CellPress

Alternative Splicing Regulatory Networks: Functions, Mechanisms, and Evolution

Jernej Ule[1,2,*] and Benjamin J. Blencowe[3,4,*]
[1]The Francis Crick Institute, London NW1 1AT, UK
[2]Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, Queen Square, London WC1N 3BG, UK
[3]Donnelly Centre, University of Toronto, Toronto, ON M5S 3E1, Canada
[4]Department of Molecular Genetics, University of Toronto, Toronto, ON, M5S 3E1, Canada
*Correspondence: jernej.ule@crick.ac.uk (J.U.), b.blencowe@utoronto.ca (B.J.B.)
https://doi.org/10.1016/j.molcel.2019.09.017

High-throughput sequencing-based methods and their applications in the study of transcriptomes have revolutionized our understanding of alternative splicing. Networks of functionally coordinated and biologically important alternative splicing events continue to be discovered in an ever-increasing diversity of cell types in the context of physiologically normal and disease states. These studies have been complemented by efforts directed at defining sequence codes governing splicing and their cognate *trans*-acting factors, which have illuminated important combinatorial principles of regulation. Additional studies have revealed critical roles of position-dependent, multivalent protein-RNA interactions that direct splicing outcomes. Investigations of evolutionary changes in RNA binding proteins, splice variants, and associated *cis* elements have further shed light on the emergence, mechanisms, and functions of splicing networks. Progress in these areas has emphasized the need for a coordinated, community-based effort to systematically address the functions of individual splice variants associated with normal and disease biology.

Transcripts from nearly all human protein-coding genes undergo one or more forms of alternative splicing, such as inclusion or skipping of individual "cassette" exons, switching between alternative 5′ and 3′ splice sites, differential retention of introns, mutually exclusive splicing of adjacent exons, and other, more complex patterns of splice site selection (Pan et al., 2008; Wang et al., 2008). All of these forms of splicing require the spliceosome, a megadalton machine that catalyzes splicing reactions (Wahl et al., 2009). Spliceosome formation entails a complex interplay of *trans*-acting factors, including small nuclear ribonucleoprotein particles (snRNPs, U1, U2, U4/U6, and U5) comprising small nuclear RNAs (snRNAs) and associated proteins, together with ~150 additional proteins. The formation of spliceosomes and their mechanism of action has been illuminated in remarkable detail by a series of recent cryoelectron microscopy structures, work that has been reviewed elsewhere (Kastner et al., 2019; Plaschka et al., 2019; Yan et al., 2019).

Binding of snRNPs to pre-mRNA is typically stabilized by mutual "definition" interactions across introns and exons (De Conti et al., 2013; Figure 1). Intron definition interactions predominate when introns are relatively short (e.g., in the range of up to a few hundred nucleotides), as is the case in yeast and most invertebrate species. In contrast, exon definition interactions predominate in vertebrates (Robberson et al., 1990), where introns have a median length of approximately 1 kb (Hong et al., 2006). In either case, the principles governing splice site recognition and pairing are thought to be similar. For example, current models posit that U1 snRNP binds to the 5′ splice site and communicates via bridging interactions with splicing factor 1 (SF1) and the U2 snRNP auxiliary factor (a heterodimer of U2AF1 and U2AF2) bound to the 3′ splice site and its adjacent polypyrimidine tract (Abovich and Rosbash, 1997; De Conti et al., 2013).

Additional interactions that contribute to exon and intron definition are mediated by members of the RNA recognition motif (RRM)-containing SR family of proteins (referred to below as "SR proteins") and SR-related proteins, each of which contains one or more intrinsically disordered region (IDR) rich in alternating arginine and serine residues, referred to as the "RS domain" (Figure 1). For example, it has been proposed that when SR proteins bind to exonic enhancer sequences, their RS domains interact with the RS domains of the U1 snRNP-specific 70-kDa protein (SNRNP70) and U2AF1 to promote exon definition (Wu and Maniatis, 1993). In *S. pombe*, it has been shown that intron definition is promoted by interactions between the RS domains of Rsd1 and Prp5 (orthologues of human RBM38 and DDX46), which interact with U1 and U2 snRNP, respectively (Shao et al., 2012).

Numerous additional interactions come into play to forge intron and exon definition interactions. For example, the SR-related proteins SRRM1 and SRRM2 can bridge interactions between snRNPs bound at splice sites and SR proteins bound at exonic enhancers (Eldridge et al., 1999). Moreover, phosphorylated RS domains have been reported to bind double-stranded RNA, which can promote base-pairing between snRNPs and pre-mRNA (Shen and Green, 2006). Collectively, these and additional early interactions, some of which are described later, facilitate the stable recruitment of U2 snRNP to the pre-mRNA branch site, followed by addition of U4/U6 and U5 snRNPs in the form of a tri-snRNP particle. The actions of many RNA helicases then promote rearrangements of snRNP interactions and establishment of a catalytically competent spliceosome that carries out the two *trans*-esterification reactions that lead to lariat formation, intron removal, and exon ligation (Wahl et al., 2009).

 Crown Copyright © 2019 Published by Elsevier Inc.

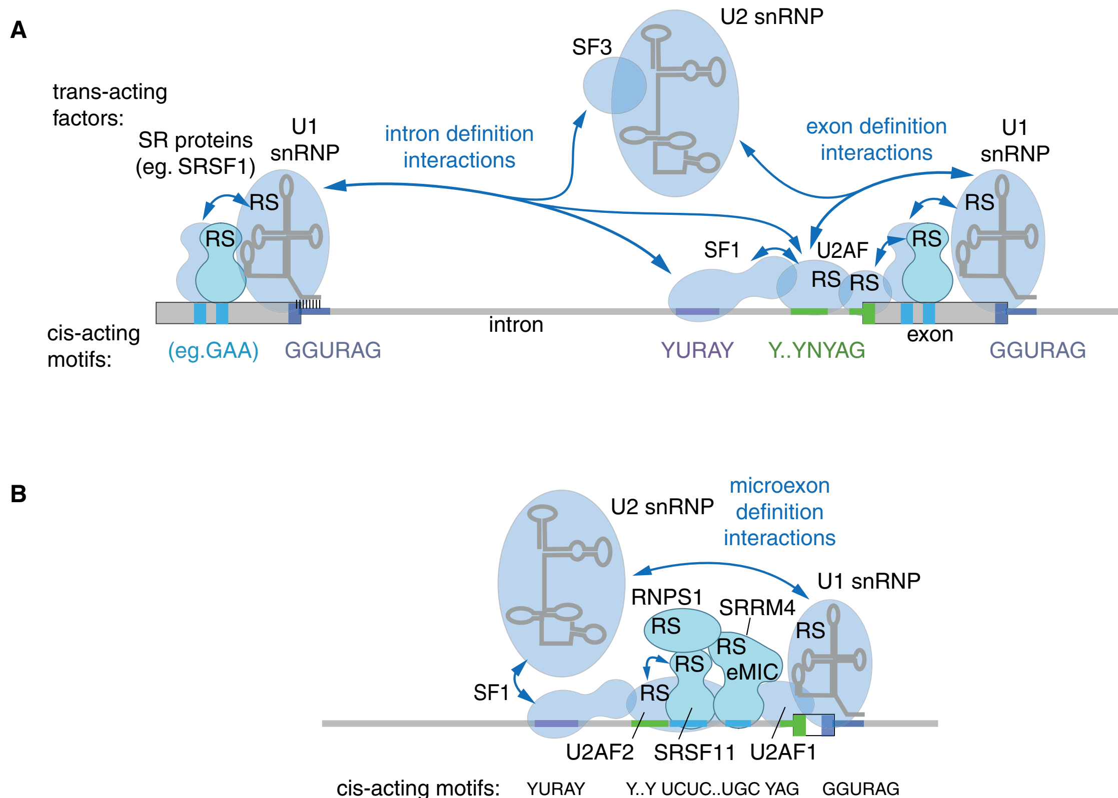

Figure 1. Exon and Intron Definition Interactions

(A) Schematic of spliceosomal components and regulatory proteins that participate in exon and intron definition and interactions between them. *Trans*-acting splicing factors are shown as blue shapes, and their names are shown next to the shape. The RS domain is marked by "RS." Blue arrows denote intron or exon definition interactions, many of which are mediated by the RS domain. Exons are represented as gray boxes, intronic RNA and snRNAs as gray lines, and *cis*-acting motifs as colored lines, with the consensus sequences of these motifs shown underneath. The pairing of U1 snRNA with the 5′ splice site is indicated by black lines.

(B) Schematic of microexon definition, shown in the same manner as described in (A).

Many types of RNA-binding proteins (RBPs) can regulate alternative splicing. In addition to SR proteins, these include the heterogeneous ribonucleoprotein (hnRNP) family of proteins as well as RBPs containing RRM, K homology domain (KH), zinc-finger, or other domains (Lunde et al., 2007). The full set of proteins that control alternative splicing is not known, although recent large-scale screens employing systematic RNAi or CRISPR-Cas-mediated ablation of genes have revealed repertoires involving a few hundred proteins that act directly or indirectly to regulate specific alternative exons (Gonatopoulos-Pournatzis et al., 2018; Han et al., 2017; Papasaikas et al., 2015; Tejedor et al., 2015). Among other unexpected factors, these studies have highlighted previously annotated DNA-binding proteins as having potential direct roles in RNA binding and splicing regulation. RBPs bind *cis* elements in introns and exons and regulate splice site selection largely by promoting or repressing definition interactions (De Conti et al., 2013; Fu and Ares, 2014). Thus, they mainly act at the early stages of spliceosome formation, although regulation can also be imparted at later stages of assembly (Wahl et al., 2009).

In this review, we highlight recent advances in the identification and characterization of networks of splicing regulation, including significant strides that have been made in the systematic analysis of RBPs and associated regulatory mechanisms through application of *in vitro* binding (Dominguez et al., 2018) and *in vivo* cross-linking and immunoprecipitation (CLIP) methods (Lee and Ule, 2018; Van Nostrand et al., 2018), proteomics (Hentze et al., 2018), functional genomics (Gonatopoulos-Pournatzis et al., 2018), and increasingly powerful computational approaches (Baeza-Centurion et al., 2019; Jaganathan et al., 2019). We review how these and other complementary approaches are further providing unprecedented new insights into the evolution of mechanisms governing alternative splicing as well as how disruption of these mechanisms causes or contributes to human diseases and disorders. Finally, we conclude by discussing challenges for the field that lie ahead.

Biological Significance of Alternative Splicing Regulatory Networks

The development and application of custom microarrays and, later, high-throughput RNA sequencing (RNA-seq) methods, revealed the extraordinary complexity of regulated alternative splicing in metazoans, particularly in vertebrate species (Blencowe, 2015; Fu and Ares, 2014). Recent transcriptome sequencing efforts involving both short- and long-read technologies are increasingly focusing on specialized cell types and

individual cells from different organs. To date, dynamic alternative splicing networks have been detected in embryonic stem and precursor cells, during differentiation or reprogramming of various cell lineages as well as epithelial-mesenchymal transitions, and in adult organs such as the brain, heart, skeletal muscle, liver, kidney, adipose tissue, and testes and in the immune system (Baralle and Giudice, 2017; Bhate et al., 2015; Gabut et al., 2011; Han et al., 2013; Irimia et al., 2014; Kalsotra and Cooper, 2011; Licatalosi and Darnell, 2010; Mallory et al., 2015; Tapial et al., 2017; Wong et al., 2013; Zhang et al., 2016). Additional regulated alternative splicing networks have been detected in association with specific physiologic states of cells, such as thermal regulation and the stress response (Boutz et al., 2015; Gotic et al., 2016; Low et al., 2008; Preußner et al., 2017). Many regulatory RBPs function in a cell-, tissue-, or condition-specific manner and are capable of coordinately regulating functionally coherent "networks" of exons and introns (Braunschweig et al., 2013; Licatalosi and Darnell, 2010). Thus, our understanding of the repertoires of detected splice variants as well as other forms of transcript variation across cellular conditions in the context of normal and disease physiology continues to rise dramatically.

Notably, regulated alternative exons that overlap protein-coding sequences are often located within predicted IDRs that are coincident with sites of post-translational modifications and protein-protein interactions, and the role of alternative splicing in diversifying protein interaction capabilities has been demonstrated experimentally (Buljan et al., 2012; Ellis et al., 2012; Yang et al., 2016). An important and likely general function of alternative splicing networks is therefore to control protein-protein interactions to impart important cell-, tissue-, and condition-specific functions of widely expressed genes. In addition to remodeling the IDRs, a smaller number of conserved events in alternative splicing networks directly overlap critical modular protein domains and affect various important protein functions, such as those involved in control of transcription and chromatin, as reviewed elsewhere (Irimia and Blencowe, 2012; Kelemen et al., 2013; Porter et al., 2018). The identification and characterization of such events highlights the capacity of alternative splicing networks to have a broad effect on physiology through their ability to cross-talk with orthogonal gene regulatory layers.

A Neuronal Microexon Network

Comparative vertebrate transcriptomics of a common set of organs from fish to human revealed that brain-specific alternative splicing events are among the most evolutionarily conserved (Barbosa-Morais et al., 2012; Merkin et al., 2012). Particularly striking in this regard is a network of a few hundred 3- to 27-nt neuronal microexons that represent the most highly conserved class of alternative splicing events discovered to date (Irimia et al., 2014; Li et al., 2015). These microexons predominantly preserve open reading frames and insert one to several amino acids in proteins associated with neurogenesis, axon guidance, and synaptic functions (Irimia et al., 2014; Johnson et al., 2019; Quesnel-Vallières et al., 2019; Ustianenko et al., 2017). Similar to longer exons, individual neuronal microexons can also affect orthogonal regulatory layers; for example, they alter the function of the lysine-specific histone demethylase 1A (KDM1A, also known as Lsd1), control activation domains of Mef2 family transcription factors, and regulate the activity of the cytoplasmic polyadenylation element binding protein 4 (CPEB4), which controls poly(A) tail length and translation (Ebert and Greenberg, 2013; Parras et al., 2018; Rusconi et al., 2017).

Notably, misregulation of activity-dependent splicing of microexons likely plays a causative role in autism, at least in part as a consequence of disrupted expression of the major *trans*-acting regulator of microexons, the neuronal SR-related protein nSR100/SRRM4 (Irimia et al., 2014; Quesnel-Vallières et al., 2015, 2016). SRRM4 activates splicing of microexons by binding specialized upstream intronic enhancer elements together with the SR proteins SRSF11 and RNPS1 (Gonatopoulos-Pournatzis et al., 2018; Raj et al., 2014; Figure 1B). The C-terminal IDR of SRRM4 contains an "enhancer of microexons" (eMIC) domain, which interacts with the branchpoint-binding protein SF1 and U2AF to promote recruitment of U2 snRNP and, thus, activates the earliest stages of splicing complex formation (Torres-Méndez et al., 2019). The neuronal inclusion of microexons can be further modulated by additional proteins; for example, the neuronal enriched Nova proteins repress microexons in Robo1 and Robo2 genes during the later stages of neuronal development to control navigation of commissural axons (Johnson et al., 2019). In contrast to longer neurally regulated exons that are enriched within IDRs, as described above, neural microexons significantly overlap or are adjacent to modular domains that function in mediating protein and other ligand interactions (Irimia et al., 2014). Many microexon-regulated proteins are known to form physical interactions with one another, suggesting that the coordinated inclusion of dozens of microexons likely remodels large protein interaction networks in neurons that are disrupted in autism.

Mechanisms Underlying the Regulation of Alternative Splicing Networks

Multivalency and RNP Condensation in Splicing Regulation

Key to a more general understanding of the function and regulation of alternative splicing networks is the systematic identification of corresponding splicing regulators and their cognate *cis*-acting binding sites. Most RNA-binding domains of RPBs recognize short (i.e., 3–4 nt) and degenerate motifs, and the capacity of such individual motifs to predict splicing outcomes is low. Instead, mutagenesis of minigene reporters as well as transcriptomics studies have demonstrated that many RBPs cooperatively interact with repeating or "multivalent" motifs within or proximal to alternative exons to efficiently regulate splicing (Cereda et al., 2014; Chou et al., 2000; Fu and Ares, 2014; Ule et al., 2003). Biophysical studies with purified RBPs and RNA have demonstrated that multivalent interactions can drive condensation into dynamic complexes through the phenomenon of liquid-liquid phase separation (LLPS) (Banani et al., 2017; Kato and McKnight, 2018; Li et al., 2012). Such condensation can increase the concentration of molecules in RNP condensates by two orders of magnitude compared with the surrounding liquid (Li et al., 2012), and this likely enables RBPs to bind more stably to RNA transcripts with multivalent sites compared with the interaction of a single RNA-binding

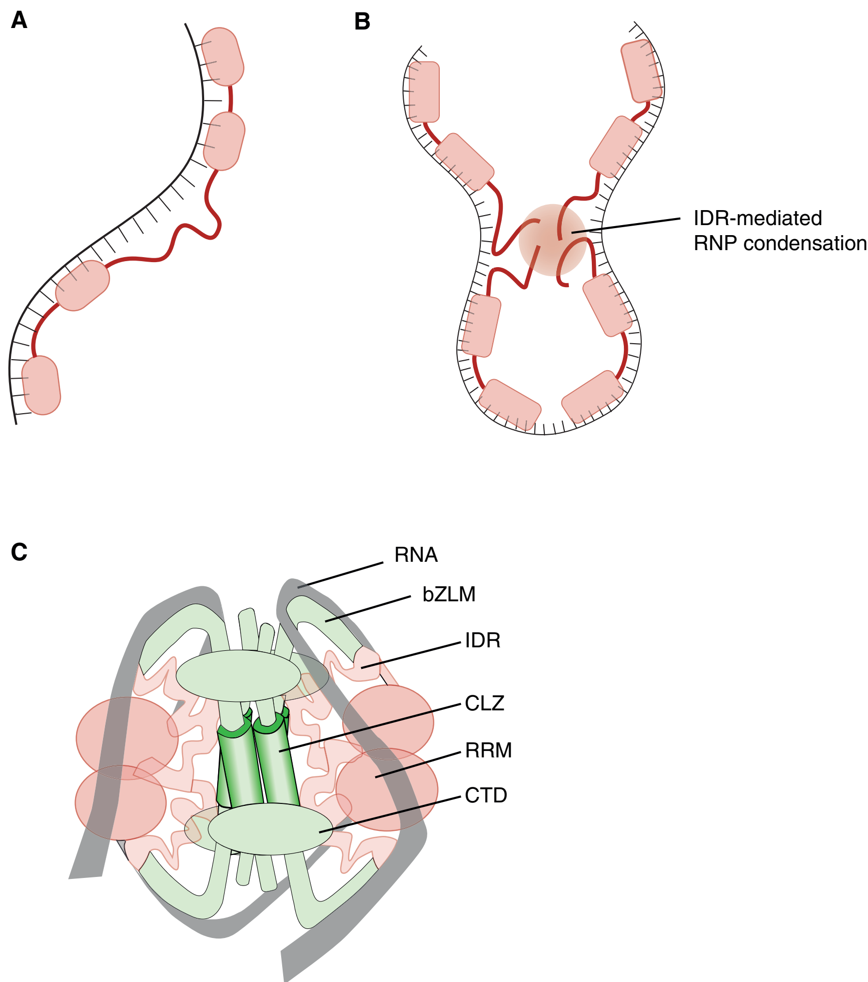

Figure 2. Multivalent RNP Assembly
Intrinsically disordered regions (IDRs) enable multiple RNA-binding domains with similar RNA specificity to recognize multivalent RNA motifs, which can be achieved in three primary ways:
(A) Domains with similar sequence specificity are connected by IDRs within a multi-domain RBP, such as PTBP1.
(B) Multiple copies of the same or different types of RBPs are brought together by multivalent interactions formed by the IDR, which can promote RNP condensation (Banani et al., 2017; Kato and McKnight, 2018). Such condensates might be better capable of recognizing multivalent RNA sequences.
(C) IDRs can connect multiple types of domains to enable homomeric assembly of an RBP into a regular structural unit. For example, hnRNPC contains a single RRM domain, but is capable of multivalent assembly on sequences containing multiple U-tracts because it forms a tetramer through the action of distinct domains. The RRM is connected via an IDR to a basic leucine zipper-like motif (bZLM), which is proposed to interact in a sequence-independent manner with the RNA that wraps around the tetramer, followed by a C-terminal leucine zipper-like domain (CLZ) that forms a stable coiled-coil tetramer, and the C-terminal domain (CTD) is proposed to regulate protein-RNA interactions (adapted from Whitson et al., 2005).

domain of an RBP with an individual RNA motif. For example, incubation of purified PTBP1 with an RNA oligonucleotide containing multiple cytidine and uridine (CU) motifs leads to LLPS under *in vitro* conditions (Li et al., 2012). PTBP1 contains four RRM domains, each of which can bind a short CU-rich motif (Chou et al., 2000; Oberstrass et al., 2005), and many other RBPs contain multiple domains with similar sequence specificity (Lunde et al., 2007; Figure 2A), which likely enables their condensation on cognate multivalent RNA sequences.

Even RBPs that contain only one or two RNA-binding domains can undergo RNP condensation on RNAs with multivalent motifs if their IDRs promote homomultimerization. This is often achieved through short, multivalent protein-protein interacting motifs in IDRs, such as repeating amino acids, short linear motifs, and/or dynamic secondary structures that are capable of relatively promiscuous and weak homomeric interactions (Banani et al., 2017; Kato and McKnight, 2018). Multiple recent studies have demonstrated that RNP condensation mediated by IDRs and the multivalent binding properties of RBPs can function in transcriptome-wide assembly and function of splicing regulators (Attig et al., 2018; Gueroussov et al., 2017; Ying et al., 2017; Zhou et al., 2019; Figure 2B). For instance, the tyrosine-rich IDR of RBFOX can mediate LLPS *in vitro*, whereas in cells it is crucial for the interaction of RBFOX with a large RNP complex referred to as large assembly of splicing regulators (LASR), which contains eight RBPs, including hnRNPC, hnRNPH, hnRNPM, and MATR3 (Damianov et al., 2016; Ying et al., 2017). Interestingly, the IDR is not required for sequence-specific binding of RBFOX to RNA but for its assembly into higher-order complexes that regulate a subset of its target alternative exons. Notably, the IDR of RBFOX also contributes to formation of RNA granules upon stress as part of a cellular adaptive response (Kucherenko and Shcherbata, 2018), indicating that the multivalent interactions formed by the IDR can contribute to assembly of multiple types of RNP condensates.

Another recent study has revealed that IDRs of many RBPs, including those of most members of the hnRNP A and D families, contain glycine and tyrosine repeats that can promote LLPS *in vitro* and promote binding to multivalent RNA sites to globally regulate alternative splicing (Gueroussov et al., 2017). Interestingly, the C-terminal IDRs of hnRNP A and D family proteins were found to contain conserved exons that are alternatively spliced in mammals but constitutively spliced in other vertebrate species. Differential inclusion of these exons controls the formation of tyrosine-dependent multivalent hnRNP assemblies that, in turn, function to globally regulate splicing. Thus, IDRs in RBPs contribute to regulation of alternative splicing and are themselves often regulated through alternative splicing. Notably, as proposed for transcriptional control (Hnisz et al., 2017), RNP

CellPress

condensation could increase the responsiveness of regulatory networks to local concentrations and modifications of IDR-containing proteins, which, in turn, may play an important role in mediating inputs from external and internal signals.

RNA Position Dependence of Splicing Regulation

Analysis of RBP binding profiles around co-regulated alternative splicing events has been an effective means of unravelling regulatory mechanisms and can be summarized by three main insights. First, SR proteins typically enhance splicing when binding between other spliceosomal components because their RS domains generally mediate heteromeric interactions with other RS domain-containing splicing factors in a manner that promotes combinatorial assembly of the spliceosome on RNA elements, including the branchpoint, splice sites, and enhancer motifs. SR proteins typically bind to exonic enhancer sequences to activate splicing (Fu and Ares, 2014), but in the case of microexons, nSR100/SRRM4 binds to intronic enhancer sequences upstream of the 3′ splice site (Raj et al., 2014; Figure 1B). When binding at these positions, SR proteins generally oppose the repressive effects of hnRNPs (Chen and Manley, 2009); for example, SRRM4 opposes the repressive effects of PTBP1 on the inclusion of microexons (Gonatopoulos-Pournatzis et al., 2018; Li et al., 2015; Raj et al., 2014). In contrast to their general enhancing activities, some characterized SR proteins have been reported to repress splicing when binding to intronic sequences downstream of target exons (Erkelenz et al., 2013; Ibrahim et al., 2005; Kanopka et al., 1996). It remains to be determined whether such reciprocal patterns of SR proteins commonly contribute to splicing regulation across the transcriptome.

Second, computational studies have been used to construct "RNA maps" of RBP activity in splicing regulation by evaluating the position of their binding around exons. Initially, studies of Nova proteins revealed RNA maps accounting for their dual effects on splicing in the brain that depend on their binding position on pre-mRNA, defined either by analysis of multivalent motifs or with CLIP data (Licatalosi et al., 2008; Ule et al., 2006). The multivalency of RNA motifs has been exploited to further develop computational approaches to identify regulatory motifs and RNA maps at co-regulated exons, recently with the additional context of the predicted RNA structure (Cereda et al., 2014; Dominguez et al., 2018; Feng et al., 2019). Through the efforts of many laboratories, RNA maps of numerous additional RBPs have been determined around regulated exons, revealing their position-dependent splicing regulation (Van Nostrand et al., 2018; Witten and Ule, 2011). For instance, Nova, PTBP1, and TDP-43 tend to repress exon inclusion when their binding overlaps an exon or splice site but activate splicing when bound to intronic sequences downstream of the 5′ splice site (Llorian et al., 2010; Tollervey et al., 2011; Ule et al., 2006; Xue et al., 2009; Figures 3A and 3B). These RBPs generally rely on IDRs that connect multiple domains with similar RNA specificity (as in the case of Nova or PTBP1) or promote formation of homomeric RBP complexes (as in the case of TDP-43), which gives these RBPs the flexibility to recognize variably spaced multivalent motifs (Figure 2). Therefore, these RBPs can bind to multivalent motifs that are located between splice sites and SR protein-binding sites, which can lead to efficient competition for spliceosome assembly on pre-mRNA (Figure 3A).

The mechanisms of enhancing effects of non-SR proteins have been explored in depth for TIA1, which interacts with the U1 snRNP-specific C protein to recruit U1 snRNP to the 5′ splice site (Förch et al., 2002), and PTBP1, which has been proposed to interact with the stem loop 4 of U1 snRNA to stimulate docking of the U1 snRNP in a productive or nonproductive conformation depending on the position of PTBP1 binding on pre-mRNA (Hamid and Makeyev, 2017; Sharma et al., 2011; Figure 3B). Interestingly, stem loop 4 of U1 snRNA also interacts with the SF3a component of the U2 snRNP, which can promote intron or exon definition (Sharma et al., 2014). The position-dependent rules governing alternative splicing are similar across hnRNPs and many other RBPs in various types of cells and tissues in species such as *C. elegans*, *Drosophila*, mouse, and human (Ajith et al., 2016; Brooks et al., 2011; Kuroyanagi et al., 2013; Wang et al., 2012; Witten and Ule, 2011). These diverse RBPs recognize a range of different RNA motifs and have limited sequence homology with each other; thus, it remains to be determined whether they operate through common mechanisms, such as employing interactions with different components of the U1 snRNP.

Third, RBPs can also follow a reciprocal position dependence when regulating an alternative exon indirectly via the adjacent exon. For instance, although Nova and TIA proteins enhance inclusion when binding downstream of an alternative exon, they can also repress inclusion of an alternative exon when binding downstream of a preceding constitutive exon (Ule et al., 2006; Wang et al., 2010; Figure 3C). Similar reciprocity at adjacent exons can be seen in the RNA maps of several other RBPs studied by the Encyclopedia of DNA Elements (ENCODE) project (Van Nostrand et al., 2018). Reciprocal effects of regulation are commonly seen at adjacent splice sites as a result of splice site competition because silencer sequences are most effective on a splice site when a competing splice site is present nearby (Yu et al., 2008). Competition between splice sites of adjacent exons can also control splicing outcomes if both introns that flank the alternative exon are available when the splicing choice is made. Indeed, splicing of alternative exons is often somewhat delayed after transcription, which might enable such competition (Drexler et al., 2019; Kim et al., 2017). Moreover, studies of Nova-regulated exons showed that excision of introns is often asymmetric so that a specific intron is spliced first in the pathway to exon inclusion, and Nova tends to bind this intron when directly regulating exon inclusion (Ule et al., 2006). However, Nova can also indirectly repress inclusion of an alternative exon by enhancing the 5′ splice site at the preceding exon, perhaps through competition with the exon inclusion pathway, which requires initial splicing of the intron downstream of the alternative exon (Figure 3C). Interestingly, splice site competition has also been shown recently to cause a non-linearity in the genotype-phenotype map of exonic mutations (Baeza-Centurion et al., 2019). The broader importance of the order of intron excision and splice site competition in splicing regulation and its interplay with multivalency and positioning of *cis*-acting motifs remain to be fully established.

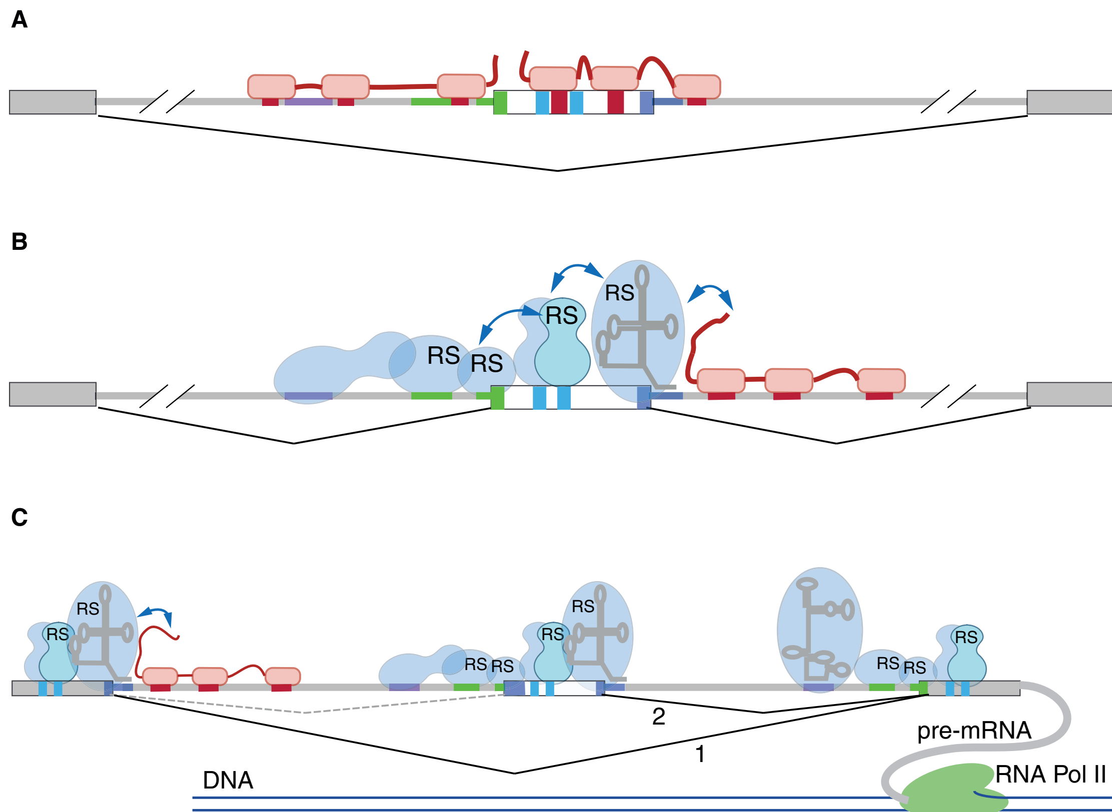

Figure 3. Alternative Splicing Regulation Mechanisms
The schematized multivalent RBPs correspondto Nova proteins, which contain three KH domains and act according to the principles shown in the figure (Ule et al., 2006). Many other RBPs have been shown to follow similar principles.
(A) Multivalent motifs (marked with red lines) that are intercalated between splice sites and enhancing motifs recruit repressive RBPs that can compete for pre-mRNA binding with other splicing factors. The RNA and *cis*-acting motifs are shown as in Figure 1A.
(B) If multivalent motifs are located downstream of the exon, then many RBPs can bind to support exon definition, most likely by interacting with the U1 snRNP. The RNA, RBPs, and *cis*-acting motifs are shown as in Figure 1A.
(C) RBPs can indirectly regulate an alternative exon by enhancing the 5′ splice site of a preceding exon, which promotes exon skipping (pathway 1). This likely requires an asymmetric pathway to exon inclusion that relies on the initial removal of the intron downstream of the alternative exon (pathway 2). In such a case, RBPs likely promote skipping by changing the competition between pathways 1 and 2.

Regulatory Roles of Cryptic Splice Sites and Exons

Cryptic Splice Sites and Intronic Decoys

One of the most remarkable feats of the cell is its ability to accurately recognize and differentially regulate correct splice sites from the myriad sequences that match splice site consensus sequences but are not selected to form mature mRNA. These cryptic sites can be distinguished from canonical splice sites in part because their positioning is not compatible with efficient definition interactions. Moreover, they are often flanked by a high density of splicing silencer motifs that bind to various hnRNPs that repress their recognition (Sibley et al., 2016; Wang et al., 2004; Zhang et al., 2008). For instance, cryptic exons can be repressed by hnRNPC when binding to U-tracts in antisense Alu elements (Zarnack et al., 2013), by Nova when binding to long clusters of YCAY motifs (Eom et al., 2013), and by TDP-43 or hnRNPL when binding UG or CA repeats, respectively (Ling et al., 2015; McClory et al., 2018).

Derepression of cryptic splice sites in human diseases can be caused either by mutations in *cis*-acting silencing elements or deregulation of *trans*-acting repressors (Kahles et al., 2018; Ling et al., 2015; Sibley et al., 2016; Zarnack et al., 2013). Mutations in the core splicing machinery can also lead to derepression of cryptic splice sites and exons, as shown by analysis of cryptic 3′ splice sites induced by cancer-associated *SF3B1* mutations (Darman et al., 2015; DeBoever et al., 2015). Interestingly, a *Caenorhabditis elegans* genetic screen recently identified alleles of the core spliceosome component Prp8 that specifically alter cryptic splicing frequency (Mayerle et al., 2019). Notably, alterations in the accuracy of splice site recognition can lead to substantial transcriptome heterogeneity in cancer, with consequences for the generation of neoantigens of possible relevance in immunotherapy (Kahles et al., 2018; https://genome.cshlp.org/content/early/2019/09/13/gr.248922.119). Thus, it is becoming clear that, aside from roles in ensuring transcriptome integrity, control of cryptic alternative splicing events can also contribute to regulation of gene expression, whereas their perturbation can lead to disease or can serve as a biomarker or therapeutic target.

Cryptic splice sites and binding sites for splicing factors can also act as intronic decoys that compete with canonical splice

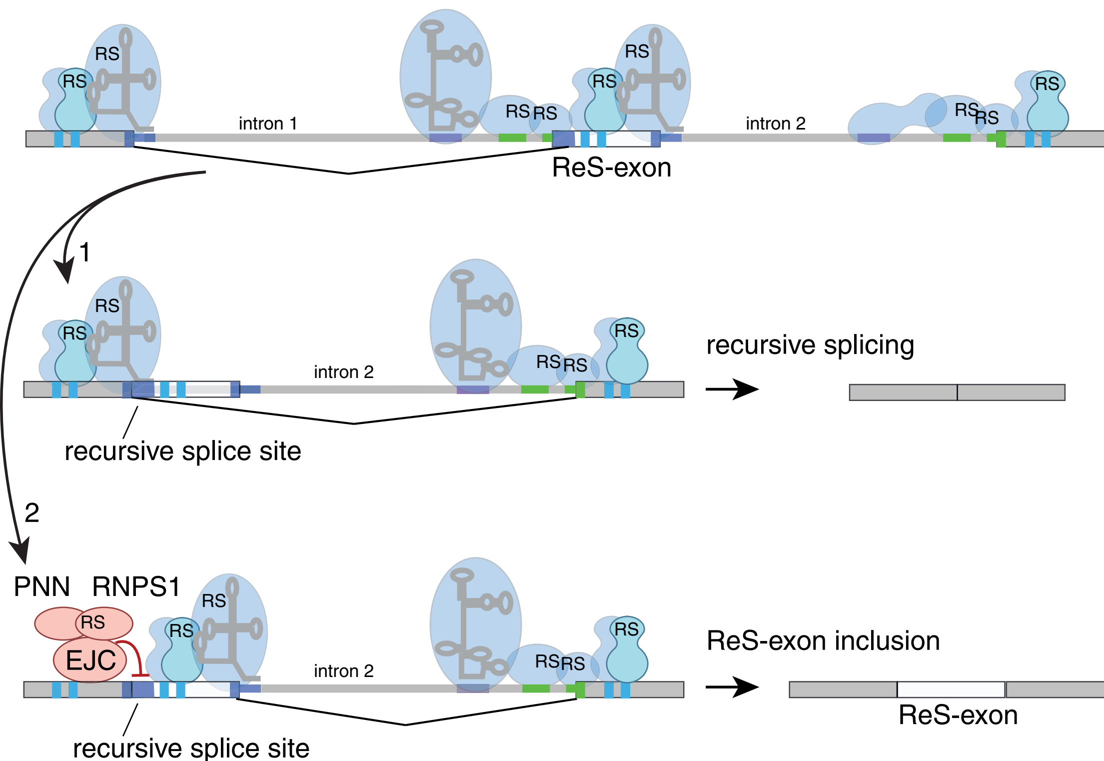

Figure 4. Recursive Splicing and Its Regulation
Schematic of the mechanism underlying recursive splicing and its regulation by the exon junction complex (EJC). For recursive splicing to be possible, an ReS-exon needs to be defined to enable splicing of the intron upstream of the ReS-exon (labeled here as "intron 1"). This splicing event reconstitutes a new 5′ splice site at the exon-exon junction, and normally also leads to deposition of the EJC on the preceding exon, which can result in two alternative outcomes. If spliceosome assembles on the reconstituted 5′ splice-site, then it can lead to ReS-exon removal through recursive splicing (as shown by arrow 1). However, in the case of canonical ReS-exons, the EJC tends to efficiently repress recognition of the reconstituted 5′ splice-site, promoting ReS-exon inclusion (as shown by arrow 2).

sites for spliceosomal recruitment and thereby affect splicing of adjacent introns or exons (Coté et al., 2001; Parra et al., 2018; Sutandy et al., 2018). The activity of such intronic decoys is tightly regulated because they tend to be flanked by splicing silencer motifs (Wang et al., 2004, 2006), whereas cryptic splicing enhancer motifs are often sequestered into double-stranded RNA (Hiller et al., 2007). Splicing factors such as U2AF2 can bind to numerous intronic decoys, where they can promote use of a cryptic splice site and interfere with splicing at the immediate downstream 3 splice site (Coté et al., 2001; Shao et al., 2014; Zarnack et al., 2013). Many RBPs can bind to intronic decoys to negatively or positively regulate U2AF2 binding, thereby indirectly regulating splicing decisions (Howard et al., 2018; Sutandy et al., 2018; Zarnack et al., 2013). The potential for splicing regulation through intronic decoys is likely to be greatest in long introns, which are enriched in genes expressed in the brain (Sibley et al., 2015) and for which cell-type-specific CLIP studies are particularly valuable for elucidating competitive and cooperative binding mechanisms and functions of RBPs (Saito et al., 2019; Ule et al., 2018).

Recursive Splicing and Its Regulation

Recursive splicing (ReS) is a process by which introns are excised in multiple steps (Hatton et al., 1998). It is present most often in long introns of *Drosophila* and human genes and has been associated with increased splicing accuracy (Burnette et al., 2005; Duff et al., 2015; Pai et al., 2018; Sibley et al., 2015). It is also required for skipping of cryptic exons that form during ReS (hereafter referred to as "ReS-exons"; previously referred to in the literature as RS-exons; Joseph et al., 2018; Sibley et al., 2015; Figure 4). ReS-exons are initially defined by the spliceosome and spliced to a preceding exon, which generates a "reconstituted" 5′ splice site that is then used in the second splicing event to "discard" the ReS-exon (Sibley et al., 2015). In human genes, most cryptic ReS-exons are present in long first introns of neuronal genes. Interestingly, alternative promoters commonly arise from long introns, leading to production of a new first exon, which is not capable of reconstituting a strong 5′ site after the first step of recursive splicing, an event that leads to inclusion of the ReS-exon. Cryptic ReS-exons contain stop codons in all three frames, and their inclusion thus elicits nonsense-mediated decay (NMD) and terminates translation. In this way, recursive splicing serves as a mechanism for quality control of transcripts produced from alternative promoters by preventing production of aberrant proteins with altered N termini (Sibley et al., 2015).

Not all ReS-exons are cryptic; hundreds of annotated ReS-exons are included in human mRNAs because their recursive splicing is repressed by the exon junction complex (EJC) (Blazquez et al., 2018; Figure 4). The EJC comprises several proteins that associate with sequences ∼24 nt upstream of spliced

junctions and has been implicated previously in mRNA turnover, export, translation, as well as splicing (Le Hir et al., 2016). It is not entirely clear why the EJC can repress recursive splicing of canonical but not cryptic ReS-exons, although the relative strengths of the canonical and reconstituted 5′ splice sites clearly play a role (Blazquez et al., 2018; Sibley et al., 2015). In addition to repressing recursive splicing, the EJC can also repress cryptic 5′ and 3′ splice sites located near exon-exon junctions (Blazquez et al., 2018; Boehm et al., 2018); when such a cryptic 5′ splice site is located close to the start of an exon, its recognition leads to inclusion of cryptic "microexon" sequences. It is intriguing that RNPS1, an auxiliary EJC component, can repress inclusion of such cryptic microexons, whereas, on the other hand, it promotes splicing of canonical neural microexons (Gonatopoulos-Pournatzis et al., 2018).

It is also interesting to consider that the repressive efficiency of core and auxiliary EJC components likely varies between tissues, which could lead to regulated recursive splicing of canonical exons. Notably, deficient EJC activity in mice has the strongest effect on the brain, where it leads to skipping of ReS-exons in genes with neurodevelopmental functions, likely contributing to a microcephaly phenotype (Blazquez et al., 2018). Moreover, several human neurodevelopmental disorders are associated with mutations in EJC components (McMahon et al., 2016), and it is plausible that deregulated recursive splicing might contribute to these disorders.

Emergence and Evolution of Alternative Splicing

The degenerate nature of splice sites provides opportunities for alternative splicing regulation, which, as described above, is often achieved through the action of *cis*-acting splicing enhancer and silencer sequences (Fu and Ares, 2014). Analysis of these *cis* elements by machine learning approaches can infer context-dependent and combinatorial mechanisms to derive "splicing codes" that predict splice site selection in a genomic sequence and, in some cases, also regulation of alternative splicing across cell types and tissues (Baeza-Centurion et al., 2019; Barash et al., 2010; Bretschneider et al., 2018; Jaganathan et al., 2019; Louadi et al., 2019; Xiong et al., 2015). Notably, a splicing code inferred from mouse data can predict splicing patterns in other vertebrate species with reasonable accuracy (Barbosa-Morais et al., 2012), in agreement with the conserved nature of the binding specificities of orthologous RBPs and their cognate *cis*-acting elements (Irimia et al., 2011; Jelen et al., 2007; Oddo et al., 2016; Solana et al., 2016).

During the early stages of genome sequencing projects, a prediction was made that differences in transcriptome diversity, rather than the absolute numbers of protein coding genes, might more strongly account for the vast range of biological complexity and phenotypic attributes across metazoan species (Ewing and Green, 2000). Analyses of expressed sequence tags and microarray profiling studies subsequently provided initial evidence that alternative splicing patterns have diverged rapidly among species (Modrek and Lee, 2003; Pan et al., 2004). Moreover, comparative RNA-seq analyses of transcriptomes of diverse organ types in vertebrate species spanning ∼350 million years of evolution have revealed that, although organ-dependent gene expression programs have remained relatively conserved, patterns of alternative splicing have diverged remarkably rapidly such that, overall, alternative splicing patterns are more similar between organs within a species than they are between the same organs of different species (Barbosa-Morais et al., 2012; Merkin et al., 2012).

The evolutionary origin of alternative splicing can be traced to the last eukaryotic common ancestor, which contained introns and the spliceosome (Collins and Penny, 2005; Csuros et al., 2011). The role of alternative splicing in proteome expansion appears to be particularly important in animals, where subsets of regulated alternative exons are strongly enriched for frame preservation, which is not observed in plants or other eukaryotes (Grau-Bové et al., 2018). Moreover, the frequency of alternative splicing through exon skipping has increased during animal evolution, with the highest skipping frequencies detected in the primate nervous system (Barbosa-Morais et al., 2012; Kim et al., 2007; McGuire et al., 2008). These increases are in part likely to be a consequence of changes in the gene architecture of animal genomes, particularly the increased density of introns, shorter exons, and heterogeneous strengths of splice sites (Csuros et al., 2011; Grau-Bové et al., 2018).

Related to these observations, biochemical and cryoelectron microscopy (cryo-EM) studies have demonstrated that, compared with the yeast spliceosome, the human spliceosome contains more regulatory components and is also considerably more dynamic (Kastner et al., 2019; Plaschka et al., 2019; Shi, 2017; Yan et al., 2019). Moreover, the structure of the human post-catalytic spliceosome revealed that it has co-opted additional metazoan-specific RBPs, which are implicated in alternative splicing, to regulate 3′ splice site selection and exon ligation (Fica et al., 2019). For example, Cactin, SDE2, and NKAP stabilize the exon ligation conformation of the spliceosome (Fica et al., 2019), and their orthologs are known to regulate alternative splicing in *S. pombe* (Lorenzi et al., 2015; Thakran et al., 2018). Thus, the human spliceosome likely allows more plasticity and RNA-specific regulation at multiple steps of the splicing reaction.

In 1977, Francois Jacob coined the phrase "evolutionary tinkering" to propose that mutations accumulate gradually in genes while minimizing disruption of existing functions (Jacob, 1977). Alternative splicing provides a remarkably fertile ground for such tinkering, particularly through evolutionary reassortment of *cis*-acting elements (Barbosa-Morais et al., 2012; Deveson et al., 2018; Merkin et al., 2012), but also through changes in *trans*-acting regulators, especially in the IDRs of RBPs. Intronic sequences derived from transposable elements (TEs) are also a particularly common source of *cis*-acting elements that vary across evolution and can regulate splicing (Gal-Mark et al., 2009; Keren et al., 2010; Figure 5A). For example, hnRNPC binds to antisense Alu sequences that are prevalent in introns and thereby represses splicing of thousands of Alu-derived exons (Zarnack et al., 2013). hnRNPC forms a tetramer that can further assemble into higher-order hnRNP particles (Skoglund et al., 1983; Whitson et al., 2005). Each tetramer contains four RRM domains in a conformation that is ideally suited for binding to two U-tracts that are present within the antisense Alu sequence (Figure 2C). Binding of hnRNPC to these U-tracts is crucial for it to achieve an affinity required to displace U2AF2, which is essential to repress the use of adjacent cryptic 3′ splice sites (Zarnack et al., 2013).

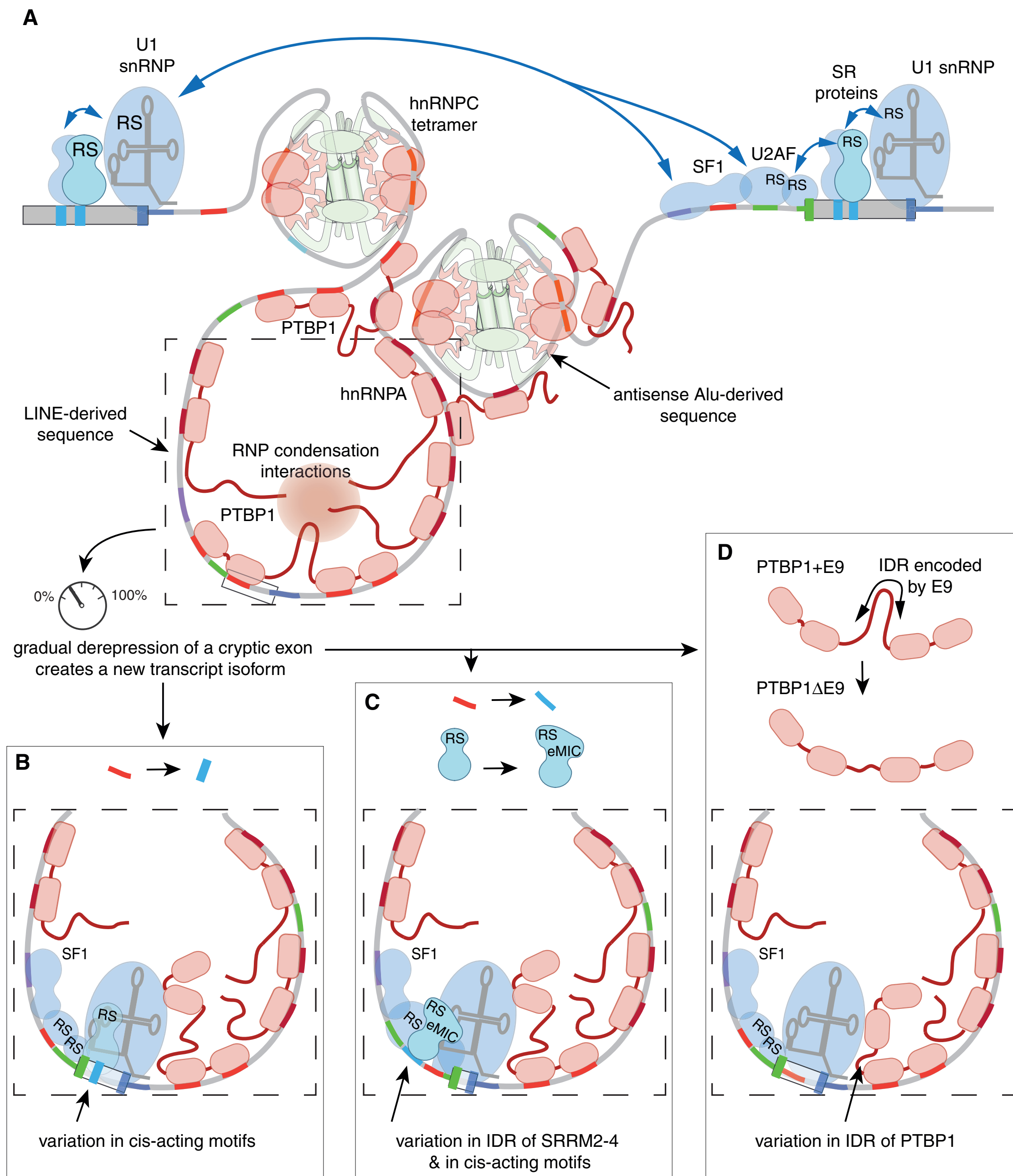

Figure 5. Emergence and Evolution of Alternative Splicing Networks

(A) IDR-mediated interactions can promote assembly of multivalent RNPs that keep introns in a repressed state while allowing intron and exon definition interactions (marked in blue lines). Multivalent motifs are indicated as red lines, while blue and green lines represent cryptic splice sites that are efficiently repressed by the multivalent RNPs. Many multivalent RNA sequences are derived from transposable elements (TEs), such as Alus, that often assemble the hnRNPC tetramer, or LINEs, which assemble the PTBP1/MATR3 complex. IDRs enable interactions that promote RNP condensation, such as homomeric contacts of hnRNPA or the heteromeric contact between PTBP1 and MATR3. Multi-domain RBPs might be able to further contribute to condensation of intronic RNA by binding to motifs that are present on distal RNA regions, as exemplified here for PTBP1. The RNA and *cis*-acting motifs are shown as in Figure 1A.

(B–D) Evolutionary variation can decrease the repressive environment, thus enabling splicing factors to assemble on the newly emerging exon. The decrease is usually gradual, leading to a change in the proportion of transcript isoforms, which minimizes disruption of the existing gene functions. This can be achieved through multiple means.

(B) Mutations that gradually decrease the multivalency of repressive motifs and/or introduce binding sites for SR proteins.

(C) Variations in IDRs, such as addition of the eMIC domain to the IDR of an ancestral SRRM ortholog, rendered them capable of binding upstream of microexons to promote their definition (Torres-Méndez et al., 2019). This led to further evolutionary variation in *cis*-acting motifs that shaped the species-specific splicing patterns of microexons.

(D) The repressive capacity of PTBP1 can be decreased by a splicing change that shortens the IDR that links the RRM domains, which, in turn, facilitates activation of a mammalian brain-specific alternative splicing program (Gueroussov et al., 2015).

Similar to Alu elements, the vast majority of long interspersed nuclear elements (LINEs) are evolutionary relics that, with few exceptions, are transcribed as part of host genes, most often within the longest introns. Most LINEs have acquired a vast number of mutations, some of which have created new functional elements, such as enhancers, promoters, or exons (Chuong et al., 2017). Interestingly, comparisons of mammalian genomes revealed that LINEs bind differently to RBPs depending on how evolutionarily old they are (Attig et al., 2018). Repressive proteins such as PTBP1 and MATR3 favor evolutionarily young LINE elements because these are longer and more multivalent. For example, an antisense LINE1 sequence contains many dozens of UC-rich motifs dispersed over hundreds of nucleotides. PTBP1 and MATR3 both recognize UC-rich motifs, and they directly interact, mediated by a region of the IDR in MATR3, which is required to ensure that they cooperate rather than compete when binding multivalent RNA-binding sites (Attig et al., 2018; Coelho et al., 2015). MATR3 is required for efficient recruitment of PTBP1 to LINEs, indicating that cooperative binding of the two proteins is particularly important when condensation on long multivalent RNA is required. Notably, the evolutionary young LINEs are depleted from a ±1 kb vicinity of constitutive exons both in mouse and human genes, indicating that the repression of multivalent RNPs formed on LINEs can spread to the surrounding sequences in RNA. Thus, multivalent RNPs that assemble on intronic RNA derived from TEs can make large portions of the intron inaccessible to other proteins, maintaining the intron in a repressed state (Figure 5A). The flexibility of multivalent assembly provided by IDRs likely enables the repressive RNPs to tolerate changes in the positioning of binding motifs introduced by genetic drift so that they can maintain introns in a repressed state in spite of genetic variation.

With evolutionary age, the number of repressive multivalent binding motifs in Alus and LINEs decreases, and these changes correlate with decreased binding of repressive RBPs and increased binding of splicing factors such as U2AF2 (Attig et al., 2016, 2018; Figure 5B). Comparison between TEs within and across genomes has demonstrated that exon emergence proceeds through a gradual loss in the multivalency of repressive motifs. In this way, new exons initially emerge as minor transcript isoforms, minimizing any potential disruptive effects on gene functions and decreasing negative selection against such new exons. Thus, multivalent RNPs presumably enable the newly emerging cryptic exons to persist in populations so that cryptic exons can undergo further evolutionary tinkering through mutations that gradually weaken their repression, enabling evolution of new transcript isoforms (Attig and Ule, 2019). However, because of the large numbers of cryptic exons in our transcriptome, occasionally a mutation strongly induces inclusion of a cryptic exon, which can cause a variety of human diseases (Sibley et al., 2016).

Finally, variations in IDRs also commonly diversify splicing regulatory capacities between evolutionary lineages. For example, a new exon that extended an IDR in the serine and arginine repetitive matrix (SRRM) family of splicing factors led to the birth of microexons in bilaterians (Figure 5C). This can be traced to a differential alternative splicing event within the IDR that created the eMIC domain in an ancestral invertebrate Srrm ortholog, which originated the neuronal microexon program (Torres-Méndez et al., 2019). Gene duplication and specialization of emerging SRRM2–SRRM4 orthologs subsequently resulted in further evolution of vertebrate microexon regulation. Moreover, IDRs of other proteins can also be a force for innovation; an alternative exon that encodes a linker region between the RRMs of PTBP1 is specifically skipped in mammals, where it reduces the repressive activity of PTBP1 to facilitate activation of a mammalian brain-specific alternative splicing program (Gueroussov et al., 2015; Figure 5D). As mentioned earlier, lineage-dependent alternative splicing events often change the IDRs of hnRNPs to shape their global regulatory properties (Gueroussov et al., 2017). Taken together, it is becoming clear that alternative splicing has been particularly important for enriching proteomic complexity in animals in ways that have provided an expanded toolkit for evolution.

Function versus Noise or Evolutionary Fodder?

As the number of alternative splicing events detected in large-scale sequencing studies continues to rise, it has been argued that only a minor fraction of splice variants are regulated or translated or are of functional importance (Tress et al., 2017). These arguments stem in large part from computational analyses suggesting that the majority of annotated alternative exons are evolving at neutral rates and also the poor detection frequency of peptides corresponding to alternative splicing variants in analyses of shotgun mass spectrometry data. However, it is well established that such data are severely underrepresented for multiple technical reasons that necessitate careful modeling of false-negative rates (Blencowe, 2017; Nilsen and Graveley, 2010). For example, trypsin-only proteomics is predicted (i.e., based on an *in silico* analysis) to miss detection of the majority of splice junction-spanning peptides (Wang et al., 2018), and mass spectrometry (MS) analysis methods, such as those employed by Tress et al. (2017), which required detection of the same peptide at least twice in two or more independent datasets, are heavily biased against detection of differentially regulated alternative splicing events even though their functionality is supported by enrichment for frame preservation and evolutionary conservation (Brown et al., 2014; Fagnani et al., 2007; Gerstein et al., 2014; Sugnet et al., 2006; Wang et al., 2008).

Moreover, approximately 75% of human exon-skipping events detected by RNA-seq data in transcripts with medium to high abundance were detected in ribosome profiling data (Weatheritt et al., 2016), and another recent study has provided evidence for widespread translation of neutrally evolving peptide sequences (Ruiz-Orera et al., 2018). As mentioned above, even TE-derived exons that are at the earliest stages of emergence and are poorly conserved might have unforeseen roles under specific physiological states. For example, increased inclusion of TE-derived exons was observed upon UV irradiation in key cell cycle checkpoint regulators, and because most of these exons are out of frame or contain premature stop codons and, thus, can prevent translation of full-length proteins or cause NMD of mRNA, they may contribute to cell cycle arrest until the DNA damage process is complete (Avgan et al., 2019). Thus, alternative splicing events lie on an evolving

spectrum of regulation and functionality; therefore, it is very challenging to draw a line between those that are functional or non-functional.

Despite the significant challenges associated with assigning functional roles to individual alternative splicing events, more rapid progress in this area will ultimately come from the development of new functional genomic approaches. For example, CRISPR-based systems have the potential to afford systematic ablation of alternative splicing events in screens coupled to phenotypic readouts and are expected to provide assessments regarding the extent of functionally important splicing variants (Yuan et al., 2018). It is envisioned that such screens will initially focus on alternative splicing events in pathway-specific genes where an associated phenotypic readout is known or can be predicted. Such screens will provide a foundation for further in-depth studies and will also facilitate the predictions of phenotypic effects of genetic variation and disease mutations that frequently affect the splicing process (Climente-González et al., 2017; Jaganathan et al., 2019; Ohno et al., 2018; Park et al., 2018; Sterne-Weiler and Sanford, 2014).

Conclusions and Future Perspectives

This review provides a focused account of recent developments in our understanding of the mechanisms, functions, and evolution of alternative splicing regulatory networks. As the number of new ''omics'' technologies continues to grow, generating new types of transcriptomic datasets with which to derive the principles of gene regulation, it is becoming increasingly apparent that underexplored alternative splicing mechanisms await further investigation. For example, an increasing arsenal of omics methods is being used to map different types of RNA modifications comprising the ''epitranscriptome,'' and an important area for future study will be to determine how these may affect alternative splicing as well as other steps in post-transcriptional gene regulation (Haussmann et al., 2016; Lence et al., 2016; Pacini and Koziol, 2018; Shi et al., 2019; Zhou et al., 2019). Similarly, methods for transcriptome-wide mapping of spliceosomal assembly and RNA-RNA contacts, including both intra- and inter-molecular contacts, will be important for understanding how RNA structures regulate the formation of functional RNP complexes and the availability of splice sites or the contacts between splice sites, which can facilitate either regular splicing or back-splicing during circular RNA (circRNA) biogenesis (Aw et al., 2016; Briese et al., 2019; Burke et al., 2018; Chen et al., 2018; Kristensen et al., 2019; Lu et al., 2016; Nguyen et al., 2016; Sharma et al., 2016). Such mapping studies and complementary methods are beginning to uncover new roles for non-coding RNA (ncRNA) products of splicing, such as excised introns and circRNA isoforms (Kristensen et al., 2019; Morgan et al., 2019; Parenteau et al., 2019). New roles of *trans*-acting ncRNAs in splicing mechanisms continue to be unraveled, such as in masking functional sites on RNAs or sequestering splicing factors (Modic et al., 2019; Romero-Barrios et al., 2018). These studies are expected to illuminate new roles of autoregulation, crossregulation, and feedback networks in controlling splicing regulatory networks in the context of development and disease (Jangi and Sharp, 2014).

For a deeper mechanistic understanding, the application of cryo-EM and complementary methods will be critically important to further elucidate the structures of splicing regulatory complexes and their roles in initial definition reactions as well as in the subsequent early stages of spliceosome formation. Moreover, increased application of single cell profiling, long-read sequencing of full-length transcripts, as well as methods to study RNP condensates and measure coupled steps in post-transcriptional gene regulation are expected to further unravel the complexity of alternative splicing and its coordination with transcription and other regulatory processes (Anvar et al., 2018; Ding and Elowitz, 2019; Fiszbein et al., 2019; Guo et al., 2019; Wan and Larson, 2018). An important collective goal of these studies will be to derive more sophisticated predictive codes for alternative splicing regulation that will enable modeling of alternative splicing decisions in a broader range of cell types and to more accurately predict the effect of normal and disease variation on such decisions (Siegfried and Karni, 2018). Such advances will complement emerging functional genomic and machine learning approaches for interrogating the regulation and roles of individual splice variants (Gonatopoulos-Pournatzis et al., 2018; Narykov et al., 2018). The resulting information will facilitate further development of strategies for therapeutic targeting of the splicing process, for example, through the use of small molecules, antisense oligonucleotides, and CRISPR-based approaches for editing *cis* elements or modulating splicing regulation (Fong et al., 2019; Konermann et al., 2018; Lee and Abdel-Wahab, 2016; Wang et al., 2019; Yuan et al., 2018).

Finally, we emphasize that the time has come to establish general guidelines for a coordinated, community-based effort to systematically annotate and integrate important information regarding alternative splicing regulation and function into public data resources (Chakrabarti et al., 2018; Tapial et al., 2017; Van Nostrand et al., 2018). Such a step, which must involve cooperation with journals to ensure that new data are curated according to rigorous standards as they appear, will help achieve the ultimate goal of generating an exon- and intron-resolution understanding of gene function and regulation.

ACKNOWLEDGMENTS

We thank our lab members, in particular Rasim Barutcu, Ulrich Braunschweig, Hannes Bretschneider, Thomas Gonatopoulos-Pournatzis, Martina Hallegger, Flora Lee, Lorea Blazquez, Anob Chakrabarti, Rupert Faraway, Paulo Gameiro, Hong Han, and Jonathan Roth, as well as Juan Valcarcel, Brent Graveley, Manuel Irimia, Kiyoshi Nagai, Chris Smith, and Julian König for helpful discussions and critical review of the manuscript. We are grateful to Joe Brock for assistance with the figures. J.U. also thanks Agnieszka and Gustav for inspiration because the review was drafted around the time of Gustav's birth. Research in B.J.B.'s group is supported by grants from the Canadian Institutes of Health Research, the Simons Foundation, and the Canada First Research Excellence Medicine by Design Program. Research in J.U.'s group is supported by grants from the European Research Council (617837-Translate) and the Wellcome Trust (103760/Z/14/Z). B.J.B. holds the Banbury Chair in Medical Research at the University of Toronto. The Francis Crick Institute receives its core funding from Cancer Research UK (FC001002), the UK Medical Research Council (FC001002), and the Wellcome Trust (FC001002).

REFERENCES

Abovich, N., and Rosbash, M. (1997). Cross-intron bridging interactions in the yeast commitment complex are conserved in mammals. Cell *89*, 403–412.

Ajith, S., Gazzara, M.R., Cole, B.S., Shankarling, G., Martinez, N.M., Mallory, M.J., and Lynch, K.W. (2016). Position-dependent activity of CELF2 in the regulation of splicing and implications for signal-responsive regulation in T cells. RNA Biol. *13*, 569–581.

Anvar, S.Y., Allard, G., Tseng, E., Sheynkman, G.M., de Klerk, E., Vermaat, M., Yin, R.H., Johansson, H.E., Ariyurek, Y., den Dunnen, J.T., et al. (2018). Full-length mRNA sequencing uncovers a widespread coupling between transcription initiation and mRNA processing. Genome Biol. *19*, 46.

Attig, J., and Ule, J. (2019). Genomic Accumulation of Retrotransposons Was Facilitated by Repressive RNA-Binding Proteins: A Hypothesis. BioEssays *41*, e1800132.

Attig, J., Ruiz de Los Mozos, I., Haberman, N., Wang, Z., Emmett, W., Zarnack, K., König, J., and Ule, J. (2016). Splicing repression allows the gradual emergence of new Alu-exons in primate evolution. eLife *5*, e19545.

Attig, J., Agostini, F., Gooding, C., Chakrabarti, A.M., Singh, A., Haberman, N., Zagalak, J.A., Emmett, W., Smith, C.W.J., Luscombe, N.M., and Ule, J. (2018). Heteromeric RNP Assembly at LINEs Controls Lineage-Specific RNA Processing. Cell *174*, 1067–1081.e17.

Avgan, N., Wang, J.I., Fernandez-Chamorro, J., and Weatheritt, R.J. (2019). Multilayered control of exon acquisition permits the emergence of novel forms of regulatory control. Genome Biol. *20*, 141.

Aw, J.G.A., Shen, Y., Wilm, A., Sun, M., Lim, X.N., Boon, K.L., Tapsin, S., Chan, Y.S., Tan, C.P., Sim, A.Y.L., et al. (2016). In Vivo Mapping of Eukaryotic RNA Interactomes Reveals Principles of Higher-Order Organization and Regulation. Mol. Cell *62*, 603–617.

Baeza-Centurion, P., Miñana, B., Schmiedel, J.M., Valcárcel, J., and Lehner, B. (2019). Combinatorial Genetics Reveals a Scaling Law for the Effects of Mutations on Splicing. Cell *176*, 549–563.e23.

Banani, S.F., Lee, H.O., Hyman, A.A., and Rosen, M.K. (2017). Biomolecular condensates: organizers of cellular biochemistry. Nat. Rev. Mol. Cell Biol. *18*, 285–298.

Baralle, F.E., and Giudice, J. (2017). Alternative splicing as a regulator of development and tissue identity. Nat. Rev. Mol. Cell Biol. *18*, 437–451.

Barash, Y., Calarco, J.A., Gao, W., Pan, Q., Wang, X., Shai, O., Blencowe, B.J., and Frey, B.J. (2010). Deciphering the splicing code. Nature *465*, 53–59.

Barbosa-Morais, N.L., Irimia, M., Pan, Q., Xiong, H.Y., Gueroussov, S., Lee, L.J., Slobodeniuc, V., Kutter, C., Watt, S., Colak, R., et al. (2012). The evolutionary landscape of alternative splicing in vertebrate species. Science *338*, 1587–1593.

Bhate, A., Parker, D.J., Bebee, T.W., Ahn, J., Arif, W., Rashan, E.H., Chorghade, S., Chau, A., Lee, J.H., Anakk, S., et al. (2015). ESRP2 controls an adult splicing programme in hepatocytes to support postnatal liver maturation. Nat. Commun. *6*, 8768.

Blazquez, L., Emmett, W., Faraway, R., Pineda, J.M.B., Bajew, S., Gohr, A., Haberman, N., Sibley, C.R., Bradley, R.K., Irimia, M., and Ule, J. (2018). Exon Junction Complex Shapes the Transcriptome by Repressing Recursive Splicing. Mol. Cell *72*, 496–509.e9.

Blencowe, B.J. (2015). Reflections for the 20th anniversary issue of RNA journal. RNA *21*, 573–575.

Blencowe, B.J. (2017). The Relationship between Alternative Splicing and Proteomic Complexity. Trends Biochem. Sci. *42*, 407–408.

Boehm, V., Britto-Borges, T., Steckelberg, A.L., Singh, K.K., Gerbracht, J.V., Gueney, E., Blazquez, L., Altmüller, J., Dieterich, C., and Gehring, N.H. (2018). Exon Junction Complexes Suppress Spurious Splice Sites to Safeguard Transcriptome Integrity. Mol. Cell *72*, 482–495.e7.

Boutz, P.L., Bhutkar, A., and Sharp, P.A. (2015). Detained introns are a novel, widespread class of post-transcriptionally spliced introns. Genes Dev. *29*, 63–80.

Braunschweig, U., Gueroussov, S., Plocik, A.M., Graveley, B.R., and Blencowe, B.J. (2013). Dynamic integration of splicing within gene regulatory pathways. Cell *152*, 1252–1269.

Bretschneider, H., Gandhi, S., Deshwar, A.G., Zuberi, K., and Frey, B.J. (2018). COSSMO: predicting competitive alternative splice site selection using deep learning. Bioinformatics *34*, i429–i437.

Briese, M., Haberman, N., Sibley, C.R., Faraway, R., Elser, A.S., Chakrabarti, A.M., Wang, Z., König, J., Perera, D., Wickramasinghe, V.O., et al. (2019). A systems view of spliceosomal assembly and branchpoints with iCLIP. Nat. Struct. Mol. Biol. *26*, 930–940.

Brooks, A.N., Yang, L., Duff, M.O., Hansen, K.D., Park, J.W., Dudoit, S., Brenner, S.E., and Graveley, B.R. (2011). Conservation of an RNA regulatory map between Drosophila and mammals. Genome Res. *21*, 193–202.

Brown, J.B., Boley, N., Eisman, R., May, G.E., Stoiber, M.H., Duff, M.O., Booth, B.W., Wen, J., Park, S., Suzuki, A.M., et al. (2014). Diversity and dynamics of the Drosophila transcriptome. Nature *512*, 393–399.

Buljan, M., Chalancon, G., Eustermann, S., Wagner, G.P., Fuxreiter, M., Bateman, A., and Babu, M.M. (2012). Tissue-specific splicing of disordered segments that embed binding motifs rewires protein interaction networks. Mol. Cell *46*, 871–883.

Burke, J.E., Longhurst, A.D., Merkurjev, D., Sales-Lee, J., Rao, B., Moresco, J.J., Yates, J.R., 3rd, Li, J.J., and Madhani, H.D. (2018). Spliceosome Profiling Visualizes Operations of a Dynamic RNP at Nucleotide Resolution. Cell *173*, 1014–1030.e17.

Burnette, J.M., Miyamoto-Sato, E., Schaub, M.A., Conklin, J., and Lopez, A.J. (2005). Subdivision of large introns in Drosophila by recursive splicing at non-exonic elements. Genetics *170*, 661–674.

Cereda, M., Pozzoli, U., Rot, G., Juvan, P., Schweitzer, A., Clark, T., and Ule, J. (2014). RNAmotifs: prediction of multivalent RNA motifs that control alternative splicing. Genome Biol. *15*, R20.

Chakrabarti, A.M., Haberman, N., Praznik, A., Luscombe, N.M., and Ule, J. (2018). Data Science Issues in Studying Protein–RNA Interactions with CLIP Technologies. Annu. Rev. Biomed. Data Sci. *1*, 235–261.

Chen, M., and Manley, J.L. (2009). Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches. Nat. Rev. Mol. Cell Biol. *10*, 741–754.

Chen, W., Moore, J., Ozadam, H., Shulha, H.P., Rhind, N., Weng, Z., and Moore, M.J. (2018). Transcriptome-wide Interrogation of the Functional Intronome by Spliceosome Profiling. Cell *173*, 1031–1044.e13.

Chou, M.Y., Underwood, J.G., Nikolic, J., Luu, M.H., and Black, D.L. (2000). Multisite RNA binding and release of polypyrimidine tract binding protein during the regulation of c-src neural-specific splicing. Mol. Cell *5*, 949–957.

Chuong, E.B., Elde, N.C., and Feschotte, C. (2017). Regulatory activities of transposable elements: from conflicts to benefits. Nat. Rev. Genet. *18*, 71–86.

Climente-González, H., Porta-Pardo, E., Godzik, A., and Eyras, E. (2017). The Functional Impact of Alternative Splicing in Cancer. Cell Rep. *20*, 2215–2226.

Coelho, M.B., Attig, J., Bellora, N., König, J., Hallegger, M., Kayikci, M., Eyras, E., Ule, J., and Smith, C.W.J. (2015). Nuclear matrix protein Matrin3 regulates alternative splicing and forms overlapping regulatory networks with PTB. EMBO J. *34*, 653–668.

Collins, L., and Penny, D. (2005). Complex spliceosomal organization ancestral to extant eukaryotes. Mol. Biol. Evol. *22*, 1053–1066.

Coté, J., Dupuis, S., Jiang, Z., and Wu, J.Y. (2001). Caspase-2 pre-mRNA alternative splicing: Identification of an intronic element containing a decoy 3′ acceptor site. Proc. Natl. Acad. Sci. USA *98*, 938–943.

Csuros, M., Rogozin, I.B., and Koonin, E.V. (2011). A detailed history of intron-rich eukaryotic ancestors inferred from a global survey of 100 complete genomes. PLoS Comput. Biol. *7*, e1002150.

Damianov, A., Ying, Y., Lin, C.H., Lee, J.A., Tran, D., Vashisht, A.A., Bahrami-Samani, E., Xing, Y., Martin, K.C., Wohlschlegel, J.A., and Black, D.L. (2016). Rbfox Proteins Regulate Splicing as Part of a Large Multiprotein Complex LASR. Cell *165*, 606–619.

Darman, R.B., Seiler, M., Agrawal, A.A., Lim, K.H., Peng, S., Aird, D., Bailey, S.L., Bhavsar, E.B., Chan, B., Colla, S., et al. (2015). Cancer-Associated

CellPress

SF3B1 Hotspot Mutations Induce Cryptic 3′ Splice Site Selection through Use of a Different Branch Point. Cell Rep. *13*, 1033–1045.

De Conti, L., Baralle, M., and Buratti, E. (2013). Exon and intron definition in pre-mRNA splicing. Wiley Interdiscip. Rev. RNA *4*, 49–60.

DeBoever, C., Ghia, E.M., Shepard, P.J., Rassenti, L., Barrett, C.L., Jepsen, K., Jamieson, C.H.M., Carson, D., Kipps, T.J., and Frazer, K.A. (2015). Transcriptome sequencing reveals potential mechanism of cryptic 3′ splice site selection in SF3B1-mutated cancers. PLoS Comput. Biol. *11*, e1004105.

Deveson, I.W., Brunck, M.E., Blackburn, J., Tseng, E., Hon, T., Clark, T.A., Clark, M.B., Crawford, J., Dinger, M.E., Nielsen, L.K., et al. (2018). Universal Alternative Splicing of Noncoding Exons. Cell Syst. *6*, 245–255.e5.

Ding, F., and Elowitz, M.B. (2019). Constitutive splicing and economies of scale in gene expression. Nat. Struct. Mol. Biol. *26*, 424–432.

Dominguez, D., Freese, P., Alexis, M.S., Su, A., Hochman, M., Palden, T., Bazile, C., Lambert, N.J., Van Nostrand, E.L., Pratt, G.A., et al. (2018). Sequence, Structure, and Context Preferences of Human RNA Binding Proteins. Mol. Cell *70*, 854–867.e9.

Drexler, H.L., Choquet, K., and Stirling Churchman, L. (2019). Human co-transcriptional splicing kinetics and coordination revealed by direct nascent RNA sequencing. bioRxiv. https://doi.org/10.1101/611020.

Duff, M.O., Olson, S., Wei, X., Garrett, S.C., Osman, A., Bolisetty, M., Plocik, A., Celniker, S.E., and Graveley, B.R. (2015). Genome-wide identification of zero nucleotide recursive splicing in Drosophila. Nature *521*, 376–379.

Ebert, D.H., and Greenberg, M.E. (2013). Activity-dependent neuronal signalling and autism spectrum disorder. Nature *493*, 327–337.

Eldridge, A.G., Li, Y., Sharp, P.A., and Blencowe, B.J. (1999). The SRm160/300 splicing coactivator is required for exon-enhancer function. Proc. Natl. Acad. Sci. USA *96*, 6125–6130.

Ellis, J.D., Barrios-Rodiles, M., Colak, R., Irimia, M., Kim, T., Calarco, J.A., Wang, X., Pan, Q., O'Hanlon, D., Kim, P.M., et al. (2012). Tissue-specific alternative splicing remodels protein-protein interaction networks. Mol. Cell *46*, 884–892.

Eom, T., Zhang, C., Wang, H., Lay, K., Fak, J., Noebels, J.L., and Darnell, R.B. (2013). NOVA-dependent regulation of cryptic NMD exons controls synaptic protein levels after seizure. eLife *2*, e00178.

Erkelenz, S., Mueller, W.F., Evans, M.S., Busch, A., Schöneweis, K., Hertel, K.J., and Schaal, H. (2013). Position-dependent splicing activation and repression by SR and hnRNP proteins rely on common mechanisms. RNA *19*, 96–102.

Ewing, B., and Green, P. (2000). Analysis of expressed sequence tags indicates 35,000 human genes. Nat. Genet. *25*, 232–234.

Fagnani, M., Barash, Y., Ip, J.Y., Misquitta, C., Pan, Q., Saltzman, A.L., Shai, O., Lee, L., Rozenhek, A., Mohammad, N., et al. (2007). Functional coordination of alternative splicing in the mammalian central nervous system. Genome Biol. *8*, R108.

Feng, H., Bao, S., Rahman, M.A., Weyn-Vanhentenryck, S.M., Khan, A., Wong, J., Shah, A., Flynn, E.D., Krainer, A.R., and Zhang, C. (2019). Modeling RNA-Binding Protein Specificity In Vivo by Precisely Registering Protein-RNA Crosslink Sites. Mol. Cell *74*, 1189–1204.e6.

Fica, S.M., Oubridge, C., Wilkinson, M.E., Newman, A.J., and Nagai, K. (2019). A human postcatalytic spliceosome structure reveals essential roles of metazoan factors for exon ligation. Science *363*, 710–714.

Fiszbein, A., Krick, K.S., and Burge, C.B. (2019). Exon-mediated activation of transcription starts. bioRxiv. https://doi.org/10.1101/565184.

Fong, J.Y., Pignata, L., Goy, P.A., Kawabata, K.C., Lee, S.C.W., Koh, C.M., Musiani, D., Massignani, E., Kotini, A.G., Penson, A., et al. (2019). Therapeutic Targeting of RNA Splicing Catalysis through Inhibition of Protein Arginine Methylation. Cancer Cell *36*, 194–209.e9.

Förch, P., Puig, O., Martínez, C., Séraphin, B., and Valcárcel, J. (2002). The splicing regulator TIA-1 interacts with U1-C to promote U1 snRNP recruitment to 5′ splice sites. EMBO J. *21*, 6882–6892.

Fu, X.D., and Ares, M., Jr. (2014). Context-dependent control of alternative splicing by RNA-binding proteins. Nat. Rev. Genet. *15*, 689–701.

Gabut, M., Samavarchi-Tehrani, P., Wang, X., Slobodeniuc, V., O'Hanlon, D., Sung, H.K., Alvarez, M., Talukder, S., Pan, Q., Mazzoni, E.O., et al. (2011). An alternative splicing switch regulates embryonic stem cell pluripotency and reprogramming. Cell *147*, 132–146.

Gal-Mark, N., Schwartz, S., Ram, O., Eyras, E., and Ast, G. (2009). The pivotal roles of TIA proteins in 5′ splice-site selection of alu exons and across evolution. PLoS Genet. *5*, e1000717.

Gerstein, M.B., Rozowsky, J., Yan, K.K., Wang, D., Cheng, C., Brown, J.B., Davis, C.A., Hillier, L., Sisu, C., Li, J.J., et al. (2014). Comparative analysis of the transcriptome across distant species. Nature *512*, 445–448.

Gonatopoulos-Pournatzis, T., Wu, M., Braunschweig, U., Roth, J., Han, H., Best, A.J., Raj, B., Aregger, M., O'Hanlon, D., Ellis, J.D., et al. (2018). Genome-wide CRISPR-Cas9 Interrogation of Splicing Networks Reveals a Mechanism for Recognition of Autism-Misregulated Neuronal Microexons. Mol. Cell *72*, 510–524.e12.

Gotic, I., Omidi, S., Fleury-Olela, F., Molina, N., Naef, F., and Schibler, U. (2016). Temperature regulates splicing efficiency of the cold-inducible RNA-binding protein gene Cirbp. Genes Dev. *30*, 2005–2017.

Grau-Bové, X., Ruiz-Trillo, I., and Irimia, M. (2018). Origin of exon skipping-rich transcriptomes in animals driven by evolution of gene architecture. Genome Biol. *19*, 135.

Gueroussov, S., Gonatopoulos-Pournatzis, T., Irimia, M., Raj, B., Lin, Z.Y., Gingras, A.C., and Blencowe, B.J. (2015). An alternative splicing event amplifies evolutionary differences between vertebrates. Science *349*, 868–873.

Gueroussov, S., Weatheritt, R.J., O'Hanlon, D., Lin, Z.Y., Narula, A., Gingras, A.C., and Blencowe, B.J. (2017). Regulatory Expansion in Mammals of Multivalent hnRNP Assemblies that Globally Control Alternative Splicing. Cell *170*, 324–339.e23.

Guo, Y.E., Manteiga, J.C., Henninger, J.E., Sabari, B.R., Dall'Agnese, A., Hannett, N.M., Spille, J.H., Afeyan, L.K., Zamudio, A.V., Shrinivas, K., et al. (2019). Pol II phosphorylation regulates a switch between transcriptional and splicing condensates. Nature *572*, 543–548.

Hamid, F.M., and Makeyev, E.V. (2017). A mechanism underlying position-specific regulation of alternative splicing. Nucleic Acids Res. *45*, 12455–12468.

Han, H., Irimia, M., Ross, P.J., Sung, H.K., Alipanahi, B., David, L., Golipour, A., Gabut, M., Michael, I.P., Nachman, E.N., et al. (2013). MBNL proteins repress ES-cell-specific alternative splicing and reprogramming. Nature *498*, 241–245.

Han, H., Braunschweig, U., Gonatopoulos-Pournatzis, T., Weatheritt, R.J., Hirsch, C.L., Ha, K.C.H., Radovani, E., Nabeel-Shah, S., Sterne-Weiler, T., Wang, J., et al. (2017). Multilayered Control of Alternative Splicing Regulatory Networks by Transcription Factors. Mol. Cell *65*, 539–553.e7.

Hatton, A.R., Subramaniam, V., and Lopez, A.J. (1998). Generation of alternative Ultrabithorax isoforms and stepwise removal of a large intron by resplicing at exon-exon junctions. Mol. Cell *2*, 787–796.

Haussmann, I.U., Bodi, Z., Sanchez-Moran, E., Mongan, N.P., Archer, N., Fray, R.G., and Soller, M. (2016). m^6A potentiates Sxl alternative pre-mRNA splicing for robust Drosophila sex determination. Nature *540*, 301–304.

Hentze, M.W., Castello, A., Schwarzl, T., and Preiss, T. (2018). A brave new world of RNA-binding proteins. Nat. Rev. Mol. Cell Biol. *19*, 327–341.

Hiller, M., Zhang, Z., Backofen, R., and Stamm, S. (2007). Pre-mRNA secondary structures influence exon recognition. PLoS Genet. *3*, e204.

Hnisz, D., Shrinivas, K., Young, R.A., Chakraborty, A.K., and Sharp, P.A. (2017). A Phase Separation Model for Transcriptional Control. Cell *169*, 13–23.

Hong, X., Scofield, D.G., and Lynch, M. (2006). Intron size, abundance, and distribution within untranslated regions of genes. Mol. Biol. Evol. *23*, 2392–2404.

Howard, J.M., Lin, H., Wallace, A.J., Kim, G., Draper, J.M., Haeussler, M., Katzman, S., Toloue, M., Liu, Y., and Sanford, J.R. (2018). HNRNPA1

promotes recognition of splice site decoys by U2AF2 in vivo. Genome Res. *28*, 689–698.

Ibrahim, E.C., Schaal, T.D., Hertel, K.J., Reed, R., and Maniatis, T. (2005). Serine/arginine-rich protein-dependent suppression of exon skipping by exonic splicing enhancers. Proc. Natl. Acad. Sci. USA *102*, 5002–5007.

Irimia, M., and Blencowe, B.J. (2012). Alternative splicing: decoding an expansive regulatory layer. Curr. Opin. Cell Biol. *24*, 323–332.

Irimia, M., Denuc, A., Burguera, D., Somorjai, I., Martín-Durán, J.M., Genikhovich, G., Jimenez-Delgado, S., Technau, U., Roy, S.W., Marfany, G., and Garcia-Fernàndez, J. (2011). Stepwise assembly of the Nova-regulated alternative splicing network in the vertebrate brain. Proc. Natl. Acad. Sci. USA *108*, 5319–5324.

Irimia, M., Weatheritt, R.J., Ellis, J.D., Parikshak, N.N., Gonatopoulos-Pournatzis, T., Babor, M., Quesnel-Vallières, M., Tapial, J., Raj, B., O'Hanlon, D., et al. (2014). A highly conserved program of neuronal microexons is misregulated in autistic brains. Cell *159*, 1511–1523.

Jacob, F. (1977). Evolution and tinkering. Science *196*, 1161–1166.

Jaganathan, K., Kyriazopoulou Panagiotopoulou, S., McRae, J.F., Darbandi, S.F., Knowles, D., Li, Y.I., Kosmicki, J.A., Arbelaez, J., Cui, W., Schwartz, G.B., et al. (2019). Predicting Splicing from Primary Sequence with Deep Learning. Cell *176*, 535–548.e24.

Jangi, M., and Sharp, P.A. (2014). Building robust transcriptomes with master splicing factors. Cell *159*, 487–498.

Jelen, N., Ule, J., Zivin, M., and Darnell, R.B. (2007). Evolution of Nova-dependent splicing regulation in the brain. PLoS Genet. *3*, 1838–1847.

Johnson, V., Junge, H.J., and Chen, Z. (2019). Temporal regulation of axonal repulsion by alternative splicing of a conserved microexon in mammalian *Robo1* and *Robo2*. eLife *8*, e46042.

Joseph, B., Kondo, S., and Lai, E.C. (2018). Short cryptic exons mediate recursive splicing in Drosophila. Nat. Struct. Mol. Biol. *25*, 365–371.

Kahles, A., Lehmann, K.V., Toussaint, N.C., Hüser, M., Stark, S.G., Sachsenberg, T., Stegle, O., Kohlbacher, O., Sander, C., and Rätsch, G.; Cancer Genome Atlas Research Network (2018). Comprehensive Analysis of Alternative Splicing Across Tumors from 8,705 Patients. Cancer Cell *34*, 211–224.e6.

Kalsotra, A., and Cooper, T.A. (2011). Functional consequences of developmentally regulated alternative splicing. Nat. Rev. Genet. *12*, 715–729.

Kanopka, A., Mühlemann, O., and Akusjärvi, G. (1996). Inhibition by SR proteins of splicing of a regulated adenovirus pre-mRNA. Nature *381*, 535–538.

Kastner, B., Will, C.L., Stark, H., and Lührmann, R. (2019). Structural Insights into Nuclear pre-mRNA Splicing in Higher Eukaryotes. Cold Spring Harb. Perspect. Biol. a032417.

Kato, M., and McKnight, S.L. (2018). A Solid-State Conceptualization of Information Transfer from Gene to Message to Protein. Annu. Rev. Biochem. *87*, 351–390.

Kelemen, O., Convertini, P., Zhang, Z., Wen, Y., Shen, M., Falaleeva, M., and Stamm, S. (2013). Function of alternative splicing. Gene *514*, 1–30.

Keren, H., Lev-Maor, G., and Ast, G. (2010). Alternative splicing and evolution: diversification, exon definition and function. Nat. Rev. Genet. *11*, 345–355.

Kim, E., Magen, A., and Ast, G. (2007). Different levels of alternative splicing among eukaryotes. Nucleic Acids Res. *35*, 125–131.

Kim, S.W., Taggart, A.J., Heintzelman, C., Cygan, K.J., Hull, C.G., Wang, J., Shrestha, B., and Fairbrother, W.G. (2017). Widespread intra-dependencies in the removal of introns from human transcripts. Nucleic Acids Res. *45*, 9503–9513.

Konermann, S., Lotfy, P., Brideau, N.J., Oki, J., Shokhirev, M.N., and Hsu, P.D. (2018). Transcriptome Engineering with RNA-Targeting Type VI-D CRISPR Effectors. Cell *173*, 665–676.e14.

Kristensen, L.S., Andersen, M.S., Stagsted, L.V.W., Ebbesen, K.K., Hansen, T.B., and Kjems, J. (2019). The biogenesis, biology and characterization of circular RNAs. Nat. Rev. Genet. Published online August 8, 2019. https://doi.org/10.1038/s41576-019-0158-7.

Kucherenko, M.M., and Shcherbata, H.R. (2018). Stress-dependent miR-980 regulation of Rbfox1/A2bp1 promotes ribonucleoprotein granule formation and cell survival. Nat. Commun. *9*, 312.

Kuroyanagi, H., Watanabe, Y., Suzuki, Y., and Hagiwara, M. (2013). Position-dependent and neuron-specific splicing regulation by the CELF family RNA-binding protein UNC-75 in Caenorhabditis elegans. Nucleic Acids Res. *41*, 4015–4025.

Le Hir, H., Saulière, J., and Wang, Z. (2016). The exon junction complex as a node of post-transcriptional networks. Nat. Rev. Mol. Cell Biol. *17*, 41–54.

Lee, S.C.W., and Abdel-Wahab, O. (2016). Therapeutic targeting of splicing in cancer. Nat. Med. *22*, 976–986.

Lee, F.C.Y., and Ule, J. (2018). Advances in CLIP Technologies for Studies of Protein-RNA Interactions. Mol. Cell *69*, 354–369.

Lence, T., Akhtar, J., Bayer, M., Schmid, K., Spindler, L., Ho, C.H., Kreim, N., Andrade-Navarro, M.A., Poeck, B., Helm, M., and Roignant, J.Y. (2016). m^6A modulates neuronal functions and sex determination in Drosophila. Nature *540*, 242–247.

Li, P., Banjade, S., Cheng, H.C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J.V., King, D.S., Banani, S.F., et al. (2012). Phase transitions in the assembly of multivalent signalling proteins. Nature *483*, 336–340.

Li, Y.I., Sanchez-Pulido, L., Haerty, W., and Ponting, C.P. (2015). RBFOX and PTBP1 proteins regulate the alternative splicing of micro-exons in human brain transcripts. Genome Res. *25*, 1–13.

Licatalosi, D.D., and Darnell, R.B. (2010). RNA processing and its regulation: global insights into biological networks. Nat. Rev. Genet. *11*, 75–87.

Licatalosi, D.D., Mele, A., Fak, J.J., Ule, J., Kayikci, M., Chi, S.W., Clark, T.A., Schweitzer, A.C., Blume, J.E., Wang, X., et al. (2008). HITS-CLIP yields genome-wide insights into brain alternative RNA processing. Nature *456*, 464–469.

Ling, J.P., Pletnikova, O., Troncoso, J.C., and Wong, P.C. (2015). TDP-43 repression of nonconserved cryptic exons is compromised in ALS-FTD. Science *349*, 650–655.

Llorian, M., Schwartz, S., Clark, T.A., Hollander, D., Tan, L.Y., Spellman, R., Gordon, A., Schweitzer, A.C., de la Grange, P., Ast, G., and Smith, C.W. (2010). Position-dependent alternative splicing activity revealed by global profiling of alternative splicing events regulated by PTB. Nat. Struct. Mol. Biol. *17*, 1114–1123.

Lorenzi, L.E., Bah, A., Wischnewski, H., Shchepachev, V., Soneson, C., Santagostino, M., and Azzalin, C.M. (2015). Fission yeast Cactin restricts telomere transcription and elongation by controlling Rap1 levels. EMBO J. *34*, 115–129.

Louadi, Z., Oubounyt, M., Tayara, H., and Chong, K.T. (2019). Deep Splicing Code: Classifying Alternative Splicing Events Using Deep Learning. Genes (Basel) *10*, E587.

Low, K.H., Lim, C., Ko, H.W., and Edery, I. (2008). Natural variation in the splice site strength of a clock gene and species-specific thermal adaptation. Neuron *60*, 1054–1067.

Lu, Z., Zhang, Q.C., Lee, B., Flynn, R.A., Smith, M.A., Robinson, J.T., Davidovich, C., Gooding, A.R., Goodrich, K.J., Mattick, J.S., et al. (2016). RNA Duplex Map in Living Cells Reveals Higher-Order Transcriptome Structure. Cell *165*, 1267–1279.

Lunde, B.M., Moore, C., and Varani, G. (2007). RNA-binding proteins: modular design for efficient function. Nat. Rev. Mol. Cell Biol. *8*, 479–490.

Mallory, M.J., Allon, S.J., Qiu, J., Gazzara, M.R., Tapescu, I., Martinez, N.M., Fu, X.D., and Lynch, K.W. (2015). Induced transcription and stability of CELF2 mRNA drives widespread alternative splicing during T-cell signaling. Proc. Natl. Acad. Sci. USA *112*, E2139–E2148.

Mayerle, M., Yitiz, S., Soulette, C., Rogel, L.E., Ramirez, A., Ragle, J.M., Katzman, S., Guthrie, C., and Zahler, A.M. (2019). Prp8 impacts cryptic but not alternative splicing frequency. Proc. Natl. Acad. Sci. USA *116*, 2193–2199.

McClory, S.P., Lynch, K.W., and Ling, J.P. (2018). HnRNP L represses cryptic exons. RNA *24*, 761–768.

McGuire, A.M., Pearson, M.D., Neafsey, D.E., and Galagan, J.E. (2008). Cross-kingdom patterns of alternative splicing and splice recognition. Genome Biol. *9*, R50.

McMahon, J.J., Miller, E.E., and Silver, D.L. (2016). The exon junction complex in neural development and neurodevelopmental disease. Int. J. Dev. Neurosci. *55*, 117–123.

Merkin, J., Russell, C., Chen, P., and Burge, C.B. (2012). Evolutionary dynamics of gene and isoform regulation in Mammalian tissues. Science *338*, 1593–1599.

Modic, M., Grosch, M., Rot, G., Schirge, S., Lepko, T., Yamazaki, T., Lee, F.C.Y., Rusha, E., Shaposhnikov, D., Palo, M., et al. (2019). Cross-Regulation between TDP-43 and Paraspeckles Promotes Pluripotency-Differentiation Transition. Mol. Cell *74*, 951–965.e13.

Modrek, B., and Lee, C.J. (2003). Alternative splicing in the human, mouse and rat genomes is associated with an increased frequency of exon creation and/or loss. Nat. Genet. *34*, 177–180.

Morgan, J.T., Fink, G.R., and Bartel, D.P. (2019). Excised linear introns regulate growth in yeast. Nature *565*, 606–611.

Narykov, O., Johnson, N., and Korkin, D. (2018). Determining rewiring effects of alternatively spliced isoforms on protein-protein interactions using a computational approach. bioRxiv. https://doi.org/10.1101/256834.

Nguyen, T.C., Cao, X., Yu, P., Xiao, S., Lu, J., Biase, F.H., Sridhar, B., Huang, N., Zhang, K., and Zhong, S. (2016). Mapping RNA-RNA interactome and RNA structure in vivo by MARIO. Nat. Commun. *7*, 12023.

Nilsen, T.W., and Graveley, B.R. (2010). Expansion of the eukaryotic proteome by alternative splicing. Nature *463*, 457–463.

Oberstrass, F.C., Auweter, S.D., Erat, M., Hargous, Y., Henning, A., Wenter, P., Reymond, L., Amir-Ahmady, B., Pitsch, S., Black, D.L., and Allain, F.H. (2005). Structure of PTB bound to RNA: specific binding and implications for splicing regulation. Science *309*, 2054–2057.

Oddo, J.C., Saxena, T., McConnell, O.L., Berglund, J.A., and Wang, E.T. (2016). Conservation of context-dependent splicing activity in distant Muscleblind homologs. Nucleic Acids Res. *44*, 8352–8362.

Ohno, K., Takeda, J.I., and Masuda, A. (2018). Rules and tools to predict the splicing effects of exonic and intronic mutations. Wiley Interdiscip. Rev. RNA *9*.

Pacini, C., and Koziol, M.J. (2018). Bioinformatics challenges and perspectives when studying the effect of epigenetic modifications on alternative splicing. Philos. Trans. R. Soc. Lond. B Biol. Sci. *373*, 20170073.

Pai, A.A., Paggi, J.M., Yan, P., Adelman, K., and Burge, C.B. (2018). Numerous recursive sites contribute to accuracy of splicing in long introns in flies. PLoS Genet. *14*, e1007588.

Pan, Q., Shai, O., Misquitta, C., Zhang, W., Saltzman, A.L., Mohammad, N., Babak, T., Siu, H., Hughes, T.R., Morris, Q.D., et al. (2004). Revealing global regulatory features of mammalian alternative splicing using a quantitative microarray platform. Mol. Cell *16*, 929–941.

Pan, Q., Shai, O., Lee, L.J., Frey, B.J., and Blencowe, B.J. (2008). Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat. Genet. *40*, 1413–1415.

Papasaikas, P., Tejedor, J.R., Vigevani, L., and Valcárcel, J. (2015). Functional splicing network reveals extensive regulatory potential of the core spliceosomal machinery. Mol. Cell *57*, 7–22.

Parenteau, J., Maignon, L., Berthoumieux, M., Catala, M., Gagnon, V., and Abou Elela, S. (2019). Introns are mediators of cell response to starvation. Nature *565*, 612–617.

Park, E., Pan, Z., Zhang, Z., Lin, L., and Xing, Y. (2018). The Expanding Landscape of Alternative Splicing Variation in Human Populations. Am. J. Hum. Genet. *102*, 11–26.

Parra, M., Booth, B.W., Weiszmann, R., Yee, B., Yeo, G.W., Brown, J.B., Celniker, S.E., and Conboy, J.G. (2018). An important class of intron retention events in human erythroblasts is regulated by cryptic exons proposed to function as splicing decoys. RNA *24*, 1255–1265.

Parras, A., Anta, H., Santos-Galindo, M., Swarup, V., Elorza, A., Nieto-González, J.L., Picó, S., Hernández, I.H., Díaz-Hernández, J.I., Belloc, E., et al. (2018). Autism-like phenotype and risk gene mRNA deadenylation by CPEB4 mis-splicing. Nature *560*, 441–446.

Plaschka, C., Newman, A.J., and Nagai, K. (2019). Structural Basis of Nuclear pre-mRNA Splicing: Lessons from Yeast. Cold Spring Harb. Perspect. Biol. *11*, a032391.

Porter, R.S., Jaamour, F., and Iwase, S. (2018). Neuron-specific alternative splicing of transcriptional machineries: Implications for neurodevelopmental disorders. Mol. Cell. Neurosci. *87*, 35–45.

Preußner, M., Goldammer, G., Neumann, A., Haltenhof, T., Rautenstrauch, P., Müller-McNicoll, M., and Heyd, F. (2017). Body Temperature Cycles Control Rhythmic Alternative Splicing in Mammals. Mol. Cell *67*, 433–446.e4.

Quesnel-Vallières, M., Irimia, M., Cordes, S.P., and Blencowe, B.J. (2015). Essential roles for the splicing regulator nSR100/SRRM4 during nervous system development. Genes Dev. *29*, 746–759.

Quesnel-Vallières, M., Dargaei, Z., Irimia, M., Gonatopoulos-Pournatzis, T., Ip, J.Y., Wu, M., Sterne-Weiler, T., Nakagawa, S., Woodin, M.A., Blencowe, B.J., and Cordes, S.P. (2016). Misregulation of an Activity-Dependent Splicing Network as a Common Mechanism Underlying Autism Spectrum Disorders. Mol. Cell *64*, 1023–1034.

Quesnel-Vallières, M., Weatheritt, R.J., Cordes, S.P., and Blencowe, B.J. (2019). Autism spectrum disorder: insights into convergent mechanisms from transcriptomics. Nat. Rev. Genet. *20*, 51–63.

Raj, B., Irimia, M., Braunschweig, U., Sterne-Weiler, T., O'Hanlon, D., Lin, Z.Y., Chen, G.I., Easton, L.E., Ule, J., Gingras, A.C., et al. (2014). A global regulatory mechanism for activating an exon network required for neurogenesis. Mol. Cell *56*, 90–103.

Robberson, B.L., Cote, G.J., and Berget, S.M. (1990). Exon definition may facilitate splice site selection in RNAs with multiple exons. Mol. Cell. Biol. *10*, 84–94.

Romero-Barrios, N., Legascue, M.F., Benhamed, M., Ariel, F., and Crespi, M. (2018). Splicing regulation by long noncoding RNAs. Nucleic Acids Res. *46*, 2169–2184.

Ruiz-Orera, J., Verdaguer-Grau, P., Villanueva-Cañas, J.L., Messeguer, X., and Albà, M.M. (2018). Translation of neutrally evolving peptides provides a basis for de novo gene evolution. Nat. Ecol. Evol. *2*, 890–896.

Rusconi, F., Grillo, B., Toffolo, E., Mattevi, A., and Battaglioli, E. (2017). NeuroLSD1: Splicing-Generated Epigenetic Enhancer of Neuroplasticity. Trends Neurosci. *40*, 28–38.

Saito, Y., Yuan, Y., Zucker-Scharff, I., Fak, J.J., Jereb, S., Tajima, Y., Licatalosi, D.D., and Darnell, R.B. (2019). Differential NOVA2-Mediated Splicing in Excitatory and Inhibitory Neurons Regulates Cortical Development and Cerebellar Function. Neuron *101*, 707–720.e5.

Shao, W., Kim, H.S., Cao, Y., Xu, Y.Z., and Query, C.C. (2012). A U1-U2 snRNP interaction network during intron definition. Mol. Cell. Biol. *32*, 470–478.

Shao, C., Yang, B., Wu, T., Huang, J., Tang, P., Zhou, Y., Zhou, J., Qiu, J., Jiang, L., Li, H., et al. (2014). Mechanisms for U2AF to define 3′ splice sites and regulate alternative splicing in the human genome. Nat. Struct. Mol. Biol. *21*, 997–1005.

Sharma, S., Maris, C., Allain, F.H.T., and Black, D.L. (2011). U1 snRNA directly interacts with polypyrimidine tract-binding protein during splicing repression. Mol. Cell *41*, 579–588.

Sharma, S., Wongpalee, S.P., Vashisht, A., Wohlschlegel, J.A., and Black, D.L. (2014). Stem-loop 4 of U1 snRNA is essential for splicing and interacts with the U2 snRNP-specific SF3A1 protein during spliceosome assembly. Genes Dev. *28*, 2518–2531.

Sharma, E., Sterne-Weiler, T., O'Hanlon, D., and Blencowe, B.J. (2016). Global Mapping of Human RNA-RNA Interactions. Mol. Cell *62*, 618–626.

Shen, H., and Green, M.R. (2006). RS domains contact splicing signals and promote splicing by a common mechanism in yeast through humans. Genes Dev. *20*, 1755–1765.

Shi, Y. (2017). Mechanistic insights into precursor messenger RNA splicing by the spliceosome. Nat. Rev. Mol. Cell Biol. *18*, 655–670.

Shi, H., Wei, J., and He, C. (2019). Where, When, and How: Context-Dependent Functions of RNA Methylation Writers, Readers, and Erasers. Mol. Cell *74*, 640–650.

Sibley, C.R., Emmett, W., Blazquez, L., Faro, A., Haberman, N., Briese, M., Trabzuni, D., Ryten, M., Weale, M.E., Hardy, J., et al. (2015). Recursive splicing in long vertebrate genes. Nature *521*, 371–375.

Sibley, C.R., Blazquez, L., and Ule, J. (2016). Lessons from non-canonical splicing. Nat. Rev. Genet. *17*, 407–421.

Siegfried, Z., and Karni, R. (2018). The role of alternative splicing in cancer drug resistance. Curr. Opin. Genet. Dev. *48*, 16–21.

Skoglund, U., Andersson, K., Björkroth, B., Lamb, M.M., and Daneholt, B. (1983). Visualization of the formation and transport of a specific hnRNP particle. Cell *34*, 847–855.

Solana, J., Irimia, M., Ayoub, S., Orejuela, M.R., Zywitza, V., Jens, M., Tapial, J., Ray, D., Morris, Q., Hughes, T.R., et al. (2016). Conserved functional antagonism of CELF and MBNL proteins controls stem cell-specific alternative splicing in planarians. eLife *5*, e16797.

Sterne-Weiler, T., and Sanford, J.R. (2014). Exon identity crisis: disease-causing mutations that disrupt the splicing code. Genome Biol. *15*, 201.

Sugnet, C.W., Srinivasan, K., Clark, T.A., O'Brien, G., Cline, M.S., Wang, H., Williams, A., Kulp, D., Blume, J.E., Haussler, D., and Ares, M., Jr. (2006). Unusual intron conservation near tissue-regulated exons found by splicing microarrays. PLoS Comput. Biol. *2*, e4.

Sutandy, F.X.R., Ebersberger, S., Huang, L., Busch, A., Bach, M., Kang, H.S., Fallmann, J., Maticzka, D., Backofen, R., Stadler, P.F., et al. (2018). In vitro iCLIP-based modeling uncovers how the splicing factor U2AF2 relies on regulation by cofactors. Genome Res. *28*, 699–713.

Tapial, J., Ha, K.C.H., Sterne-Weiler, T., Gohr, A., Braunschweig, U., Hermoso-Pulido, A., Quesnel-Vallières, M., Permanyer, J., Sodaei, R., Marquez, Y., et al. (2017). An atlas of alternative splicing profiles and functional associations reveals new regulatory programs and genes that simultaneously express multiple major isoforms. Genome Res. *27*, 1759–1768.

Tejedor, J.R., Papasaikas, P., and Valcárcel, J. (2015). Genome-wide identification of Fas/CD95 alternative splicing regulators reveals links with iron homeostasis. Mol. Cell *57*, 23–38.

Thakran, P., Pandit, P.A., Datta, S., Kolathur, K.K., Pleiss, J.A., and Mishra, S.K. (2018). Sde2 is an intron-specific pre-mRNA splicing regulator activated by ubiquitin-like processing. EMBO J. *37*, 89–101.

Tollervey, J.R., Curk, T., Rogelj, B., Briese, M., Cereda, M., Kayikci, M., König, J., Hortobágyi, T., Nishimura, A.L., Zupunski, V., et al. (2011). Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. Nat. Neurosci. *14*, 452–458.

Torres-Méndez, A., Bonnal, S., Marquez, Y., Roth, J., Iglesias, M., Permanyer, J., Almudí, I., O'Hanlon, D., Guitart, T., Soller, M., et al. (2019). A novel protein domain in an ancestral splicing factor drove the evolution of neural microexons. Nat. Ecol. Evol. *3*, 691–701.

Tress, M.L., Abascal, F., and Valencia, A. (2017). Alternative Splicing May Not Be the Key to Proteome Complexity. Trends Biochem. Sci. *42*, 98–110.

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

Ule, J., Stefani, G., Mele, A., Ruggiu, M., Wang, X., Taneri, B., Gaasterland, T., Blencowe, B.J., and Darnell, R.B. (2006). An RNA map predicting Nova-dependent splicing regulation. Nature *444*, 580–586.

Ule, J., Hwang, H.W., and Darnell, R.B. (2018). The Future of Cross-Linking and Immunoprecipitation (CLIP). Cold Spring Harb. Perspect. Biol. *10*, a032243.

Ustianenko, D., Weyn-Vanhentenryck, S.M., and Zhang, C. (2017). Microexons: discovery, regulation, and function. Wiley Interdiscip. Rev. RNA *8*.

Van Nostrand, E.L., Freese, P., Pratt, G.A., Wang, X., Wei, X., Xiao, R., Blue, S.M., Chen, J.Y., Cody, N.A.L., Dominguez, D., et al. (2018). A Large-Scale Binding and Functional Map of Human RNA Binding Proteins. bioRxiv. https://doi.org/10.1101/179648.

Wahl, M.C., Will, C.L., and Lührmann, R. (2009). The spliceosome: design principles of a dynamic RNP machine. Cell *136*, 701–718.

Wan, Y., and Larson, D.R. (2018). Splicing heterogeneity: separating signal from noise. Genome Biol. *19*, 86.

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

Wang, Z., Xiao, X., Van Nostrand, E., and Burge, C.B. (2006). General and specific functions of exonic splicing silencers in splicing control. Mol. Cell *23*, 61–70.

Wang, E.T., Sandberg, R., Luo, S., Khrebtukova, I., Zhang, L., Mayr, C., Kingsmore, S.F., Schroth, G.P., and Burge, C.B. (2008). Alternative isoform regulation in human tissue transcriptomes. Nature *456*, 470–476.

Wang, Z., Kayikci, M., Briese, M., Zarnack, K., Luscombe, N.M., Rot, G., Zupan, B., Curk, T., and Ule, J. (2010). iCLIP predicts the dual splicing effects of TIA-RNA interactions. PLoS Biol. *8*, e1000530.

Wang, E.T., Cody, N.A.L., Jog, S., Biancolella, M., Wang, T.T., Treacy, D.J., Luo, S., Schroth, G.P., Housman, D.E., Reddy, S., et al. (2012). Transcriptome-wide regulation of pre-mRNA splicing and mRNA localization by muscleblind proteins. Cell *150*, 710–724.

Wang, X., Codreanu, S.G., Wen, B., Li, K., Chambers, M.C., Liebler, D.C., and Zhang, B. (2018). Detection of Proteome Diversity Resulted from Alternative Splicing is Limited by Trypsin Cleavage Specificity. Mol. Cell. Proteomics *17*, 422–430.

Wang, E., Lu, S.X., Pastore, A., Chen, X., Imig, J., Chun-Wei Lee, S., Hockemeyer, K., Ghebrechristos, Y.E., Yoshimi, A., Inoue, D., et al. (2019). Targeting an RNA-Binding Protein Network in Acute Myeloid Leukemia. Cancer Cell *35*, 369–384.e7.

Weatheritt, R.J., Sterne-Weiler, T., and Blencowe, B.J. (2016). The ribosome-engaged landscape of alternative splicing. Nat. Struct. Mol. Biol. *23*, 1117–1123.

Whitson, S.R., LeStourgeon, W.M., and Krezel, A.M. (2005). Solution structure of the symmetric coiled coil tetramer formed by the oligomerization domain of hnRNP C: implications for biological function. J. Mol. Biol. *350*, 319–337.

Witten, J.T., and Ule, J. (2011). Understanding splicing regulation through RNA splicing maps. Trends Genet. *27*, 89–97.

Wong, J.J.L., Ritchie, W., Ebner, O.A., Selbach, M., Wong, J.W.H., Huang, Y., Gao, D., Pinello, N., Gonzalez, M., Baidya, K., et al. (2013). Orchestrated intron retention regulates normal granulocyte differentiation. Cell *154*, 583–595.

Wu, J.Y., and Maniatis, T. (1993). Specific interactions between proteins implicated in splice site selection and regulated alternative splicing. Cell *75*, 1061–1070.

Xiong, H.Y., Alipanahi, B., Lee, L.J., Bretschneider, H., Merico, D., Yuen, R.K.C., Hua, Y., Gueroussov, S., Najafabadi, H.S., Hughes, T.R., et al. (2015). RNA splicing. The human splicing code reveals new insights into the genetic determinants of disease. Science *347*, 1254806.

Xue, Y., Zhou, Y., Wu, T., Zhu, T., Ji, X., Kwon, Y.S., Zhang, C., Yeo, G., Black, D.L., Sun, H., et al. (2009). Genome-wide analysis of PTB-RNA interactions reveals a strategy used by the general splicing repressor to modulate exon inclusion or skipping. Mol. Cell *36*, 996–1006.

Yan, C., Wan, R., and Shi, Y. (2019). Molecular Mechanisms of pre-mRNA Splicing through Structural Biology of the Spliceosome. Cold Spring Harb. Perspect. Biol. *11*, a032409.

Yang, X., Coulombe-Huntington, J., Kang, S., Sheynkman, G.M., Hao, T., Richardson, A., Sun, S., Yang, F., Shen, Y.A., Murray, R.R., et al. (2016). Widespread Expansion of Protein Interaction Capabilities by Alternative Splicing. Cell *164*, 805–817.

CellPress

Ying, Y., Wang, X.J., Vuong, C.K., Lin, C.H., Damianov, A., and Black, D.L. (2017). Splicing Activation by Rbfox Requires Self-Aggregation through Its Tyrosine-Rich Domain. Cell *170*, 312–323.e10.

Yu, Y., Maroney, P.A., Denker, J.A., Zhang, X.H.F., Dybkov, O., Lührmann, R., Jankowsky, E., Chasin, L.A., and Nilsen, T.W. (2008). Dynamic regulation of alternative splicing by silencers that modulate 5′ splice site competition. Cell *135*, 1224–1236.

Yuan, J., Ma, Y., Huang, T., Chen, Y., Peng, Y., Li, B., Li, J., Zhang, Y., Song, B., Sun, X., et al. (2018). Genetic Modulation of RNA Splicing with a CRISPR-Guided Cytidine Deaminase. Mol. Cell *72*, 380–394.e7.

Zarnack, K., König, J., Tajnik, M., Martincorena, I., Eustermann, S., Stévant, I., Reyes, A., Anders, S., Luscombe, N.M., and Ule, J. (2013). Direct competition between hnRNP C and U2AF65 protects the transcriptome from the exonization of Alu elements. Cell *152*, 453–466.

Zhang, C., Li, W.H., Krainer, A.R., and Zhang, M.Q. (2008). RNA landscape of evolution for optimal exon and intron discrimination. Proc. Natl. Acad. Sci. USA *105*, 5797–5802.

Zhang, X., Chen, M.H., Wu, X., Kodani, A., Fan, J., Doan, R., Ozawa, M., Ma, J., Yoshida, N., Reiter, J.F., et al. (2016). Cell-Type-Specific Alternative Splicing Governs Cell Fate in the Developing Cerebral Cortex. Cell *166*, 1147–1162.e15.

Zhou, K.I., Shi, H., Lyu, R., Wylder, A.C., Matuszek, Ż., Pan, J.N., He, C., Parisien, M., and Pan, T. (2019). Regulation of Co-transcriptional Pre-mRNA Splicing by m^6A through the Low-Complexity Protein hnRNPG. Mol. Cell, S1097-2765(19)30535-0.