL, Ye W, Kimberly WT, Wolfe MS, Selkoe DJ. Assembly of the gamma-secretase complex involves early formation of an intermediate subcomplex of Aph-1 and nicastrin. The Journal of biological chemistry. 2003; 278:37213–37222. [PubMed: 12857757]
140. Morais VA, Crystal AS, Pijak DS, Carlin D, Costa J, Lee VM, Doms RW. The transmembrane domain region of nicastrin mediates direct interactions with APH-1 and the gamma-secretase complex. The Journal of biological chemistry. 2003; 278:43284–43291. [PubMed: 12917438]
141. Shirotani K, Edbauer D, Kostka M, Steiner H, Haass C. Immature nicastrin stabilizes APH-1 independent of PEN-2 and presenilin: identification of nicastrin mutants that selectively interact with APH-1. J Neurochem. 2004; 89:1520–1527. [PubMed: 15189355]
142. Fraering PC, LaVoie MJ, Ye W, Ostaszewski BL, Kimberly WT, Selkoe DJ, Wolfe MS. Detergent-dependent dissociation of active gamma-secretase reveals an interaction between Pen-2 and PS1-NTF and offers a model for subunit organization within the complex. Biochemistry-Us. 2004; 43:323–333.
143. Shirotani K, Edbauer D, Prokop S, Haass C, Steiner H. Identification of distinct gamma-secretase complexes with different APH-1 variants. The Journal of biological chemistry. 2004; 279:41340–41345. [PubMed: 15286082]
144. Saito S, Takahashi-Sasaki N, Araki W. Identification and characterization of a novel human APH-1b splice variant lacking exon 4. Biochem Biophys Res Commun. 2005; 330:1068–1072. [PubMed: 15823552]
145. Lee SF, Shah S, Yu C, Wigley WC, Li H, Lim M, Pedersen K, Han W, Thomas P, Lundkvist J, Hao YH, Yu G. A conserved GXXXG motif in APH-1 is critical for assembly and activity of the gamma-secretase complex. The Journal of biological chemistry. 2004; 279:4144–4152. [PubMed: 14627705]
146. Zubenko GS, Hughes HB, Stiffler JS, Hurtt MR, Kaplan BB. A genome survey for novel Alzheimer disease risk loci: results at 10-cM resolution. Genomics. 1998; 50:121–128. [PubMed: 9653640]
147. Kehoe P, Wavrant-De Vrieze F, Crook R, Wu WS, Holmans P, Fenton I, Spurlock G, Norton N, Williams H, Williams N, Lovestone S, Perez-Tur J, Hutton M, Chartier-Harlin MC, Shears S, Roehl K, Booth J, Van Voorst W, Ramic D, Williams J, Goate A, Hardy J, Owen MJ. A full genome scan for late onset Alzheimer's disease. Hum Mol Genet. 1999; 8:237–245. [PubMed: 9931331]
148. Confaloni A, Crestini A, Albani D, Piscopo P, Campeggi LM, Terreni L, Tartaglia M, Forloni G. Rat nicastrin gene: cDNA isolation, mRNA variants and expression pattern analysis. Brain Res Mol Brain Res. 2005; 136:12–22. [PubMed: 15893582]
149. Mitsuda N, Yamagata HD, Zhong W, Aoto M, Akatsu H, Uekawa N, Kamino K, Taguchi K, Yamamoto T, Maruyama M, Kosaka K, Takeda M, Kondo I, Miki T. A novel alternative splice variant of nicastrin and its implication in Alzheimer disease. Life Sci. 2006; 78:2444–2448. [PubMed: 16303145]
150. Capell A, Kaether C, Edbauer D, Shirotani K, Merkl S, Steiner H, Haass C. Nicastrin interacts with gamma-secretase complex components via the N-terminal part of its transmembrane domain. The Journal of biological chemistry. 2003; 278:52519–52523. [PubMed: 14602727]
151. Maillet M, Robert SJ, Lezoualc'h F. New insights into serotonin 5-HT4 receptors : a novel therapeutic target for Alzheimer's disease? Curr Alzheimer Res. 2004; 1:79–85. [PubMed: 15975071]

152. Lezoualc'h F. 5-HT4 receptor and Alzheimer's disease: the amyloid connection. Exp Neurol. 2007; 205:325–329. [PubMed: 17346704]

153. Cho S, Hu Y. Activation of 5-HT4 receptors inhibits secretion of beta-amyloid peptides and increases neuronal survival. Exp Neurol. 2007; 203:274–278. [PubMed: 16978609]

154. Robert SJ, Lezoualc'h F. Distinct functional effects of human 5-HT4 receptor isoforms on beta-amyloid secretion. Neurodegener Dis. 2008; 5:163–165. [PubMed: 18322379]

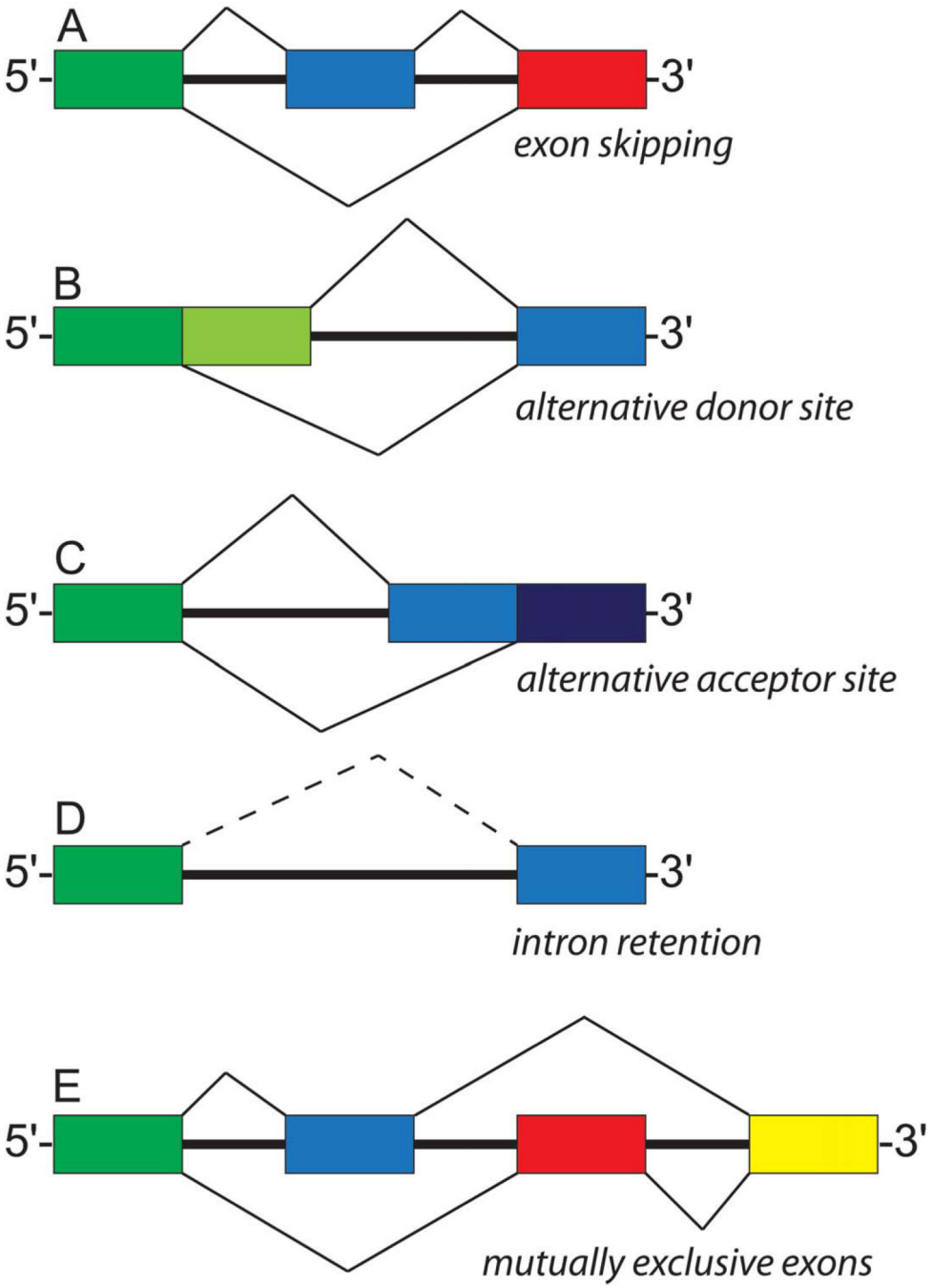

Figure 1. Modes of AS

(A) Exon skipping. **Top:** canonical splicing. **Bottom:** removal of the blue exon. **(B)** Alternative donor sites. **Top:** canonical splicing. **Bottom:** use of an alternative 5' donor site. **(C)** Alternative acceptor sites. **Top:** canonical splicing. **Bottom:** use of an alternative 3' acceptor site. **(D)** Intron retention. In cases of intron retention, the canonical splicing (dotted line) does not occur and the intron is included in the processed mRNA. **(E)** Mutually exclusive exon splicing. **Top:** inclusion of blue exon and exclusion of red exon. **Bottom:** exclusion of blue exon and inclusion of red exon.

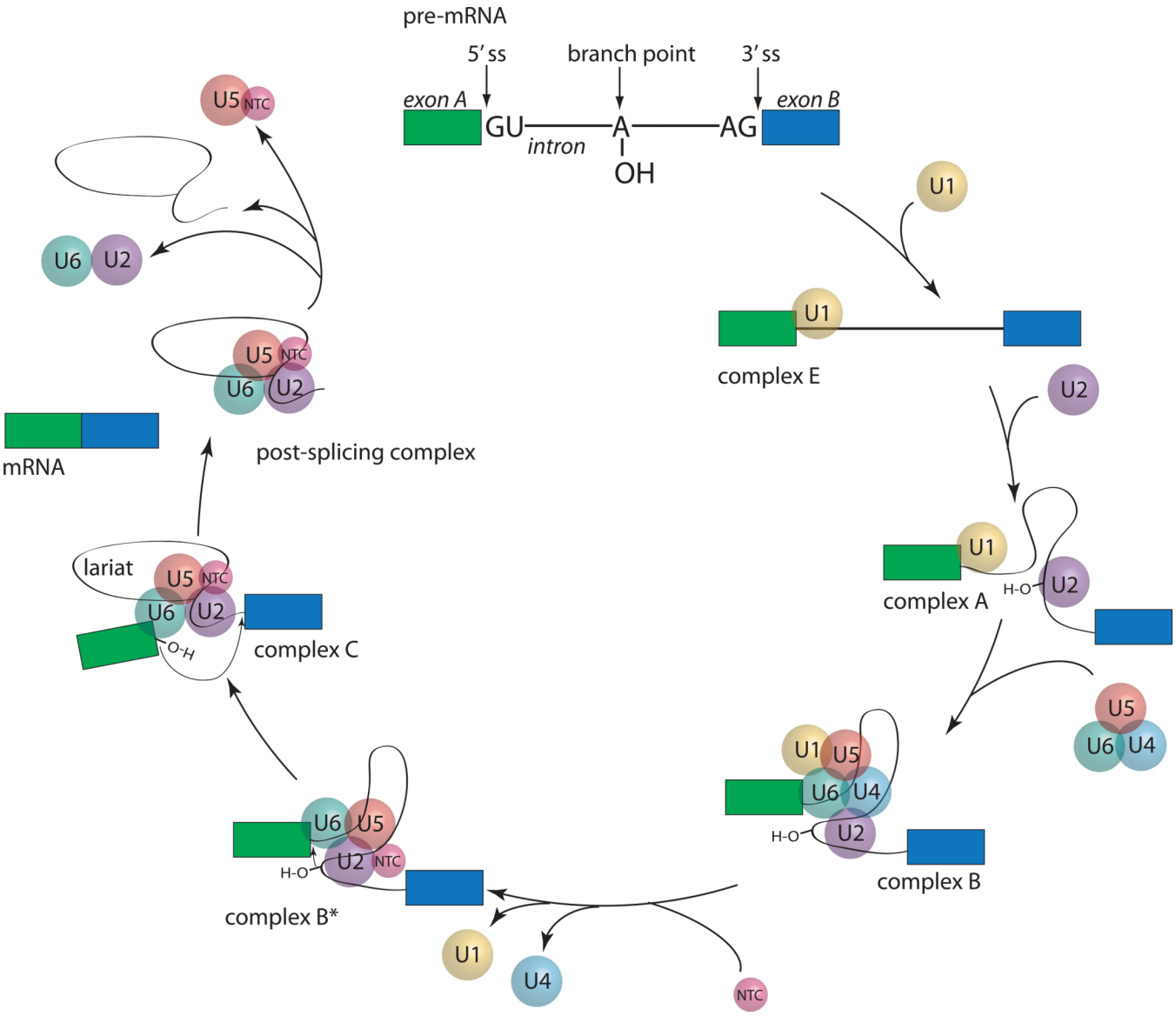

Figure 2. Spliceosome assembly and splicing reaction

From top, clockwise: U1 snRNP associates with the 5' splice site to make complex E. U2 snRNP is recruited and brings the branch site near the 5' splice site, forming complex A. The U4/U6.U5 tri-snRNP is recruited to form a pre-catalytic complex B. The arrival of the nineteen complex (NTC) and the release of U1 and U4 snRNAs mark the conversion to complex B*, an active spliceosome. Complex C forms after the first transesterification reaction and carries out the second transesterification reaction. The mature message is released and the post-splicing complex is recycled.

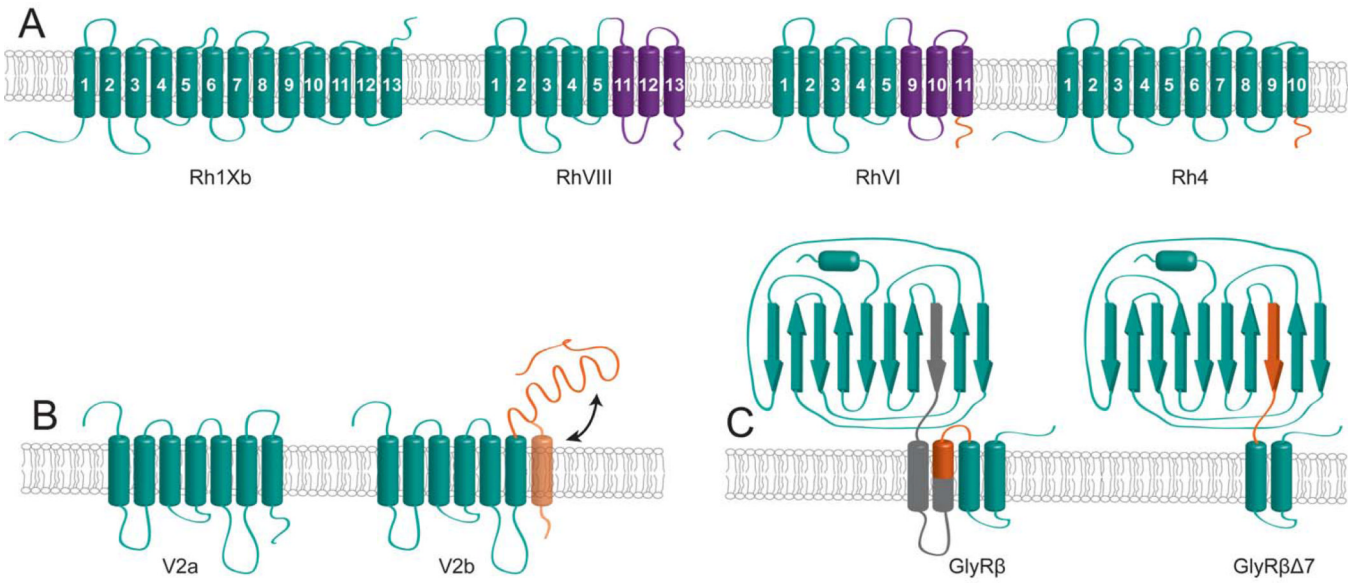

Figure 3. AS can alter MP topology

(A) The membrane topology of the RhCcEe protein is altered due to AS. RhX1b is the canonical isoform; helices are numbered per this isoform. Exon removal in RhVIII results in the removal of TM segments 6–10. The original TM segments 11–13 (purple) cross the membrane with inverted topology. RhVI, as a result of exon removal, is missing the residues that form TM segments 6–8 and also displays inverted topology in the canonical TM segments 9–11. Additionally, RhVI has another deletion due to exon removal. This deletion causes a frameshift and results in a different C-terminal sequence with a premature stop codon. The Rh4 isoform undergoes exon removal that induces a frameshift resulting in a different C-terminal sequence and a premature stop codon at the same location as in RhVI. The new C-terminal sequence generated by the frameshift in RhVI and Rh4 encodes the same 14 amino acids (orange), indicating that the sequence likely is important. **(B)** The G-protein coupled receptor V2 vasopressin receptor. V2a is a stably expressed seven-TM segment (7-TM) protein found at the plasma membrane. Conversely, V2b differs in sequence from V2a in the C-terminal region after the sixth TM segment. This alteration in the C-terminus results from the use of an alternate 3' splice site 76 base pairs downstream of the canonical 3' splice site, which causes a frameshift. The V2b protein isoform was experimentally shown to significantly populate two topologies: a 7-TM topology similar to canonical GPCRs with the C-terminus located intracellularly, and a 6-TM topology with the C-terminus oriented to the extracellular matrix. **(C)** The glycine receptor β subunit is alternatively spliced to remove exon 7 (βΔ7). The long isoform of the β subunit including exon 7 possesses has four-TM topology. The βΔ7 variant has a predicted two-TM segment topology due to the removal of the tenth β-strand in the extracellular domain, TM segment 1, the first intracellular loop, and a portion of TM segment 2 (grey). The remaining portion of TM segment 2 is predicted to substitute for the tenth β strand in the extracellular domain (orange).

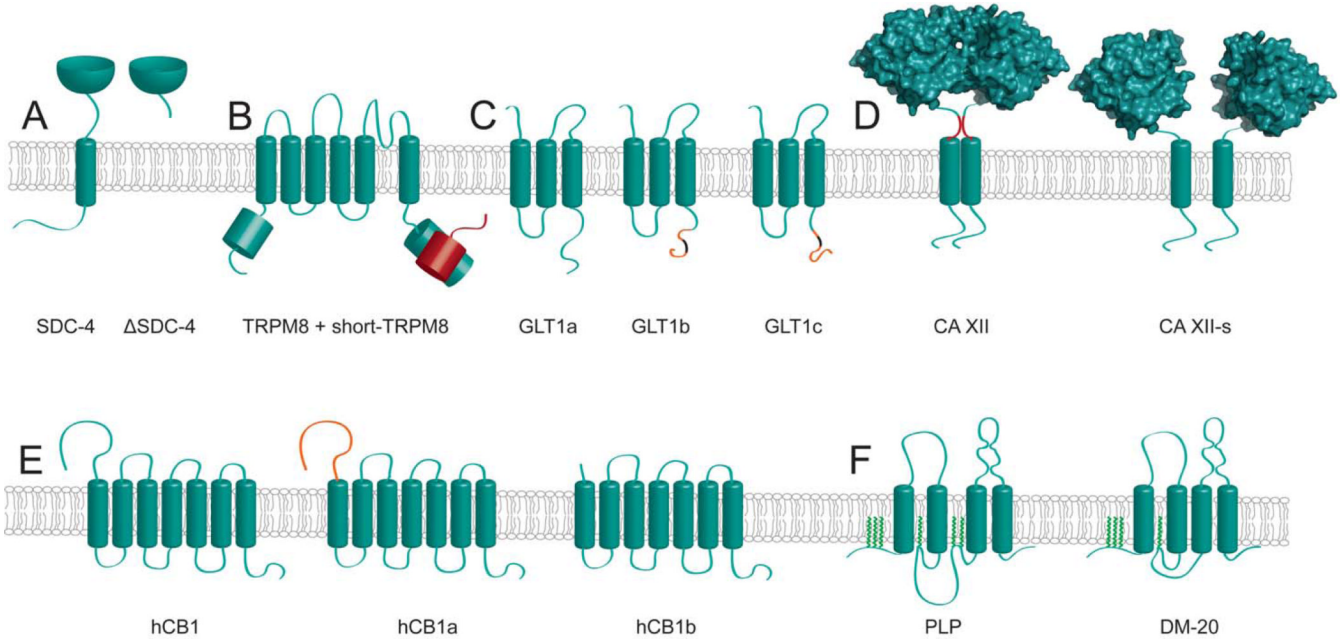

Figure 4. AS can alter MP function

(A) AS of syndecan-4 results in an mRNA that encodes only the ectodomain. This AS event may serve a similar purpose as proteolytic shedding of ectodomains. **(B)** AS of TRPM8 creates a short isoform (red) that regulates TRPM8 through interaction with the C-terminal domain, acting to stabilize the closed formation of the channel. **(C)** AS of GLT1 results in isoforms with different C-terminal tails. GLT1b and GLT1c contain different C-terminal sequences (orange), but both include a PDZ-binding motif (black) that presumably functions to recruit proteins that contain PDZ-domains. GLT1a does not possess this motif. **(D)** AS of CA XII results in the removal of a GXXXG motif (red) that mediates dimerization. This may prevent homodimerization and influence the activity of the enzyme. **(E)** AS hCB1 results in different N-termini (orange) and altered receptor responses to canonical ligands, presumably through an allosteric effect. **(F)** Alternative splicing of PLP results in removal of a portion of a loop that includes sites for post-translational lipid modifications. Removal of these sites may influence protein trafficking to lipid raft domains.

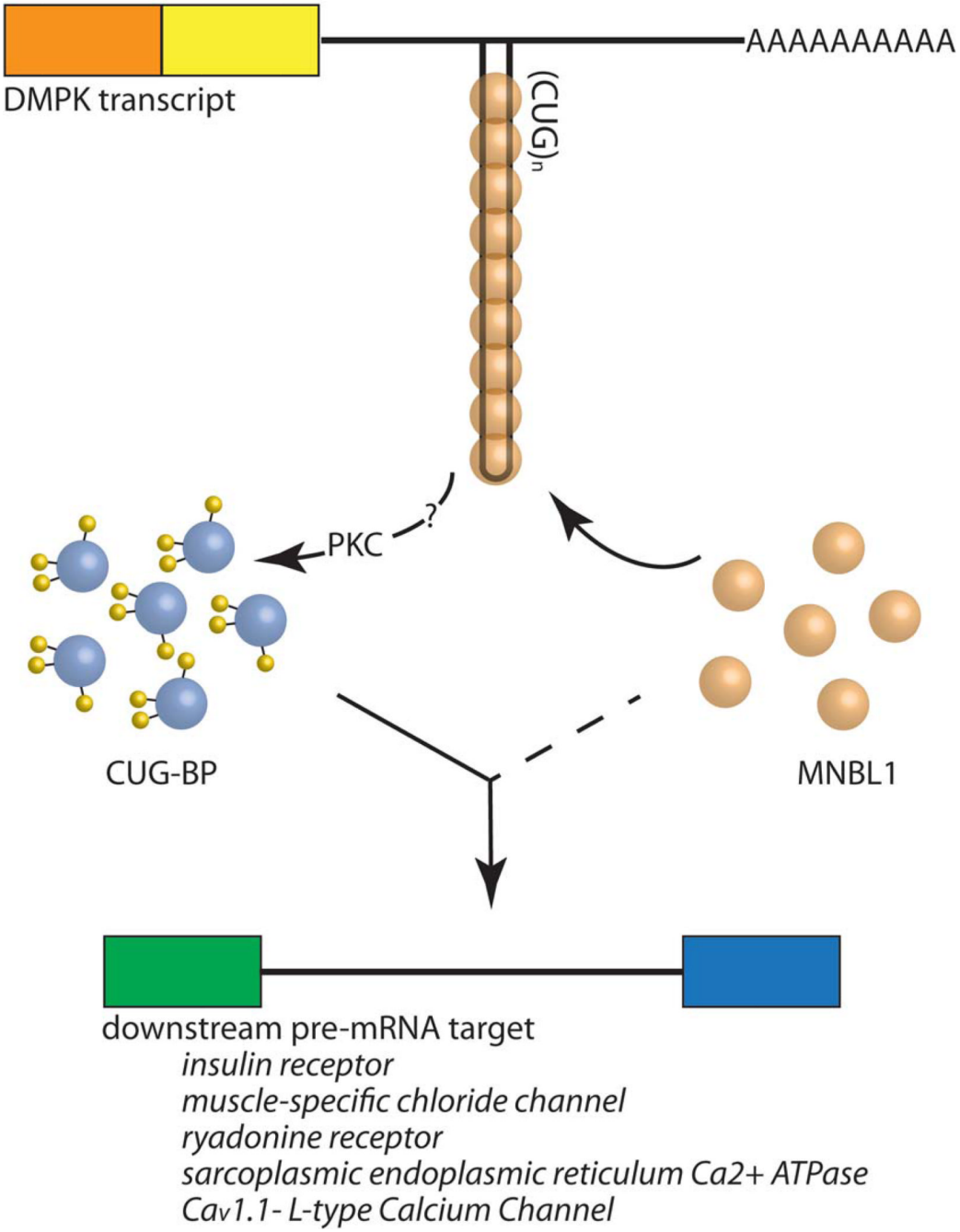

Figure 5. CUG repeats in DMPK gene cause dysregulation of AS

Mutation of the DMPK gene to include CUG repeats sequesters the MNBL1 protein and stimulates hyperphosphorylation of the CUG-BP through a PKC-mediated mechanism. These changes result in dysregulated splicing of downstream targets of MNBL1 and CUG-BP.

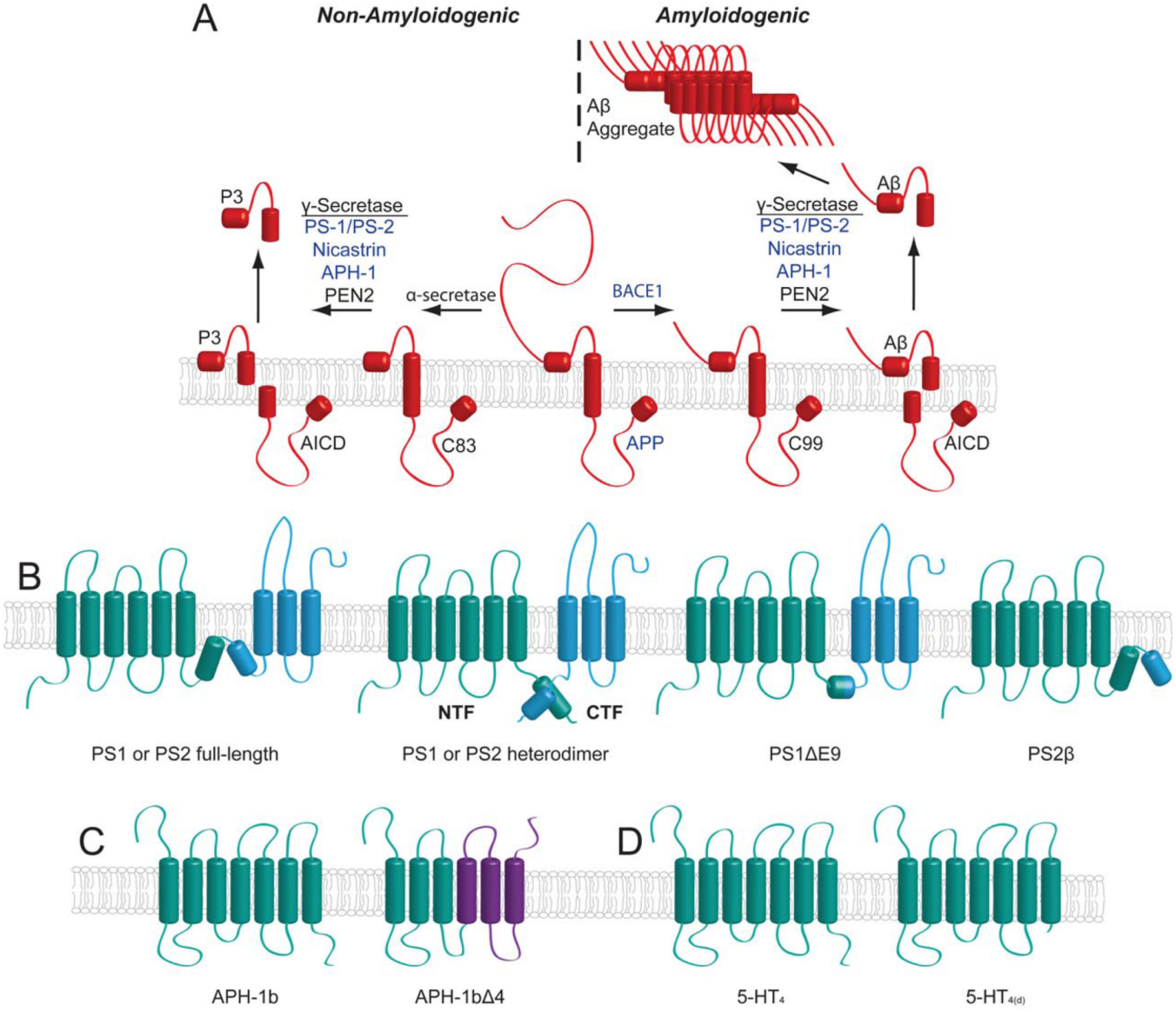

Figure 6. AS affects multiple proteins in the amyloidogenic pathway of AlzD

(A) APP processing pathways. **Left:** Non-amyloidogenic processing is initiated by α-secretase to produce C83, which is further processed by γ-secretase to produce the amyloid precursor protein intracellular domain (AICD) and the p3 peptide. **Right:** amyloidogenic processing is initiated by β-secretase to produce C99, which is further processed by γ-secretase to produce the AICD and the Aβ peptide. The Aβ peptide can form toxic oligomers and aggregates. Proteins affected by AS are highlighted in blue. **(B)** Alternative splicing of presenilin impacts γ-secretase activity. Exon 9 removal in PS1 results in a constitutively active form of the protein by considerably shortening the autoinhibitory loop and removing the endoproteolytic cleavage site. Exon 5 skipping in PS2 results in protein truncation after the autoinhibitory loop, creating a variant that is missing the CTF that is required for catalytic activity. **(C)** Exon 4 removal in APH-1b results in removal of the entire fourth TM segment, causing inverted topology of the last three TM segments (purple). This also results in the omission of the GXXXG motif that is required for γ-secretase

assembly. **(D)** AS of the 5-HT_4 receptor results in a much shorter C-terminal tail, which presumably alters the signaling properties of this GPCR.