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RNA structure and splicing regulation*

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The process of pre-mRNA splicing is fundamental to gene regulation in eukaryotes. Specific RNA sequences called introns, which do not code for amino acids, are removed during pre-mRNA splicing, and flanking sequences are spliced together as exons forming mRNAs that are exported to the cytosol. While the spliceosome, a complex macromolecular machinery, helps form the catalytic core for splicing reaction, the transesterification reaction itself is catalyzed by the pre-mRNA molecule. The transesterification reaction as well as intermediates generated during pre-mRNA splicing are surprisingly similar to those generated during self-splicing group II introns that are generally present within lower organisms, including bacteria and yeast. In eukaryotes, multiple but different combinations of cis-elements and trans-acting are associated with the removal of different introns. Thus, each individual pre-mRNA is processed through a specific combination of sequence elements (cis-elements) and interacting proteins (trans-acting factors). Depending upon the relative expression of trans-acting factors, some sequences can be either recognized as exons or removed as introns, a process called alternative-splicing.

RNA structures capable of sequestering and/or exposing cis-elements play an important role in determining the outcome of pre-mRNA splicing. RNA structure also modulates backsplicing events that give rise to circular RNAs (circRNAs). In several instances, pre-mRNA splicing is intimately linked to transcription, which in turn is modulated by a variety of factors as well as stimuli. An increasing number of genetic diseases are now being associated with splicing defects either due to the mutations of the cis-elements, mutations in a small number of spliceosomal proteins or aberrant expression of splicing factors. Hence, understanding the diverse mechanisms of pre-mRNA splicing is essential to develop effective therapies of aberrant splicing-associated diseases.

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This special issue compiles several review articles highlighting the role of RNA structure in pre-mRNA splicing. In Chapter 1, Smathers and Robart outline the parallels between splicing mechanisms of group II and spliceosomal introns by comparing recent cryo-electron microscopy (cryo-EM)-generated structures of the spliceosomal catalytic core with the crystal structures of group II intron [1]. Chapter 2 (Shenasa and Hertel) focusses on aspects of the combinatorial control that governs the recognition of exons [2]. As an example of RNA structures ruling the most complex spliced gene, Jin et al. (Chapter 3) discuss the identification, evolution and regulatory roles of RNA secondary structures in regulation of alternative splicing of the mutually exclusive exons of the *Drosophila melanogaster Down syndrome cell adhesion molecule (Dscam1)* gene [3]. In Chapter 4 (Lisowiec-Wachnicka et al.) summarizes the currently known types of structural motifs associated with regulation of alternative splicing [4]. Here, the authors also introduce the recently coined term “RNA structurome”, which refers to a broader role of RNA in gene regulation. U1 snRNP is a component of the spliceosome that performs the first step in exon recognition. In Chapter 5, Schaal et al. outline the importance and the limitations of the U1 snRNP recruitment to the 5' splice site (5'ss) and describe factors that regulate exon recognition in addition to the RNA:RNA duplex formed between the 5'ss and the U1 snRNA that regulates exon recognition [5].

Recent reports reveal abundant expression of circRNAs generated by backsplicing in human cells. In Chapter 6, Dmitri Pervouchine describes how topology impacts formation of circRNAs [6]. He also talks about instances when backsplicing events do not result into the circularization. Chapter 7 (Welden and Stamm) discusses the role of specific RNA structures in the generation of circRNAs in humans [7]. Chapter 8 (Andrews and Moss) summarizes the computational approaches to determine regulatory RNA structures that modulate alternative splicing [8]. These approaches are particularly useful for predicting unique structures formed by long-range base-pair interactions. In Chapter 9, Berglund et al. describe the role of the expanded repeat-RNA structures in aberrant splicing that leads to diseases [9]. They emphasize how depletion of splicing factors due to their sequestration by RNA structures formed by repeats could lead to the pathological conditions. Spinal muscular atrophy (SMA) is caused by the deletion of or mutations in *Survival Motor Neuron 1 (SMN1)* gene. *SMN2*, a nearly identical copy of *SMN1*, cannot compensate for the loss of *SMN1* due to skipping of exon 7. Chapter 10 (N.N. Singh and R.N. Singh) describes the role of RNA structures that dictates the usage of *SMN2* exon 7 [10]. Importantly, small compounds have potential to modulate *SMN2* exon 7 splicing through interaction of stem-loop structure (TSL2) sequestering the 5'ss of exon 7 [10]. In addition, the *SMN* pre-mRNA can form circular RNAs, due to local secondary structures [10].

Neil and Fairbrother (Chapter 11) describe the role of intronic RNA in post-transcriptional regulation [11]. Giudice et al. (Chapter 12) discuss ways to modulate alternative splicing, which has been impacted by mutations or loss of critical RNA Binding Proteins (RBPs), for therapeutic applications [12]. Chapters 13 and 14 (D. Baralle et al. and D. Elliot et al.) focus on the role of aberrant splicing in ciliopathy and lung cancer, respectively [13,14]. This line of research has a translational aspect as clinical genetic diagnosis of splicing defects allows the development of possible therapeutic targets/approaches applying principles indicated in Chapter 12. In addition, these studies contribute to a basic understanding of splicing, since

mutations frequently uncover novel regulatory elements in RNA processing. Oroz and Laurents (Chapter 15) describe general structural features of RBPs [15]. The structure of RBPs is an important player not only in normal function but also in pathological situations. The structural pathology born with the prion discovery is extending to RBPs in many neurodegenerative diseases, they present unusual protein aggregation and phase transitions, many times without specific mutations but induced by external factors like oxidative stress. Chapter 16 (Kielkopf et al.) focuses on the structure of SF3b1, one of the components of the Spliceosome Factor 3b (SF3B) complex and part of the U2 snRNP [16]. Finally, Ruth Sperling (Chapter 17) discusses the role of small non-coding RNAs in alternative splicing [17].

The guest editors would like to express their gratitude to contributors for bringing their valuable insights towards a better understanding of alternative splicing. Splicing is a complex process involving hundreds of proteins and even greater number of cis-elements as well as RNA structural elements. Because of the dynamic nature of the spliceosomal assembly, various aspects of splicing regulation are still not predictable. We hope that topics covered in this special issue will stimulate the ongoing dialogue on alternative splicing regulation mechanisms.

Chapter Number (Reference No.)	Manuscript Number	Manuscript Title	Corresponding Author
		Preface	Singh, Baralle, Stamm
1	BBAGRM_2018_426	The mechanism of splicing as told by group II introns: ancestors of the spliceosome	Aaron Robart
2	BBAGRM_2018_434_R1	Combinatorial Regulation of Alternative Splicing	Klemens Hertel
3	BBAGRM_2018_433_R1	Role of RNA secondary structures in regulating Dscam alternative splicing	Yongfeng Jin
4	BBAGRM_2019_103	The regulation properties of RNA secondary structure in alternative splicing	Jolanta Lisowiec-W chnicka
5	BBAGRM_2018_424_R1	Context matters: Regulation of splice donor usage	Heiner Schaal
6	BBAGRM_2018_427_R1	Circular exonic RNAs: when RNA structure meets topology	Dmitri Pervouchine
7	BBAGRM_2019_173	Pre-mRNA structures forming circular RNAs	Stefan Stamm
8	BBAGRM_2018_429_R1	Computational approaches for the discovery of splicing regulatory RNA structures	Walter Moss
9	BBAGRM_2018_440_R1	Repeat-Associated RNA Structure and Aberrant Splicing	Andrew Berglund
10	BBAGRM_20184_23	How RNA structure dictates the usage of a critical exon of spinal muscular atrophy gene	Ravindra Singh
11	BBAGRM_2019_296	Intronic RNA: Ad'junk' Mediator of Post-Transcriptional Regulation	William Fairbrother
12	BBAGRM_2018_435_R1	More than a messenger: Alternative splicing as a therapeutic target	Jimena Giudice

Chapter Number (Reference No.)	Manuscript Number	Manuscript Title	Corresponding Author
13	BBAGRM_2019_88_R1	Splicing in the pathogenesis, diagnosis and treatment of ciliopathies	Diana Baralle
14	BBAGRM_2018_437	Alternative Splicing in Lung Cancer	David Elliott
15	BBAGRM_2018_430_R1	RNA Binding Proteins: Diversity from Microsurgeons to Cowboys	Douglas Laurents
16	BBAGRM_2018_432	Structures of SF3b1 Reveal a Dynamic Achilles Heel of Spliceosome Assembly: Implications for cancer-associated abnormalities and drug discovery	Clara Kielkopf
17	BBAGRM_2018_421_R1	Small non-coding RNA within the endogenous spliceosome and alternative splicing regulation	Ruth Sperling

Biographies

Francisco E Baralle is the Lead Scientist at the RNA Metabolism Group at Italian Liver Foundation in Trieste, Italy. He gained his BSc and PhD in Chemistry at the University of Buenos Aires, Argentina and completed his degree in Medicine and Surgery at the University of Naples, Italy. In 1974, he moved to the MRC Laboratory of Molecular Biology, Cambridge, UK, where he worked in the Division directed by Nobel Laureate Dr. Frederick Sanger. In 1980, he became an elected member of the European Molecular Biology Organization (EMBO). In 1993, he was awarded the Platinum Konex Prize for Science and Technology (Argentina) as the best scientist of the decade in Genetic and Cytology. In 2010 he was elected member of The World Academy of Sciences (TWAS), in 2014 he was awarded an MD Honoris Causae by the Faculty of Medicine of Montevideo and 2017 was elected member of the Academia Latinoamericana. In 1990, he was appointed Director of the Trieste Component of the International Center for Genetic Engineering and Biotechnology (ICGEB) and between 2004 and 2014 was the Director-General of ICGEB. Prof. Baralle is credited with several seminal studies, including the first complete sequencing of a mRNA (beta-globin) and uncovering the novel mechanisms of alternative splicing regulation. More recently, he uncovered the role of TDP43, a protein frequently present in the inclusions of the Amyotrophic Lateral Sclerosis (ALS) brain, in splicing regulation.

Ravindra N Singh is a Professor of Biomedical Sciences at Iowa State University, Ames, Iowa, USA. He received his B.Sc. (Chemistry Honors) in 1983 and M.Sc. (Biochemistry) in 1985 from Banaras Hindu University, Varanasi, India. He obtained his Ph.D. (Biochemistry) in 1993 from Russian Academy of Sciences, Pushchnino, Russia. During his graduate training in Russia, he purified and characterized several recombinant proteins expressed in *E. coli*. As a postdoctoral fellow in the laboratory of Dr. Kapil Mehta at M.D. Anderson Cancer Center, Houston, Texas (USA) from 1993 to 1995, he purified and characterized a rare transglutaminase from the filarial parasite *Brugia malayi*. During his second postdoctoral training in the laboratory of Dr. Theo Dreher at the Oregon State University, Corvallis, Oregon (USA) from 1995 to 1998, he characterized RNA motifs required for the RNA dependent RNA polymerase activity of the Turnip Yellow Mosaic Virus (TYMV). During his third and the last postdoctoral training in the laboratory of Dr. Alan Lambowitz at the University of Texas, Austin, Texas (USA) from 1998 to 2001, he characterized novel RNA motifs critical for the protein-dependent group II intron splicing. From 2001 to 2007, Dr. Singh was a tenure-track Assistant Professor at the University of Massachusetts Medical School (UMASS), Worcester, Massachusetts (USA). As an independent investigator, he chose to work on uncovering the mechanism(s) of alternative splicing of exon 7 of Survival Motor Neuron (SMN) genes that are generally associated with the spinal muscular atrophy (SMA), which is one of the leading genetic diseases of children and infants. While at UMASS, his team demonstrated the feasibility of the *in vivo* selection of an entire exon and discovered intronic splicing silencer N1 (ISS-N1) as well as the inhibitory terminal stem-loop 2 (TSL2). For the discovery of ISS-N1, Dr. Singh received 2006 Presidential Early Career Award for Scientists and Engineers (PECASE), which is the highest award given by the United States of America and The White House to the promising young investigators. Discovery of ISS-N1 led the development of nusinersen (Spinraza™), which became the first approved drug for the treatment of SMA. In 2007, he moved to Iowa State University (ISU), Ames, Iowa (USA) as a tenured Associate Professor. In 2012, he was promoted to the rank of the Full Professor. While at ISU, his group established a novel multi-exon-skipping detection assay (MESDA) and discovered a unique RNA structure formed by a long-distance interaction. His team also identified novel SMN exons and discovered a huge repertoire of SMN circular RNAs (circRNAs). In addition, his team characterized sequence and structural determinants of the RNA-SMN interactions.

Stefan Stamm is a Professor of Biochemistry at the University of Kentucky, Lexington, Kentucky, USA. His work centers on alternative splicing and disease, investigating the role

of the splicing factor tra2-beta1 in exon enhancer complexes, the function of unusual C/D box snoRNAs (SNORD115) in alternative splicing of the serotonin receptor 2C, the discovery of the YTH-domain that recognizes N6-methyl adenosine and recently the discovery of circular RNAs from the human tau locus. Currently he is testing the role of snoRNAs in Prader-Willi syndrome and the contribution of tau circular RNAs to frontotemporal dementia and Alzheimer's disease. Stefan Stamm received his Diploma in Biochemistry from the University of Hannover, Germany in 1988. As his Ph.D. work he studied neuron-specific alternative splicing at Mount Sinai Hospital, New York and Cold Spring Harbor Laboratory from 1989 to 1992, followed by a two year Post-doc with David Helfman at the Cold Spring Harbor Laboratory, again focusing on alternative splicing in the brain. From 1995 to 2001 he was an Independent group leader at the Max-Planck Institute for Neurobiology in Munich, Germany followed by an associate professorship at the University of Erlangen-Nuremberg, Germany, that ended in 2007, when he moved to Lexington Kentucky, USA.

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