[Frontiers in Bioscience 13, 1681-1690, January 1, 2008]

Alternative splicing of U12-type introns

Wen-Cheng Chang[1,2], Hung-Hsi Chen[1], Woan-Yuh Tarn[1]

[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, [2]Graduate Institute of Life Science, National Defense Medical Center, Taipei, Taiwan

TABLE OF CONTENTS

1. ABSTRACT

Precise removal of introns from metazoan precursor mRNAs is critical for gene expression. Nevertheless, alternative splicing provides a means for higher eukaryotes to increase genomic complexity and proteome diversity and to regulate certain cellular functions. The presence of rare U12-type pre-mRNA introns in eukaryotic genomes further complicates splicing events. In this review, we discuss the mechanism of U12-type intron splicing with emphasis on how the U12 spliceosome selects alternative splice sites in various U12-type intron–containing pre-mRNAs. Moreover, we propose possible roles for U12-type introns in a wide range of gene regulation through splicing regulation.

2. GENE EXPRESSION REGULATED BY PRE-MRNA SPLICING

2.1. Effects of alternative splicing on gene expression

It has been estimated that there exist ~25,000 protein-coding genes in the human genome, a number similar to that of *Arabidopsis thaliana* and only two times that of *Drosophila melanogaster* (1-3). This rather surprising fact suggests that gene number may not correlate with organismal complexity. Indeed, metazoans use several mechanisms—including post-transcriptional and post-translational modifications—to enhance the diversity of protein products encoded by a limited number of genes. These mechanisms can also tailor proteomes in individual cell types or throughout cell differentiation. The vast

majority of the human precursor mRNAs (pre-mRNAs) undergo spliceosome-catalyzed splicing that allows exon ligation with concurrent intron release. Alternative splicing of pre-mRNAs from single genes of higher eukaryotes can generate mRNA isoforms that may encode proteins having different amino acid sequences and perhaps biological functions. It has been reported that more than 74% of human multi-exon genes undergo alternative splicing and that each gene generates an average of six mRNA isoforms (4-6). Therefore, alternative splicing appears to be a major mechanism to increase proteome diversity in higher eukaryotes (7, 8).

Alternative splicing in concert with other post-transcription events also provides a means by which cells regulate mRNA expression level. Aberrant and even programmed alternative splicing may introduce premature termination codons within the coding region that can trigger subsequent mRNA degradation *via* nonsense-mediated mRNA decay (NMD). An estimate has suggested that ~40% of alternatively spliced transcripts acquire a stop codon and that more than half of such transcripts may be targeted for NMD (9). Moreover, alternative splicing can occur within untranslated regions (UTRs) of mRNAs. UTRs may contain *cis*-elements that control mRNA stability, transport or translation. Therefore, alternative splicing may also impact the fate of mRNAs by modulating post-splicing events (10, 11).

2.2. Regulation of alternative splicing

Regulation of alternative splicing involves a complex array of *cis*-elements of pre-mRNAs and various combinations of *trans*-acting factors (7, 12, 13). *Cis*-elements can act as splicing enhancers or silencers, or they can play a dual role depending on the binding of *trans*-acting factors. Multiple *cis*-elements may exist to fine tune the splicing patterns in different cell/tissue types or at different developmental stages. However, these *cis*-elements are somewhat divergent in sequence, and their interactions with corresponding splicing factors are generally weak (14). Nevertheless, these features may have some advantages such as to increase the capacity of *cis*-elements to bind different *trans*-acting factors and to provide a basis for exchanging binding factors in quick response to cellular signals (15, 16).

In general, *trans*-acting regulatory factors include two major types of RNA-binding proteins, namely serine/arginine dipeptide–rich (SR) proteins and heterogeneous ribonucleoproteins (hnRNPs) (14, 17, 18). These splicing factors may directly or indirectly associate with corresponding *cis*-elements and may collaborate with or antagonize each other to generate specific splicing patterns. An optimal balance among the levels/activities of *trans*-acting factors is critical for appropriate gene expression (19-24). For example, the dynamic balance between the muscleblind family members and their antagonizing *trans*-acting splicing factors, including polypyrimidine tract binding (PTB) protein and CUG-BP and ETR-like factors (CELFs), can determine the splicing pattern of several specific mRNAs during heart development (25).

3. DISEASES CAUSED BY MISREGULATION OF SPLICING

3.1. Genetic effects

Genetic mutations in cis-elements of pre-mRNAs or alterations of trans-acting factors may result in misregulation of pre-mRNA splicing and thereby generate aberrant splicing products, leading to clinical manifestations (26-29). At least 15% of point mutations at intron-exon junctions cause splicing defects leading to genetic diseases (30). However, mutations within regulatory cis-elements may also affect splice site selection or exon utilization, indicating that the impact of genetic mutations on diseases in the above analysis is underestimated (31, 32). The autosomal recessive disorder spinal muscular atrophy (SMA) is representative of splicing defects primarily resulting from an exonic silent mutation. SMA is caused by mutations or deletion of the survival motor neuron 1 (SMN1) gene (33). However, the duplicate of human SMN1, SMN2, fails to produce sufficient amounts of full-length functional SMN protein and thus cannot fully compensate for the loss of SMN1 (34). SMN2 differs from SMN1 only by a few nucleotide changes; the silent mutation at position 6 of SMN2 exon 7 is responsible for exon 7 skipping in most SMN2 transcripts, thus yielding the exon 7-truncated protein (35-37). This nucleotide change may likely destroy a splicing enhancer or create a splicing silencer in SMN2, thus reducing the efficiency of exon 7 inclusion (38, 39). In addition, several lines of evidence including the case of SMN indicate that cis-elements involving secondary structure formation may regulate alternative splicing (34). Moreover, a type of genetic diversity called single nucleotide polymorphism (SNP) can occur in pre-mRNA cis-elements, causing cell dysfunction and modulate the extent of disease. One such example is the alternative splicing of the Wilms tumor suppressor (WT1) gene in Frasier syndrome. WT1 mRNA isoforms are produced by differential use of the two closely located 5' splice sites downstream of exon 9 (40). A downstream 5' splice site mutation in Frasier syndrome individuals shifts splicing towards the upstream 5' splice site, and thus alters the ratio of the mRNA isoforms, which leads to pathogenic effects. This observation indicates that maintenance of an appropriate isoform ratio is important for cell function (41, 42).

Moreover, single nucleotide polymorphisms (SNPs), a type of genetic diversity, in pre-mRNA cis-elements may cause differences in splicing efficiency or accuracy between alleles and individuals. Thus, SNPs can also cause variations in the ratio of mRNA isoforms and thereby influence the penetrance, process or severity of human diseases (26). Numerous cases support this view; for example, splicing variations caused by SNPs in the neural cell adhesion molecule (NCAM) and neuregulin-1 receptor ErbB4 genes have been implicated in neuronal disorders such as schizophrenia (43, 44). It is also widely accepted that inter-individual differences in splicing patterns can impact drug efficacy, and thus modulation of pre-mRNA splicing may have potential pharmacological values (45).

3.2. Trans-acting effects

Many lines of evidence have suggested that *trans*-acting splicing factors contribute to the onset or progression of human diseases, such as cancer and sporadic neurological diseases including Alzheimer's disease, frontotemporal dementia and amyotropic lateral sclerosis. Indeed, aberrant splicing patterns of specific genes have been observed in several of these diseases (28, 46-51). However, lack of somatic mutations in known disease susceptibility genes suggests that modifications of splicing factors are responsible for the pathogenesis of certain acquired diseases (52-54). It is well established that cellular signaling profoundly affects the control of alternative splicing by modulating post-translational modifications—primarily phosphorylation—of splicing regulatory factors (55, 56). Such modifications can determine the abundance, localization or activity of individual factors and hence modulate the ratios of cooperative vs. antagonistic factors. Evidence has indicated that altered expression or phosphorylation levels of serine/arginine dipeptide-rich proteins (SR proteins) correlates with various splicing patterns of CD44 during breast cancer tumorigenesis (57). It is likely that signaling pathway perturbation in cancerous cells causes alterations in covalent modifications/respecification of splicing regulatory factors and ultimately leads to changes in splicing patterns. Thus, cis- and trans-regulatory factors of pre-mRNA splicing can act together as genetic modifiers, and changes in either may cause cellular dysfunction and contribute to pathogenesis (58).

4. PRESENCE OF U12-TYPE INTRONS AND THEIR SPLICING MECHANISM

4.1. A minor class of introns: U12-type introns

The vast majority of pre-mRNA introns belong to the U2 type. Another type of the intron, namely U12-type intron, also exists in representative eukaryotic organisms, except for some lineages such as *Caenorhabditis elegans*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (59-63). U12 type-introns occur with a very low frequency; the human genome has the highest number (~700) among all species examined (61, 62).

U2- and U12-type introns differ between each other primarily in the 5' splice site and branch site consensus sequences (59). Although the terminal dinucleotides of pre-mRNA introns in general follow the GT-AG rule, approximately one-third of U12-type introns contain the termini AT-AC. The U2- and U12-introns may have arisen from two different self-splicing group II introns in separate lineages and merged in a eukaryote progenitor upon lineage fusion (59, 64). As compared to U2-type introns, U12-type introns are more conserved at their 5' splice sites and branch site sequences but appear to be spliced with less accuracy and at a slower rate (65, 66). Therefore, during evolution these disadvantages may have driven U12-type introns to convert gradually to U2-type introns.

4.2. The splicing mechanism of U12-type intron–containing pre-mRNAs

The U2- and U12-type introns are removed by distinct spliceosomes (67, 68). Except for the U5 small nuclear ribonuclear particle (snRNP), the four other snRNPs differ between these two spliceosomes. The U1, U2, and U4/U6 snRNPs in the U2 spliceosome are replaced by their low-abundance functional analogs U11, U12, and U4atac/U6atac in the U12 spliceosome (69, 70). Interestingly, the respective snRNA analogs have highly similar secondary structures despite with little sequence identity, and, moreover, they make very similar RNA-RNA interaction frameworks in individual spliceosomes (68). The most conserved part between the two sets of snRNAs in the spliceosome is the intramolecular stem-loop of U6 and U6atac, which is believed to be located near or at the catalytic center (71, 72). Thus, this striking conservation suggests that the mechanism of splicing is very similar between the U2 and U12 spliceosomes (73).

In addition, the two spliceosomes share a large number of protein factors, most of which are components of the U4/U6.U5 tri-snRNP and its U12-type counterpart (74). However, the U12 spliceosome–specific proteins are primarily associated with the U11 and U12 snRNPs, suggesting that the two types of spliceosomes have distinct features particularly in splice site recognition (75). Indeed, the U11 and U12 snRNPs form a functional di-snRNP through protein-protein and protein-snRNA interactions (76). Therefore, this di-snRNP complex may simultaneously recognize the 5' splice site and the branch site elements via base pairing with the pre-mRNA (77). Moreover, the U12 snRNP may transiently interact with the sequence immediately upstream of the 5' splice site early during spliceosome assembly (78). Thus, these features in splice site recognition may be unique to U12-type intron splicing.

Despite the similarities and differences between the two spliceosomes, each spliceosome can only catalyze the removal of its cognate introns, primarily because splice site recognition involves specific base pairing between the snRNAs and pre-mRNAs. Here, we propose that the U11 and U12 snRNPs act as a unit to specify U12-type splice sites and thereby prevent unintended splicing of U12-introns by the abundant U2 spliceosome. This view is supported by results obtained from mutational analysis of U12-type introns in several human genes (79-81). Mutations of the U12-type 5' splice site induce aberrant splicing primarily mediated by the U2 spliceosome instead of the U12 spliceosome (Figure 1), suggesting that the U12 spliceosome functions only when the cognate 5' splice site can be properly recognized. Moreover, the U11-5' splice site interaction may aid the U12 partner to locate the branch site in U12-type introns and hence define the 3' splice site for splicing.

5. ALTERNATIVE SPLICING OF U12-TYPE INTRON–CONTAINING PRE-mRNAs

Increasing evidence indicates that U12-type intron–containing pre-mRNAs also undergo various types of alternative splicing. Although the regulation of U12-type intron splicing has not been extensively studied, several reports have indicated that this splicing can be activated by both SR proteins and exonic splicing enhancers, a scenario similar to that for U2-type introns (82-85). Moreover,

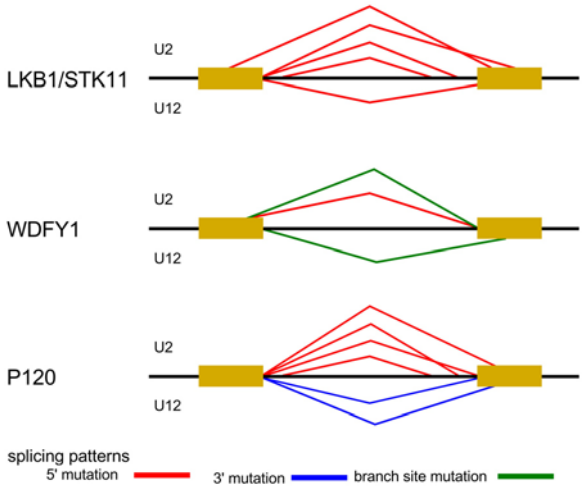

Figure 1. Alternative 5' or 3' splice site selection of U12 intron–containing pre-mRNAs. Mutations of splice site signals in the U12-type intron of the human genes, *LKB1*/*STK11*, *WDFY1* and *P120*, induce alternative 5' and/or 3' splice site utilization. Observed alternative splicing is mediated by either the U2 or the U12 spliceosome due to mutations at the 5' splice site (red), branch site (green), or 3' splice site (blue). Representative alternatively spliced patterns are shown for each case.

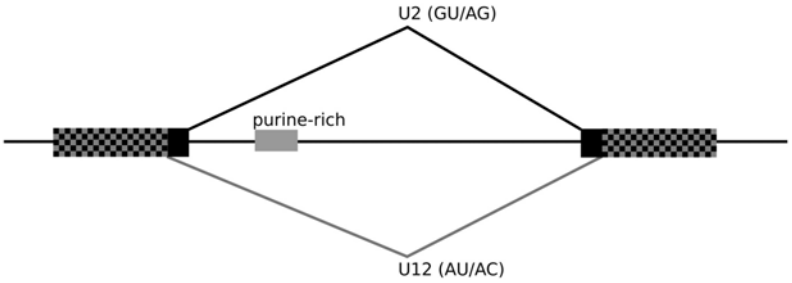

Figure 2. The "twintron" of the *Drosophila prospero* pre-mRNA. The U2-type intron (GU-AG termini) is located within the U12-type intron (AU-AC termini). The purine-rich splicing enhancer is located within the inner intron.

ccording to the "exon definition" model (86, 87), SR proteins may possibly function to bridge adjacent U12 and U2 spliceosomes during the splicing of U12-type intron pre-mRNAs. Perhaps SR proteins mediate communication between the two spliceosomes through direct contact with the 5' splice site and branch site of both types of introns (85). Finally, whether any other *trans*-acting regulatory factors also participate in U12-type intron splicing as well as how they modulate alternative splicing involving U12-type introns remains to be characterized.

5.1. An intron within an intron

The *Drosophila* gene *prospero* provides an unusual example of alternative splicing. The *prospero* pre-mRNA contains a "twintron", in which a U2-type intron (with GU-AG termini) is located within a U12-type intron (AU-AC termini) (Figure 2). Removal of the U2- or U12-type intron generates two alternatively spliced isoforms. Interestingly, expression of individual isoforms predominates at different stages during embryogenesis, indicating that a developmentally regulated factor(s) is likely involved in splicing regulation (88). Moreover, a purine-rich enhancer sequence in the inner intron appears to be required for splicing of both types of intron (85). This finding suggests that a *cis*-element can function to regulate both types of spliceosome, and that the two types of spliceosomes may compete for a similar set of regulatory factors during twintron splicing.

5.2. Exon skipping

Because splice site recognition depends on spliceosome type, coexistence of two types of introns in a pre-mRNA may potentiate the diversity of alternatively spliced mRNAs. Evidence comes from the observation that a 5' splice site mutation in a U2-type intron of the mouse gene *Scn8a* leads to exclusion of two upstream exons, between which is a U12-type intron (89). In addition, several genes contain more than one U12-type intron (90). Among these genes, the DER1-like domain family members (*DERL2* and *DERL3*) and the xanthine dehydrogenase gene (XDH) harbor two successive U12-type introns. An Expressed Sequence Tag (EST) analysis (our unpublished data) revealed a number of exon-skipped or intron-retained DERL transcripts (Figure 3), indicating that alternative or aberrant splicing of U12-type introns occurs in some circumstances. This analysis also suggested that both types of spliceosomes can share a functional 3' splice site (Figure 3, isoform 4). Moreover, retention of

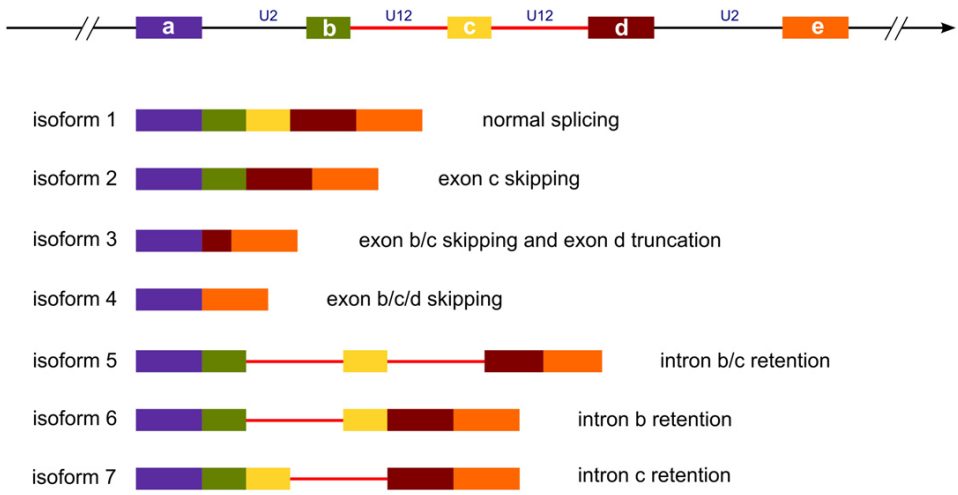

Figure 3. Alternative splicing of the human *DERL2/3* pre-mRNA. The organization of the human *DERL2*/*DERL*3 genes and their putative transcript patterns are illustrated. The data for the alternatively spliced transcripts were obtained from the EST database. The transcript patterns shown were obtained from the UCSC Genome Browser spliced EST database (http://genome.ucsc.edu).

U12-type intron(s) in mRNA isoforms suggests their lower splicing efficiency (Figure 3, isoforms 5, 6 and 7) and also supports a previous view that removal of U12-type introns may be a rate-limiting step during splicing (65, 66).

5.3. Selection of alternative 5' or 3' splice sites

A number of mutation studies have revealed clues as to how the U12 spliceosome selects splice sites. Several reports have shown that introduction of mutations in U12-type 5' splice sites can drive either type of spliceosome to use a suboptimal 5' splice site for splicing to the authentic or an alternative 3' splice site (80, 81, 91) (Figure 1). The U2 spliceosome acts dominantly in alternative splicing, likely due to its relative high abundance and low stringency with regard to 5' splice site recognition. Similar scenarios have been observed for mutations within either the branch site or 3' splice site elements (81) (Figure 1). Because most U12-type introns lack a characteristic pyrimidine tract, the branch site may dictate selection of the 3' splice site. Therefore, in the case of U12 spliceosome–activated alternative 3' splice site selection, the U12 snRNP may select a suboptimal branch site and activate a downstream AG as the 3' splice site. Nevertheless, as compared to the U12-type 5' splice site, recognition of the 3' splice site is less constrained (92). Moreover, as hypothesized above, the interaction between the U11 snRNP and 5' splice site may help the U12 snRNP to locate a branch site and thereby activate the use of a 3' splice site. Thus, while natural nucleotide alterations (mutations or SNPs) occur within a U12-type intron, a more complex splicing result may be obtained due to involvement of both types of spliceosomes (Figure 1).

5.4. U2-U12 hybrid introns

U2-U12 hybrid introns have been observed in several mitogen-activated protein kinase (MAPK) family members (81). The use of the hybrid intron strategy may provide a mechanism that drives mutually exclusive splicing to produce transcripts containing either one or the other of the exons flanking the hybrid intron (93). The two exons flanking the hybrid intron are never joined. This mechanism has its basis in the specificity of the two spliceosomes for their corresponding splice sites. In the human genome, four MAPKs, i.e. MAPK8/JNK1, MAPK9/JNK2, MAPK10/JNK3, and MAPK14/p38-alpha, contain a U2-U12 hybrid intron (Figure 4). The alternative exons in each gene share significant sequence similarity; however, the upstream exon shows higher conservation with its cognate exon in the hybrid intron-lacking MAPK members, suggesting that the downstream exon is a duplicate. Notably, the architecture of the hybrid intron differs between JNKs and p38-alpha (Figure 3). The hybrid intron of JNKs contains U12-type 5' and U2-type 3' splice sites whereas the opposite case holds for p38-alpha (Figure 4). Therefore, the U12-type intron is inevitably located downstream of the hybrid intron in JNKs but upstream of the hybrid in p38alpha (Figure 4). Some of the JNK-like MAPK members of pufferfish (*Fugu rubripes*) and zebrafish (*Danio rerio*) harbor a U12-type intron but no hybrid intron (81). Therefore, the hybrid intron may have originated from a U12-type intron via DNA rearrangement, as mutually exclusive exons are generated due to an exon duplication event (93, 94).

As shown in Figure 4, inclusion of exon 6a in JNK2 is specific to neuronal cells, whereas exon 6b–containing transcripts can be expressed in almost any cell type (54, 95). We have recently reported that exon 6b inclusion is primarily attributed to the exceptionally long and highly C/U-rich polypyrimidine tract of the hybrid intron (81). Perhaps this element recruits a ubiquitous splicing activator(s) for exon 6b inclusion. On the other hand, the RNA binding protein Nova has been implicated as a neuron-specific activator to increase the expression of exon 6a–containing transcripts (95). Thus, it would be interesting to investigate whether Nova merely promotes exon 6a utilization or can also antagonize the activity of the pyrimidine element–binding factor(s). Moreover, it would

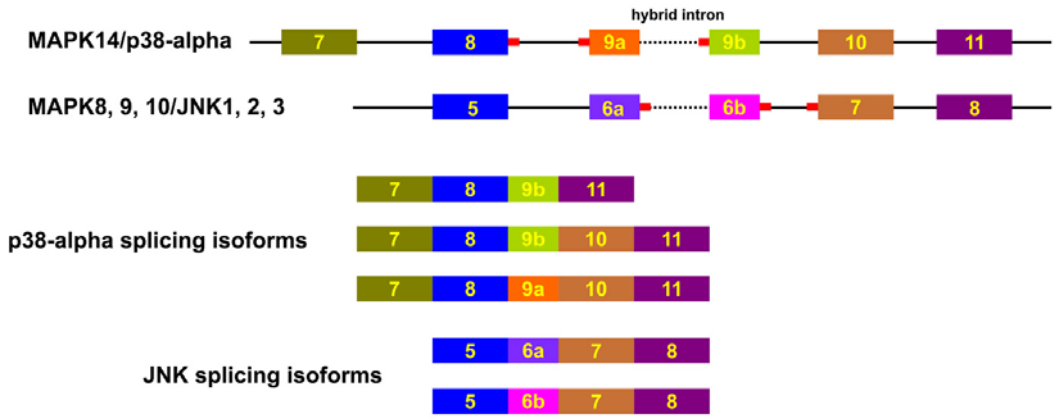

Figure 4. The U2-U12 hybrid intron in the MAPK family members. The illustration shows the gene organization of the *JNK* subfamily members JNK1, 2 and 3 (i.e., MAPK8, 9 and 10) and that of the p38-alpha (MAPK14). The hybrid intron is depicted in dashed lines; U12-type splice sites are indicated by short red lines. The transcript patterns shown were obtained from the EST database.

be valuable to understand how the splicing of the hybrid introns is regulated in other JNK/p38-alpha members.

6. POSSIBLE REGULATORY ROLES FOR U12-TYPE INTRONS

U12-type introns are found in several gene families, such as solute transporters, MAPKs, and importin-beta, likely due to gene duplication events during evolution (81). Moreover, it has been noted that U12-type introns prevail in some functionally but not genetically related genes, such as those implicated in Ras-Raf and phosphoinositol signaling pathways and in mRNA and protein metabolism (59, 81, 90). Of particular interest is a set of both constitutive and regulatory splicing factors as well as proteins involved in mRNA decay (90). Previous studies have shown that U12-type intron splicing is likely rate-limiting and is less tolerant to splice site and branch site variations as compared with U2-type introns (65, 80, 81). Perhaps through control of U12-type intron splicing, the expression of their host genes can be regulated. It is known that splicing factors can regulate the splicing of their own pre-mRNAs to generate specific alternative isoforms or modulate the expression level (23, 93, 96-99). Therefore, it is interesting to speculate whether the RNA processing factors whose genes host a U12-type intron can autoregulate their own expression by controlling the rate or accuracy of U12 intron splicing. A recent report shows that alternative splicing of SRp38 activated by an ultraconserved *cis*-element results in unproductive expression due to inclusion of a premature termination codon (99). Meanwhile, such alternative splicing prevents the use of an authentic U12-type 5' splice site but activates a cryptic U2-type site. Therefore, control of U12-type intron splicing is possibly a means by which the level of a set of splicing factors is regulated. Moreover, several genes involved in metabolism or regulation of splicing factors also host a U12-type intron; these genes encode transportins and SR protein kinases, which are involved in nuclear transport and phosphorylation of certain splicing regulators, respectively (81). Certainly, a change in expression pattern of any of these RNA processing factors would be expected to have a considerable impact on global gene expression at the level of alternative splicing regulation.

In general, the position of U12-type introns is conserved among members of a gene family due to gene duplication. An exception is the importin-beta paralogs, several of which contain one or two U12-type introns. Intriguingly, the position as well as the length of these U12-type introns varies highly among these paralogs (Table 1). Variable intron position suggests vigorous intron gain and shuffling during evolution but cannot exclude the possibility of intron loss. We presume that the presence of U12-type introns provides a means by which at least several importin-beta proteins control their expression and/or activity; such a benefit(s) may have driven importin-beta genes to acquire and maintain U12-type introns.

7. CONCLUSIONS AND PERSPECTIVES

The discovery of U12-type introns over the past dozen years has brought us insights into intron evolution and the mechanism(s) of splicing. Nevertheless, our knowledge of U12-type intron splicing and its regulation still remains at the periphery. The bioinformatic and experimental results discussed above shed light on how the U12 spliceosome recognizes splice sites in various cases of U12-type intron alternative splicing. However, questions remain as to how the U12 spliceosome competes with the more abundant U2 spliceosome to define the splice sites, particularly in the context of pre-mRNA sequences with many degenerate splice site signals, and how *trans*-acting factors coordinate the two types of spliceosomes for splicing of U12-type intron–containing pre-mRNAs.

The U12-type spliceosome is less tolerant to mutations within splice site signals. Thus, as compared with U2-type intron splicing, U12-type splicing may be prone to alternative splicing particularly when genetic variations occur within such sites. Consequently, alteration

Table 1. U12-type introns in human importin-beta family members. The list shows the position and the length of the hybrid intron and total exon number of each importion-beta paralogous gene

Gene symbol	Intron positon	Exon number	Intron length
IPO4	26	30	110
IPO9	8	24	200
IPO11	17	30	156
RANBP5	21	29	1,701
RANBP17	16	28	11,878
RANBP17	20	28	8,018
TNPO1	2	25	2,744
TNPO2	2	25	1,542
XPO4	5	22	15,715
XPO4	19	22	887
XPO5	23	32	1,763
XPO7	16	28	1,260

in splicing patterns can influence cell functions. Moreover, because a set of RNA processing factors is encoded by U12 intron–containing genes, we propose that U12-type intron splicing may regulate the expression of these proteins and subsequently amplify its effect to the expression of a wide range of other genes. Thus, U12-type introns may have a significant impact on genomic and physiological diversity, and such aspects must be tested in future studies.

8. ACKNOWLEDGEMENTS

We especially acknowledge the support from the Academia Sinica Investigator Award to W.-Y. Tarn. We also thank Dr. Tim C. Taylor for editing of the manuscript.

9. REFERENCES

1. Misra, S., M. A. Crosby, C. J. Mungall, B. B. Matthews, K. S. Campbell, P. Hradecky, Y. Huang, J. S. Kaminker, G. H. Millburn, S. E. Prochnik, C. D. Smith, J. L. Tupy, E. J. Whitfied, L. Bayraktaroglu, B. P. Berman, B. R. Bettencourt, S. E. Celniker, A. D. de Grey, R. A. Drysdale, N. L. Harris, J. Richter, S. Russo, A. J. Schroeder, S. Q. Shu, M. Stapleton, C. Yamada, M. Ashburner, W. M. Gelbart, G. M. Rubin & S. E. Lewis: Annotation of the Drosophila melanogaster euchromatic genome: a systematic review. *Genome Biol*, 3(12), RESEARCH0083 (2002)

2. International Human Genome Sequencing Consortium: Finishing the euchromatic sequence of the human genome. *Nature*, 431(7011), 931-45 (2004)

3. Haas, B. J., J. R. Wortman, C. M. Ronning, L. I. Hannick, R. K. Smith, Jr., R. Maiti, A. P. Chan, C. Yu, M. Farzad, D. Wu, O. White & C. D. Town: Complete reannotation of the Arabidopsis genome: methods, tools, protocols and the final release. *BMC Biol*, 3, 7 (2005)

4. Johnson, J. M., J. Castle, P. Garrett-Engele, Z. Kan, P. M. Loerch, C. D. Armour, R. Santos, E. E. Schadt, R. Stoughton & D. D. Shoemaker: Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. *Science*, 302(5653), 2141-4 (2003)

5. de la Grange, P., M. Dutertre, N. Martin & D. Auboeuf: FAST DB: a website resource for the study of the expression regulation of human gene products. *Nucleic Acids Res*, 33(13), 4276-84 (2005)

6. Le Texier, V., J. J. Riethoven, V. Kumanduri, C. Gopalakrishnan, F. Lopez, D. Gautheret & T. A. Thanaraj: AltTrans: transcript pattern variants annotated for both alternative splicing and alternative polyadenylation. *BMC Bioinformatics*, 7, 169 (2006)

7. Smith, C. W. & J. Valcarcel: Alternative pre-mRNA splicing: the logic of combinatorial control. *Trends Biochem Sci*, 25(8), 381-8 (2000)

8. Graveley, B. R.: Alternative splicing: increasing diversity in the proteomic world. *Trends Genet*, 17(2), 100-7 (2001)

9. Baek, D. & P. Green: Sequence conservation, relative isoform frequencies, and nonsense-mediated decay in evolutionarily conserved alternative splicing. *Proc Natl Acad Sci U S A*, 102(36), 12813-8 (2005)

10. Pesole, G., F. Mignone, C. Gissi, G. Grillo, F. Licciulli & S. Liuni: Structural and functional features of eukaryotic mRNA untranslated regions. *Gene*, 276(1-2), 73-81 (2001)

11. Hughes, T. A.: Regulation of gene expression by alternative untranslated regions. *Trends Genet*, 22(3), 119-22 (2006)

12. Maniatis, T. & R. Reed: An extensive network of coupling among gene expression machines. *Nature*, 416(6880), 499-506 (2002)

13. Maniatis, T. & B. Tasic: Alternative pre-mRNA splicing and proteome expansion in metazoans. *Nature*, 418(6894), 236-43 (2002)

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

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

16. Novoyatleva, T., Y. Tang, I. Rafalska & S. Stamm: Pre-mRNA missplicing as a cause of human disease. *Prog Mol Subcell Biol*, 44, 27-46 (2006)

17. Hastings, M. L. & A. R. Krainer: Pre-mRNA splicing in the new millennium. *Curr Opin Cell Biol*, 13(3), 302-9 (2001)

18. Singh, R. & J. Valcarcel: Building specificity with nonspecific RNA-binding proteins. *Nat Struct Mol Biol*, 12(8), 645-53 (2005)

19. Mayeda, A. & A. R. Krainer: Regulation of alternative pre-mRNA splicing by hnRNP A1 and splicing factor SF2. *Cell*, 68(2), 365-75 (1992)

20. Zahler, A. M., K. M. Neugebauer, W. S. Lane & M. B. Roth: Distinct functions of SR proteins in alternative pre-mRNA splicing. *Science*, 260(5105), 219-22 (1993)

21. Caceres, J. F., S. Stamm, D. M. Helfman & A. R. Krainer: Regulation of alternative splicing *in vivo* by overexpression of antagonistic splicing factors. *Science*, 265(5179), 1706-9 (1994)

22. Gallego, M. E., R. Gattoni, J. Stevenin, J. Marie & A. Expert-Bezancon: The SR splicing factors ASF/SF2 and SC35 have antagonistic effects on intronic enhancer-dependent splicing of the beta-tropomyosin alternative exon 6A. *EMBO J*, 16(7), 1772-84 (1997)

23. Jumaa, H. & P. J. Nielsen: The splicing factor SRp20 modifies splicing of its own mRNA and ASF/SF2 antagonizes this regulation. *EMBO J*, 16(16), 5077-85 (1997)
24. Hanamura, A., J. F. Caceres, A. Mayeda, B. R. Franza, Jr. & A. R. Krainer: Regulated tissue-specific expression of antagonistic pre-mRNA splicing factors. *RNA*, 4(4), 430-44 (1998)
25. Ladd, A. N., M. G. Stenberg, M. S. Swanson & T. A. Cooper: Dynamic balance between activation and repression regulates pre-mRNA alternative splicing during heart development. *Dev Dyn*, 233(3), 783-93 (2005)
26. Caceres, J. F. & A. R. Kornblihtt: Alternative splicing: multiple control mechanisms and involvement in human disease. *Trends Genet*, 18(4), 186-93 (2002)
27. Stoilov, P., E. Meshorer, M. Gencheva, D. Glick, H. Soreq & S. Stamm: Defects in pre-mRNA processing as causes of and predisposition to diseases. *DNA Cell Biol*, 21(11), 803-18 (2002)
28. Faustino, N. A. & T. A. Cooper: Pre-mRNA splicing and human disease. *Genes Dev*, 17(4), 419-37 (2003)
29. Blencowe, B. J.: Alternative splicing: new insights from global analyses. *Cell*, 126(1), 37-47 (2006)
30. Krawczak, M., J. Reiss & D. N. Cooper: The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. *Hum Genet*, 90(1-2), 41-54 (1992)
31. Cartegni, L., S. L. Chew & A. R. Krainer: Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet*, 3(4), 285-98 (2002)
32. Baralle, D. & M. Baralle: Splicing in action: assessing disease causing sequence changes. *J Med Genet*, 42(10), 737-48 (2005)
33. Lefebvre, S., L. Burglen, S. Reboullet, O. Clermont, P. Burlet, L. Viollet, B. Benichou, C. Cruaud, P. Millasseau, M. Zeviani & *et al.*: Identification and characterization of a spinal muscular atrophy-determining gene. *Cell*, 80(1), 155-65 (1995)
34. Butchbach, M.E., J. D. Edwards & A. H. Bugghes: Abnormal motor phenotype in the SMNDelta7 mouse model of spinal muscular atrophy. *Neurobiol Dis*, 27(2), 207-19 (2007)
35. Lorson, C. L., E. Hahnen, E. J. Androphy & B. Wirth: A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proc Natl Acad Sci U S A*, 96(11), 6307-11 (1999)
36. Monani, U. R., C. L. Lorson, D. W. Parsons, T. W. Prior, E. J. Androphy, A. H. Burghes & J. D. McPherson: A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. *Hum Mol Genet*, 8(7), 1177-83 (1999)
37. Lorson, C. L. & E. J. Androphy: An exonic enhancer is required for inclusion of an essential exon in the SMA-determining gene SMN. *Hum Mol Genet*, 9(2), 259-65 (2000)
38. Cartegni, L. & A. R. Krainer: Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. *Nat Genet*, 30(4), 377-84 (2002)
39. Kashima, T. & J. L. Manley: A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. *Nat Genet*, 34(4), 460-3 (2003)
40. Haber, D. A., R. L. Sohn, A. J. Buckler, J. Pelletier, K. M. Call & D. E. Housman: Alternative splicing and genomic structure of the Wilms tumor gene WT1. *Proc Natl Acad Sci U S A*, 88(21), 9618-22 (1991)
41. Barbaux, S., P. Niaudet, M. C. Gubler, J. P. Grunfeld, F. Jaubert, F. Kuttenn, C. N. Fekete, N. Souleyreau-Therville, E. Thibaud, M. Fellous & K. McElreavey: Donor splice-site mutations in WT1 are responsible for Frasier syndrome. *Nat Genet*, 17(4), 467-70 (1997)
42. Klamt, B., A. Koziell, F. Poulat, P. Wieacker, P. Scambler, P. Berta & M. Gessler: Frasier syndrome is caused by defective alternative splicing of WT1 leading to an altered ratio of WT1 +/-KTS splice isoforms. *Hum Mol Genet*, 7(4), 709-14 (1998)
43. Atz, M. E., B. Rollins & M. P. Vawter: NCAM1 association study of bipolar disorder and schizophrenia: polymorphisms and alternatively spliced isoforms lead to similarities and differences. *Psychiatr Genet*, 17(2), 55-67 (2007)
44. Law, A. J., J. E. Kleinman, D. R. Weinberger & C. S. Weickert: Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. *Hum Mol Genet*, 16(2), 129-41 (2007)
45. Bracco, L. & J. Kearsey: The relevance of alternative RNA splicing to pharmacogenomics. *Trends Biotechnol*, 21(8), 346-53 (2003)
46. Lin, C. L., L. A. Bristol, L. Jin, M. Dykes-Hoberg, T. Crawford, L. Clawson & J. D. Rothstein: Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. *Neuron*, 20(3), 589-602 (1998)
47. Catania, M. V., E. Aronica, B. Yankaya & D. Troost: Increased expression of neuronal nitric oxide synthase spliced variants in reactive astrocytes of amyotrophic lateral sclerosis human spinal cord. *J Neurosci*, 21(11), RC148 (2001)
48. Evin, G., M. J. Smith, A. Tziotis, C. McLean, L. Canterford, R. A. Sharples, R. Cappai, A. Weidemann, K. Beyreuther, R. G. Cotton, C. L. Masters & J. G. Culvenor: Alternative transcripts of presenilin-1 associated with frontotemporal dementia. *Neuroreport*, 13(6), 917-21 (2002)
49. Venables, J. P.: Unbalanced alternative splicing and its significance in cancer. *Bioessays*, 28(4), 378-86 (2006)
50. Newman, M., F. I. Musgrave & M. Lardelli: Alzheimer disease: amyloidogenesis, the presenilins and animal models. *Biochim Biophys Acta*, 1772(3), 285-97 (2007)
51. Scholzova, E., R. Malik, J. Sevcik & Z. Kleibl: RNA regulation and cancer development. *Cancer Lett*, 246(1-2), 12-23 (2007)
52. Stickeler, E., F. Kittrell, D. Medina & S. M. Berget: Stage-specific changes in SR splicing factors and alternative splicing in mammary tumorigenesis. *Oncogene*, 18(24), 3574-82 (1999)
53. Fischer, D. C., K. Noack, I. B. Runnebaum, D. O. Watermann, D. G. Kieback, S. Stamm & E. Stickeler: Expression of splicing factors in human ovarian cancer. *Oncol Rep*, 11(5), 1085-90 (2004)
54. Relogio, A., C. Ben-Dov, M. Baum, M. Ruggiu, C. Gemund, V. Benes, R. B. Darnell & J. Valcarcel: Alternative splicing microarrays reveal functional expression of neuron-specific regulators in Hodgkin lymphoma cells. *J Biol Chem*, 280(6), 4779-84 (2005)

55. Shin, C. & J. L. Manley: Cell signalling and the control of pre-mRNA splicing. *Nat Rev Mol Cell Biol*, 5(9), 727-38 (2004)
56. Tarn, W. Y.: Cellular signals modulate alternative splicing. *J Biomed Sci,*14(4), 517-22 (2007)
57. Watermann, D. O., Y. Tang, A. Zur Hausen, M. Jager, S. Stamm & E. Stickeler: Splicing factor Tra2-beta1 is specifically induced in breast cancer and regulates alternative splicing of the CD44 gene. *Cancer Res*, 66(9), 4774-80 (2006)
58. Nissim-Rafinia, M. & B. Kerem: The splicing machinery is a genetic modifier of disease severity. *Trends Genet*, 21(9), 480-3 (2005)
59. Burge, C. B., R. A. Padgett & P. A. Sharp: Evolutionary fates and origins of U12-type introns. *Mol Cell*, 2(6), 773-85 (1998)
60. Russell, A. G., J. M. Charette, D. F. Spencer & M. W. Gray: An early evolutionary origin for the minor spliceosome. *Nature*, 443(7113), 863-6 (2006)
61. Sheth, N., X. Roca, M. L. Hastings, T. Roeder, A. R. Krainer & R. Sachidanandam: Comprehensive splice-site analysis using comparative genomics. *Nucleic Acids Res*, 34(14), 3955-67 (2006)
62. Alioto, T. S.: U12DB: a database of orthologous U12-type spliceosomal introns. *Nucleic Acids Res*, 35(Database issue), D110-5 (2007)
63. Mount, S. M., V. Gotea, C. F. Lin, K. Hernandez & W. Makalowski: Spliceosomal small nuclear RNA genes in 11 insect genomes. *RNA*, 13(1), 5-14 (2007)
64. Lynch, M. & A. O. Richardson: The evolution of spliceosomal introns. *Curr Opin Genet Dev*, 12(6), 701-10 (2002)
65. Patel, A. A., M. McCarthy & J. A. Steitz: The splicing of U12-type introns can be a rate-limiting step in gene expression. *EMBO J*, 21(14), 3804-15 (2002)
66. Pessa, H. K., A. Ruokolainen & M. J. Frilander: The abundance of the spliceosomal snRNPs is not limiting the splicing of U12-type introns. *RNA*, 12(10), 1883-92 (2006)
67. Patel, A. A. & J. A. Steitz: Splicing double: insights from the second spliceosome. *Nat Rev Mol Cell Biol*, 4(12), 960-70 (2003)
68. Will, C. L. & R. Luhrmann: Splicing of a rare class of introns by the U12-dependent spliceosome. *Biol Chem*, 386(8), 713-24 (2005)
69. Tarn, W. Y. & J. A. Steitz: Pre-mRNA splicing: the discovery of a new spliceosome doubles the challenge. *Trends Biochem Sci*, 22(4), 132-7 (1997)
70. Burge, C. B., T. Tuschl & P. A. Sharp - Splicing of precursors to mRNAs by the spliceosomes. In: Gesteland, R.F., Cech, T. and Aktins, J.F. (eds), *The RNA World II*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1999)
71. Shukla, G. C. & R. A. Padgett: Conservation of functional features of U6atac and U12 snRNAs between vertebrates and higher plants. *RNA*, 5(4), 525-38 (1999)
72. Sashital, D. G., G. Cornilescu, C. J. McManus, D. A. Brow & S. E. Butcher: U2-U6 RNA folding reveals a group II intron-like domain and a four-helix junction. *Nat Struct Mol Biol*, 11(12), 1237-42 (2004)
73. Shukla, G. C. & R. A. Padgett: The intramolecular stem-loop structure of U6 snRNA can functionally replace the U6atac snRNA stem-loop. *RNA* , 7(1), 94-105 (2001)
74. Schneider, C., C. L. Will, O. V. Makarova, E. M. Makarov & R. Luhrmann: Human U4/U6.U5 and U4atac/U6atac.U5 tri-snRNPs exhibit similar protein compositions. *Mol Cell Biol*, 22(10), 3219-29 (2002)
75. Will, C. L., C. Schneider, M. Hossbach, H. Urlaub, R. Rauhut, S. Elbashir, T. Tuschl & R. Luhrmann: The human 18S U11/U12 snRNP contains a set of novel proteins not found in the U2-dependent spliceosome. *RNA*, 10(6), 929-41 (2004)
76. Wassarman, K. M. & J. A. Steitz: The low-abundance U11 and U12 small nuclear ribonucleoproteins (snRNPs) interact to form a two-snRNP complex. *Mol Cell Biol*, 12(3), 1276-85 (1992)
77. Frilander, M. J. & J. A. Steitz: Initial recognition of U12-dependent introns requires both U11/5' splice-site and U12/branchpoint interactions. *Genes Dev*, 13(7), 851-63 (1999)
78. Frilander, M. J. & X. Meng: Proximity of the U12 snRNA with both the 5' splice site and the branch point during early stages of spliceosome assembly. *Mol Cell Biol*, 25(12), 4813-25 (2005)
79. Kolossova, I. & R. A. Padgett: U11 snRNA interacts *in vivo* with the 5' splice site of U12-dependent (AU-AC) pre-mRNA introns. *RNA*, 3(3), 227-33 (1997)
80. Hastings, M. L., N. Resta, D. Traum, A. Stella, G. Guanti & A. R. Krainer: An LKB1 AT-AC intron mutation causes Peutz-Jeghers syndrome via splicing at noncanonical cryptic splice sites. *Nat Struct Mol Biol*, 12(1), 54-9 (2005)
81. Chang, W. C., Y. C. Chen, K. M. Lee & W. Y. Tarn: Alternative splicing and bioinformatic analysis of human U12-type introns. *Nucleic Acids Res*, 35(6), 1833-41 (2007)
82. Dietrich, R. C., G. C. Shukla, J. D. Fuller & R. A. Padgett: Alternative splicing of U12-dependent introns *in vivo* responds to purine-rich enhancers. *RNA*, 7(10), 1378-88 (2001)
83. Hastings, M. L. & A. R. Krainer: Functions of SR proteins in the U12-dependent AT-AC pre-mRNA splicing pathway. *RNA*, 7(3), 471-82 (2001)
84. Lewandowska, D., C. G. Simpson, G. P. Clark, N. S. Jennings, M. Barciszewska-Pacak, C. F. Lin, W. Makalowski, J. W. Brown & A. Jarmolowski: Determinants of plant U12-dependent intron splicing efficiency. *Plant Cell*, 16(5), 1340-52 (2004)
85. Scamborova, P., A. Wong & J. A. Steitz: An intronic enhancer regulates splicing of the twintron of Drosophila melanogaster prospero pre-mRNA by two different spliceosomes. *Mol Cell Biol*, 24(5), 1855-69 (2004)
86. Berget, S. M.: Exon recognition in vertebrate splicing. *J Biol Chem*, 270(6), 2411-4 (1995)
87. Buratti, E., M. Baralle & F. E. Baralle: Defective splicing, disease and therapy: searching for master checkpoints in exon definition. *Nucleic Acids Res*, 34(12), 3494-510 (2006)
88. Otake, L. R., P. Scamborova, C. Hashimoto & J. A. Steitz: The divergent U12-type spliceosome is required for pre-mRNA splicing and is essential for development in Drosophila. *Mol Cell*, 9(2), 439-46 (2002)
89. Buchner, D. A., M. Trudeau & M. H. Meisler: SCNM1, a putative RNA splicing factor that modifies disease severity in mice. *Science*, 301(5635), 967-9 (2003)

90. Levine, A. & R. Durbin: A computational scan for U12-dependent introns in the human genome sequence. *Nucleic Acids Res*, 29(19), 4006-13 (2001)
91. Bartosova, Z., K. Zavodna, T. Krivulcik, J. Usak, I. Mlkva, T. Kruzliak, J. Hromec, V. Usakova, I. Kopecka, P. Veres, Z. Bartosova & M. Bujalkova: STK11/LKB1 germline mutations in the first Peutz-Jeghers syndrome patients identified in Slovakia. *Neoplasma*, 54(2), 101-7 (2007)
92. Dietrich, R. C., M. J. Peris, A. S. Seyboldt & R. A. Padgett: Role of the 3' splice site in U12-dependent intron splicing. *Mol Cell Biol*, 21(6), 1942-52 (2001)
93. Smith, C. W.: Alternative splicing--when two's a crowd. *Cell*, 123(1), 1-3 (2005)
94. Letunic, I., R. R. Copley & P. Bork: Common exon duplication in animals and its role in alternative splicing. *Hum Mol Genet*, 11(13), 1561-7 (2002)
95. Ule, J., K. B. Jensen, M. Ruggiu, A. Mele, A. Ule & R. B. Darnell: CLIP identifies Nova-regulated RNA networks in the brain. *Science*, 302(5648), 1212-5 (2003)
96. Lejeune, F., Y. Cavaloc & J. Stevenin: Alternative splicing of intron 3 of the serine/arginine-rich protein 9G8 gene. Identification of flanking exonic splicing enhancers and involvement of 9G8 as a trans-acting factor. *J Biol Chem*, 276(11), 7850-8 (2001)
97. Rahman, L., V. Bliskovski, F. J. Kaye & M. Zajac-Kaye: Evolutionary conservation of a 2-kb intronic sequence flanking a tissue-specific alternative exon in the PTBP2 gene. *Genomics*, 83(1), 76-84 (2004)
98. Wollerton, M. C., C. Gooding, E. J. Wagner, M. A. Garcia-Blanco & C. W. Smith: Autoregulation of polypyrimidine tract binding protein by alternative splicing leading to nonsense-mediated decay. *Mol Cell*, 13(1), 91-100 (2004)
99. Lareau, L. F., M. Inada, R. E. Green, J. C. Wengrod & S. E. Brenner: Unproductive splicing of SR genes associated with highly conserved and ultraconserved DNA elements. *Nature*, 446(7138), 926-9 (2007)

Key Words: Alternative Splicing, U2-type intron, U12-Type Intron, U2 Spliceosome, hybrid intron, U12 Spliceosome, Splice Site Selection, Exon Skipping, Intron Retention, Review

Send correspondence to: Dr. Woan-Yuh Tarn, Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan, Tel: 8862-2652 3052, Fax: 8862-2782 9142, E-mail: wtarn@ibms.sinica.edu.tw

http://www.bioscience.org/current/vol13.htm