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# Journal of Integrated OMICS

a methodological journal

# JIOMICS

## Journal of Integrated OMICS

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Journal of Integrated OMICS, JIOMICS, provides a forum for the publication of original research papers, preliminary communications, technical notes and critical reviews in all branches of pure and applied "-omics", such as genomics, proteomics, lipidomics, metabolomics or metallomics. The manuscripts must address methodological development. Contributions are evaluated based on established guidelines, including the fundamental nature of the study, scientific novelty, and substantial improvement or advantage over existing technology or method. Original research papers on fundamental studies, and novel sensor and instrumentation development, are especially encouraged. It is expected that improvements will also be demonstrated within the context of (or with regard to) a specific biological question; ability to promote the analysis of molecular mechanisms is of particular interest. Novel or improved applications in areas such as clinical, medicinal and biological chemistry, environmental analysis, pharmacology and materials science and engineering are welcome.

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# Special Issue
# Proceeding Abstracts of the 1st International Caparica Conference in Splicing (SPLICING 2016)

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JOURNAL OF INTEGRATED OMICS

*A methodological Journal*

# SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1st INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# RBM4 promotes neuronal cell differentiation by modulating alternative splicing of signaling factors

W.-Y. Tarn, D. Dhananjaya, C.-H. Su, H.-C. Kuo, S.-I. Yu

Institute of Biomedical Sciences, Academia Sinica, 128 Academia Road, Sec. 2, Nankang, Taipei, Taiwan

**Available Online**: 31 December 2016

## ABSTRACT

**Purpose:** RBM4 plays important roles in cell differentiation and cancer progression. We have previously reported that RBM4 promotes muscle and pancreas cell differentiation via its splicing regulatory activity (J. Cell Biol., 2011; Mol. Cell. Biol., 2013). We also investigated the role of RBM4 in neuronal differentiation and migration.

**Experimental description:** We used mouse embryonal carcinoma P19 cells, mouse neuronal progenitor cell lines, and human mesenchymal cells to investigate the role of RBM4 in neuronal cell differentiation. We also examined the role of RBM4 in neuronal cell migration during cortical development.

**Results:** Using mouse P19 cells as a neural differentiation model, we found that RBM4 modulates alternative splicing of the cell-fate determining factor Numb and thereby affects the expression of neuronal differentiation factor Mash1. Accordingly, ectopic RBM4 expression in neuronal progenitor cells increased Mash1 expression and promoted cell differentiation. RBM4 is also essential for neurite outgrowth of cortical neurons *in vitro*. RBM4-induced Numb isoforms can rescue neurite outgrowth defects of RBM4-depleted neurons. The above results demonstrate that RBM4 promotes the expression of specific Numb isoforms, which contribute to neuronal cell differentiation and neurite outgrowth (Mol. Biol. Cell, 2016). More recently, we found that RBM4 could promote neuronal differentiation of mesenchymal stem cells by modulating cell metabolic status as well as promote cell migration. We have identified the targets of RBM4 that are responsible for RBM4's functions in neuron.

**Conclusions:** We demonstrated that RBM4, through alternative splicing of signaling factors, executes its functions neuronal cell differentiation and cell migration.

**Keywords:** alternative splicing, neuronal cell differentiation, glucose metabolism, neuronal migration.

**Correspondence:** Woan-Yuh Tarn, Ph.D. Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; E-mail: wtarn@ibms.sinica.edu.tw.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# The RNA-binding protein Arrest/Bruno regulates fibrillar myogenesis in *Drosophila*

M. L. Spletter[1], C. Barz[1], A. Yeroslaviz[1], O. Nikonova[1], C. Schönbauer[1], B. H. Habermann[1,2], F. Schnorrer[1,2]

[1] Muscle Dynamics group. Max Planck Institute of Biochemistry. Am Klopferspitz 18. 82152. Martinsried. Germany. [2] IBDM—Institut de Biologie du Développement de Marseille. UMR 7288 Case 907. Parc Scientifique de Luminy. 13288 Marseille Cedex 9. France.

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## ABSTRACT

*Drosophila*, like vertebrates, has multiple types of muscles with different functional properties. In adult *Drosophila*, fibrillar muscles power flight, while tubular muscles coordinate jumping and walking. These muscle types differ both in patterns of gene expression and alternative splicing, but the mechanisms generating this diversity are not well understood. Our data shows that CELF-family member Arrest/Bruno controls flight-muscle specific differentiation by regulating an alternative splicing program downstream of Spalt Major (Salm). Salm regulates a diverse set of genes that determine fibrillar muscle physiology, and Arrest targets are responsible for proper growth and maturation of the sarcomere. Loss of Arrest results in hypercontraction due to missplicing of structural components including Strn-Mlck, Mhc, Up and WupA (TnI), leading to loss of muscle fibers in adult flies. As Arrest and its sarcomeric targets are evolutionarily conserved, similar principles may regulate mammalian muscle morphogenesis.

**Purpose:** Here we investigate the genetic mechanisms underlying the differentiation of muscle types, in particular the regulation of alternative splicing during muscle-type specific development.

**Experimental description:** In this study, we used mRNA-Seq to identify differences in gene expression and exon usage between wild-type and flies with a loss-of-function of either Salm or Arrest. Building on these results, we use fly genetics and confocal microscopy to characterize muscle phenotypes and verify expression patterns.

**Results:** We show that more than 200 fibrillar-specific genes are regulated by Salm. Strikingly, we also observe that Salm promotes a fibrillar-specific program of alternative splicing. Nearly 80% of the fibrillar specific exons regulated by Salm are also regulated by Salm target RNA-binding protein Arrest/Bruno. We verify regulation of fibrillar-specific isoform expression using fosmid-GFP reporters. Functionally, loss of Arrest causes a failure in sarcomere maturation and a misregulation of myosin function resulting in flightlessness, hypercontraction and muscle loss.

**Conclusions:** We identify Arrest/Bruno as the key fibrillar muscle specific splicing factor downstream of Salm, and show that the splicing program regulated by Arrest is essential for sarcomere growth and maturation as well as muscle function.

**Keywords:** CELF, alternative splicing, Arrest/Bruno, muscle development, sarcomerogenesis, *Drosophila*.

**Acknowledgements:** We thank Anne Ephrussi for reagents and the Bloomington and VDRC stock centres for fly stocks. We are grateful to Reinhard Fässler for generous support and to Bettina Stender for technical assistance. Our work was funded by the Max Planck Society, Humboldt, EMBO (688-2011 and YIP), NIH (5F32AR062477), HFSP and ERC (FP/2007-2013 310939).

**Correspondence:** Am Klopferspitz 18, 82152 Martinsried, Germany. spletter@biochem.mpg.de

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Insights into protein splicing reaction steps by means of computational chemistry

J. I. Mujika

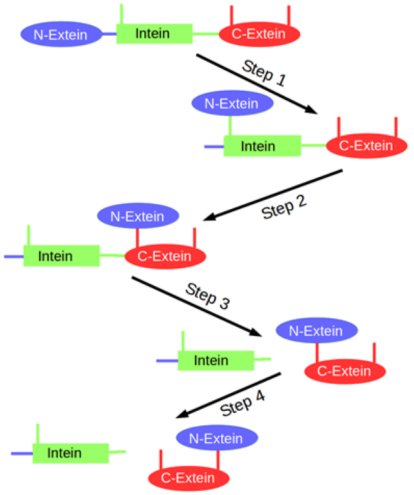
Kimika Fakultatea, University of the Basque Country (UPV/EHU) and Donostia International Physics Center (DIPC), San Sebastian (Basque Country, Spain)

**Available Online**: 31 December 2016

## ABSTRACT

Protein splicing is a post-translational process in which a biologically inactive protein is activated by the release of a segment denoted as intein and by the joining of the two flanking segments, the exteins. [1] The process is auto-catalytic, what makes inteins appealing for many applications in biotechnology, bioengineering, or medicine. Therefore, a full understanding of its underlying mechanism is crucial for an optimal application of the process in any of these areas. The canonical mechanism of the process is well established and includes four steps (see Figure). However, how each step is chemically modulated is not fully understood, and more importantly, the specific roles played by the essential amino acids are still to be elucidated.

The research presented herein was carried out by means of computational chemistry. This field of the chemistry describes the system into an atomic level and therefore it is a very useful alternative to complement the experimental studies and get access into an information difficult to achieve by other techniques. The results [2,3] reveal a detailed description of the key steps and provide a comprehensible explanation of the catalytic roles played by the conserved amino acids.

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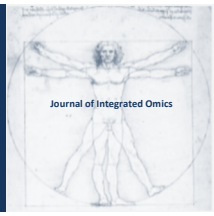
**Correspondence:** joni.mujika@ehu.eus

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# Tumor-specific modulation of alternative splicing by small molecules that target SF3B1

Gang Wu, Liying Fan, Philip M. Potter

Departments of Chemical Biology and Therapeutics and Computational Biology. St Jude Children's Research Hospital. Memphis. TN, 38105. USA

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## Abstract

**Purpose:** Recently several different classes of small molecules have been identified that interact with SF3B1. These agents induce changes in alternative splicing RNA (AS) that appear to be tumor-specific. We seek to understand the mechanism and consequences of such events in both tumor and normal cells.

**Experimental description:** Tumor cells were exposed to different agents that interact with SF3B1 and the effects on RNA splicing and the proteome have been documented. This was accomplished using a novel analysis pipeline designed to identify and validate AS isoforms in RNAseq datasets, and to correlate this data with proteomic analyses of the same samples.

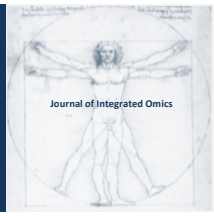
**Results:** Using our RNAseq analysis pipeline, we have developed approaches to detect, identify and quantitate unique RNA species present in datasets obtained from drug- treated cells. These results indicate that different SF3B1 targeting agents induce different AS events, in a dose- and time-dependent manner.

**Conclusions:** Our results demonstrate that the cellular responses to different spliceosome targeting agents differ, even though they interact with the same component of this complex. Additionally, RNAs obtained from AS are translated into proteins of likely unique function. As a consequence, the latter are likely to significantly modulate cellular function.

**Keywords:** SF3B1, alternative splicing, RNAseq.

**Acknowledgements:** This work was supported in part by an NIH Cancer Center Core grant CA21765, and by the American Lebanese Syrian Associated Charities (ALSAC) and St. Jude Children's Research Hospital (SJCRH).

**Correspondence:** Philip M. Potter, Department of Chemical Biology and Therapeutics, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, United States E-mail: phil.potter@stjude.org

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Detrimental exon skipping of immunoglobulin transcripts in plasma cells: towards a new splicing therapy in the treatment of plasma cell disorders

Nivine Srour, Mohamad Omar Ashi, Christophe Sirac, Michel Cogné and Laurent Delpy [1]

[1] Université de Limoges - CNRS UMR 7276, Limoges, France

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## Abstract

Aberrantly rearranged immunoglobulin (Ig) genes are frequent and usually considered sterile and innocuous due to nonsense-mediated mRNA decay. Alternative splicing can however yield internally deleted proteins from such nonproductively V(D)J-rearranged loci.

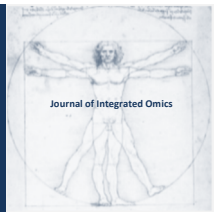
We show that nonsense codons from variable (V) Ig exons promote exon-skipping and synthesis of V domain-less Ig kappa light chains. The expression of such truncated-Ig lacking V domain impaired plasma cell differentiation and antibody responses. Truncated-Ig have intrinsic toxic effects and induce ER stress-associated apoptosis in plasma cells (PCs). Altogether, we identify a "truncated-Ig exclusion" (TIE) checkpoint dampening PC differentiation by eliminating cells expressing non-functionally rearranged Ig alleles. The TIE-checkpoint thus mediates selection of long-lived PCs with limited ER stress supporting high Ig secretion (Srour et al, J Exp Med 2016).

Based on these results, a patent was applied to increase the production of truncated-Ig lacking variable domain using antisense oligonucleotides (AON). This exon skipping therapy could open new avenues for plasma cell neoplasms (including multiple myeloma) treatment.

**Keywords:** Exon skipping, Immunoglobulin, Antisense oligonucleotides, Splicing therapy.

**Acknowledgements:** This work was supported by grants from Fondation ARC (#PGA120150202338 and SFI20121205821), Ligue Contre le Cancer (comité Haute-Vienne), Comité d'Organisation de la Recherche sur le Cancer du Limousin (CORC) and Fondation pour la Recherche Médicale.

**Correspondence:** Laurent Delpy (laurent.delpy@unilim.fr), CNRS UMR 7276, 2 rue du Dr. Marcland, F-87025 Limoges, France, Fax # 33 555 435 897; Phone # 33 519 564 214

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Going Deep: Ultra-sensitive Computational Detection of Splicing Variation from a Single and from Multiple RNA-seq Samples

L. D. Florea [1]

[1] McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, U.S.A.

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## Abstract

**Purpose:** The growing volume of RNA-seq data represents a vast resource that holds clues to gene alternative splicing. However, identifying splicing variation at great depth has been difficult: current transcript assembly methods can identify the major isoforms with very high accuracy at the expense of minor variants, or are sensitive but highly imprecise. Artifacts such as intronic reads, alignments to paralogs, and spurious matches can significantly affect the prediction. Cross-analyzing multiple data sets can provide additional power to distinguish signal from noise, however, all practical tools to date are designed to analyze one sample at a time.

**Experimental description:** Our group has developed tools for comprehensive detection and characterization of alternative splicing variation from RNA-seq data. We present two programs, the single-sample transcript assembler CLASS2 [1] and the multi-sample intron selector JULiP. CLASS2 uses read coverage information in a linear program to infer exons and build a splice graph, then selects a subset of paths in the graph using dynamic programming. JULiP employs a linear program to model intron inclusion rates and takes advantage of latent information in multiple samples to more comprehensively and accurately identify the true introns.

**Results:** CLASS2 has increased accuracy compared to other reference methods, on both simulated and real data, and can find up to twice as much splice variation compared to Cufflinks, at comparable or only slightly lower precision. It also incorporates an intronic noise filter, which makes it particularly well suited for analysis of rRNA-depleted libraries, in particular from FFPE samples, and to identify intron retention events. When applied to multi-sample RNA-seq sets, JULiP has significantly increased sensitivity, close to 90%, >30% higher compared to a single sample and >12% more than the cumulative set from all samples, whereas its precision is higher or comparable to the best of the programs. Additionally, JULiP is scalable and takes only over 1 min to analyze 100 RNA-seq data sets on a multi-computer cluster.

**Conclusions:** We described a suite of tools (http://sourceforge.net/projects/Splicebox) for accurate in depth detection of splice variation in single and multiple RNA-seq samples. Our methods are fast, scalable and accurate and can be effectively used to create comprehensive catalogs of splicing variation from the vast and varied collections of RNA-seq experiments.

**Keywords:** RNA-seq, alternative splicing, transcript assembly, linear programming.

**Acknowledgements:** NSF grants ABI-1356078 and IOS-1339134.

**References:**

[1] Song, L., S. Sabunciyan, L. Florea. (2016) CLASS2: Accurate and efficient splice variant annotation from RNA-seq reads, *Nucl. Acids Res.*, pii: gkw158 (Advance access).

**Correspondence:** 1900 E Monument St, Welch 113, Baltimore, MD 21205, USA. Email: florea@jhu.edu

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# The interplay between mRNA translation and nonsense-mediated decay in transcripts with short open reading frames

C. Onofre[1,2], J. Menezes[1,2], I. Peixeiro[1,2], C. Barbosa[1,2], L. Romão[1,2]

[1] Department of Human Genetics, Instituto Nacional de Saúde Doutor Ricardo Jorge, 1649-016 Lisboa, Portugal. [2] Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal.

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## Abstract

Mammalian nonsense-mediated mRNA decay (NMD) is a splicing- and translation-dependent surveillance pathway that recognizes and selectively degrades mRNAs carrying premature termination codons (PTCs). In addition, several studies have also implicated NMD in the regulation of steady-state levels of physiological mRNAs, and examples of natural NMD targets are transcripts containing upstream short open reading frames or long 3' untranslated regions.

The strength of the NMD response appears to reflect multiple determinants on a target mRNA. In general, the location of a PTC greater than 50 nucleotides upstream to the last exon-exon junction constitutes a major determinant of NMD. However, we have reported that human mRNAs with a PTC in close proximity to the translation initiation codon (AUG-proximal PTC), and thus, with a short open reading frame, can substantially escape NMD. Our data support a model in which cytoplasmic poly(A)-binding protein 1 (PABPC1) is brought into close proximity with an AUG-proximal PTC via interactions with the translation initiation complexes. This proximity of PABPC1 to the AUG-proximal PTC allows PABPC1 to interact with eRF3 with a consequent enhancement of the release reaction and repression of the NMD response. Here, we present strong evidence that the eIF3 is involved in delivering eIF4G-associated PABPC1 into the vicinity of the AUG-proximal PTC. In addition, we dissect the biochemical interactions of the eIF3 subunits in bridging PABPC1/eIF4G complex to the 40S ribosomal subunit. Together, our data provide a framework for understanding the mechanistic details of PTC definition and translation initiation.

**Keywords:** Nonsense-mediated mRNA decay; translation; premature termination codon (PTC).

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**Correspondence:** Departamento de Genética Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge, Av. Padre Cruz, 1649-016 LISBOA, PORTUGAL; luisa.romao@insa.min-saude.pt.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# The role of splicing factor gene mutation in craniofacial disorders

Moses Tandiono, Huw Thomas, Robert Baines, Cortland Linder, Kathryn Hentges, William Newman and Raymond O'Keefe

Faculty of Life Sciences and Manchester Centre for Genomic Medicine, The University of Manchester, United Kingdom

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## ABSTRACT

**Purpose:** The related craniofacial disorders Burn-McKeown Syndrome (BMKS) and MandibuloFacial Dysostosis Guion-Almeida type (MFDGA) are characterized by mandibular and malar hypoplasia, microcephaly, choanal atresia, external ear anomalies and other variable craniofacial and developmental defects. We have recently found that mutations that reduce expression of the U5 snRNP gene TNXL4A (DIB1) cause BMKS1. Interestingly, reduced expression of the U5 snRNP gene EFTUD2 (SNU114) causes MFDGA providing a strong link between U5 snRNP function and these craniofacial disorders, as well as a possible functional link between the Dib1 and Snu114 proteins during splicing. The mechanisms by which reduced expression of essential splicing factor genes, required for splicing all pre-mRNAs, bring about these particular craniofacial disorders are unclear. Our hypothesis is that reduced expression of DIB1 and SNU114 partially disrupts spliceosome function, resulting in missplicing of a subset of pre-mRNAs required during craniofacial development.

**Experimental description:** To address this hypothesis we have initially used yeast models of BMKS and MFDGA.

**Results:** Reduced expression of DIB1 and SNU114 in yeast does indeed cause missplicing of some pre-mRNAs but not others. In addition, we have found that reduced expression of DIB1 and SNU114 cause defects in snRNP assembly, including defects in tri-snRNP formation. We are currently investigating whether there is a functional link between Dib1 and Snu114 within the spliceosome. Growth of yeast models under a variety of conditions has identified conditions that induce ER stress as affecting growth of yeast models more than wild type cells. Related to ER stress sensitivity we have identified the intron containing gene, CNB1, as being particularly susceptible to reduced DIB1 and SNU114 expression. CNB1 codes for Calcineurin B, the regulatory subunit of calcineurin, a Ca++/calmodulin-regulated type 2B protein phosphatase. Calcineurin is important in the signaling pathway that promotes cell survival under stress.

**Conclusions:** Reduced levels of Calcineurin cause ER stress, which in turn induces apoptosis. Apoptosis of neural crest cells, at certain times and locations during development, is critically important for proper craniofacial development. We are now investigating whether patient cells are more sensitive to ER stress and setting up mouse models to determine exactly how reduced DIB1 and SNU114 expression leads to BMKS and MFDGA.

**Keywords:** U5 snRNP, yeast, Snu114, Dib1, human disease.

**Acknowledgements:** This work is funded by the BBSRC.

**Correspondence:** Faculty of Life Sciences, The University of Manchester, Michael Smith building, Oxford Road, Manchester, M13 9PT, UK. E-mail: rokeefe@manchester.ac.uk.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Transcriptional interference in human cells: exonization and intron retention

S. Raudsepp, M. Kanemägi, M. Speek

Department of Gene Technology. Faculty of Science. Tallinn University of Technology. Akadeemia tee 15. Tallinn 12618. Estonia

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## ABSTRACT

**Purpose:** Recent data suggest that retroelement insertions into exons and introns of genes induce different types of genetic disease, including cancer. Among other effects, retroelements interfere with the expression of genes by inducing alternative splicing via exon skipping and exonization using cryptic splice sites. These molecular effects may be explained by transcriptional interference (TI) and exon definition in RNA splicing. We have studied TI effects induced by L1 retrotransposon in the human *NCAM1* locus.

**Experimental description:** We analyzed the nucleosome occupation in *NCAM1* exon-intron 9 by mapping micrococcal nuclease accessible sites. To reveal TI effects, RNA polymerase II (RNAP II) pausing sites and "transcription bubble" locations, we used chromatin immunoprecipitation and probing with potassium permanganate coupled with ligation-mediated PCR.

**Results:** Our results show that changes in nucleosome positioning could result in changes in TI, that is intron retention and polyadenylation, induced by L1. In the analysis of the selected gene, we determined the locations of transcription bubbles and RNAP II occupancy upstream to the L1. These results show that RNAP II transcriptional pausing takes place at discrete regions in accordance with experimentally determined locations of transcription bubbles.

**Conclusions:** We conclude that nucleosome occupation plays a key role in the TI induced by L1 retrotransposon. The two TI effects (intron retention and cryptic polyadenylation) observed in our analysis are consistent with RNAP II pausing in the intron upstream to L1. Therefore, we believe that intronic L1 affects RNAP II elongation by regulating gene transcription through "sitting-duck" and/or roadblock mechanism.

**Keywords:** Transcriptional interference, L1 retrotransposon, intron retention, nucleosome occupation.

**Correspondence:** Mart Speek, Dept. of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, rm 129, Tallinn 12618, Estonia. Email: mart.speek@ttu.ee.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Intein PRP8 in pathogenic fungi: evolutionary aspects and splicing evaluation

Eduardo Bagagli[1], Hans Garcia-Garces[1] and Raquel Cordeiro Theodoro[2]

[1] Dept of Microbiology and Immunology, IBB-UNESP, Botucatu, SP, Brazil. [2] Dept of Cellular Biology and Genetics, UFRN, Natal, RN, Brazil

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## ABSTRACT

Inteins are intervening DNA sequences that are translated in frame with the host coding gene and then self-excised through protein splicing, which also ligates the N and C terminals of the host protein by peptide bonds, restoring its functionality. Besides their splicing domain, inteins might also contain an internal homing endonuclease (HE) domain, making them mobile elements. Inteins are usually found at conserved sites of housekeeping proteins that have vital functions in the cell, such as DNA and RNA polymerases, aminoacyl tRNA synthetases, recombinases, topoisomerases, helicases and essential components of the spliceosome, including the protein Prp8. Some extant hypotheses for this distribution are supported by the mobility of inteins by the homing cycle, while the inteins have been considered sophisticated parasitic genetic elements. One example of a domesticated intein is well known in Saccharomyces cerevisiae, namely the HO gene that has gained a well-defined function in the yeasts, by acting as site-specific endonuclease that initiates mating-type interconversion. Some recent works also indicate a possible function for inteins in the post-translational regulation of gene expression. Nevertheless, we are far from a complete understanding of why inteins have persisted in different housekeeping host proteins, mainly in unicellular organisms, over millions of years. For practical purposes, inteins proved to be valuable not only for protein biotechnology and phylogenetic studies, but also promising as a therapeutic drug target.

The PRP8 intein occurs in the protein Prp8, the main component of eukaryotic spliceosome, implicated in the editing of pre-messenger RNAs. This intein has been found in important fungal pathogens, including *Cryptococcus neoformans* (Basidiomycota, Tremellales), *Aspergillus fumigatus* (Ascomycota, Eurotiales), several members of the families *Artrodermataceae* and *Ajellomycetaceae* (Ascomycota, Onygenales), such as the dermatophytes species, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Emmonsia parva*, *Paracoccidioides brasiliensis* and *P. lutzii*. In fact, the PRP8 intein profiles, which might occur in a mini (containing just the splicing domain) or full condition (containing the splicing and the HE domains) in these fungal lineages, have been used as a simple and efficient molecular marker for phylogenetically defining and/or distinguishing the related and cryptic species that occur in these fungal groups.

The splicing function of the PPR8 inteins from *B. dermatitidis*, *E. parva*, *P. brasiliensis* and *P. lutzii* has been evaluated by our group, in a non-native recombinant protein context by inserting the intein between a Maltose Binding Protein and a Thioreodoxin, in E. coli cells. All PRP8 inteins proved to be active in this model, suggesting their usefulness for screening drugs that inhibit the intein excision in these important fungal pathogens.

**Keywords:** Intein, PRP8, spliceosome, self-splicing, Onygenales fungi

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**Correspondence:** Eduardo Bagagli, Dept Microbiol and Imunol, IBB/UNESP, Botucatu, SP, Brazil. CEP 18.618-970.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Epithelial splicing regulatory proteins ESRP1 and ESRP2 correlate with splice signatures and outcome in human colorectal cancer

Klaus Holzmann[1], Abigail J. Deloria1, Philip Kienzl[1], Justyna Łopatecka[1], Lingeng Lu[2], Brigitte Marian[1], Stefan Stättner[3]

[1] Division of Cancer Research, Department of Medicine I, Comprehensive Cancer Center, Medical University Vienna, Borschkegasse 8a, 1090 Vienna, Austria. [2] Department of Chronic Disease Epidemiology, Yale School of Public Health, School of Medicine, Yale Cancer Center, Yale University, 60 College Street, New Haven, CT 06520-0834 USA. [3] Department of Surgery, Paracelsus Medical University Salzburg, Salzburg, Austria.

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## Abstract

**Purpose:** Master splice regulators ESRP1 and ESRP2 are implicated in alternative mRNA splicing programs important for epithelial-mesenchymal transition (EMT) and tumor progression. ESRP1 was identified in colorectal cancer (CRC) as tumor suppressor, but the prognostic value of ESRPs and relation with mesenchymal splice variants is not clear.

**Experimental description:** Tumor tissue and adjacent non-neoplastic colorectal epithelium were analyzed by qPCR from 68 CRC cases and compared with FGFR, CD44, ENAH and CTNND1(p120-catenin) gene splice patterns, clinical data and outcome. ESRP1 expression was evaluated by immunohistochemistry.

**Results:** Here, we identified reduced expression of both ESRPs in primary CRC tissue, associated with shorter patient survival and switch to mesenchymal gene expression signature. We observed in CRC cell models with conditional ESRP1 expression similar splice signatures of fibroblast growth factor receptors FGFR1-3 and genes important for EMT. ESRP1 was validated in silico as independent prognostic marker by public gene expression data from CRC patients of The Cancer Genome Atlas project.

**Conclusions:** Our study supports the role of ESRPs as tumor suppressors in CRC, important as conflicting associations with breast and pancreatic cancer patient outcome exists. We concluded that ESRPs are promising candidate biomarkers for early detection, diagnosis and prognosis of CRC.

**Keywords:** colorectal cancer, epithelial splicing regulatory protein, overall survival, prognostic marker

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**Correspondence:** Klaus Holzmann, Borschkegasse 8a, 1090 Vienna, Austria, email: klaus.holzmann@meduniwien.ac.at

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Frequent in vivo detection of HIV-1 RNAs of the 1 kb class using diverse 3' splice sites

Elena Delgado[1], Yolanda Vega[1], Cristina Carrera[1], Carolina Arcones[1], Ana Mariño[2], Hortensia Álvarez[2], Antonio Ocampo[3], Celia Miralles[3], Sonia Pérez-Castro[3], Miguel Thomson[1]

[1] HIV Biology and Variability Unit. Centro Nacional de Microbiología. Instituto de Salud Carlos III. Carretera Majadahonda-Pozuelo, Km. 2. 28220 Majadahonda, Madrid, Spain. [2] Hospital Arquitecto Marcide. Ferrol, A Coruña, Spain. [3] Complejo Hospitalario Universitario de Vigo. Vigo, Pontevedra, Spain.

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## ABSTRACT

**Purpose:** All HIV-1 RNAs are transcribed from a single promoter and are processed through a complex splicing mechanism resulting in a great diversity of transcripts. According to splicing events involved in their generation, HIV-1 RNAs have been usually classified in three major categories: unspliced (~9 kb); singly spliced (~4 kb); and doubly spliced (~2 kb). We recently described a fourth RNA class, of ~1 kb, using 3' splice sites (3'ss) near the 3' end of the viral genome, within Nef coding sequence. Here we examine in vivo splice site usage for generation of HIV-1 1-kb RNAs.

**Experimental description:** Total RNA was extracted either from peripheral blood mononuclear cells (PBMCs) (n=14) or from immunomagnetically-separated CD4+CD25+ lymphocytes (n=19) from HIV-1-infected individuals. HIV-1 1-kb RNAs were amplified by RT-PCR followed by nested PCR using primers recognizing sequences near both ends of the HIV-1 genome. Sequences of PCR products were obtained either by direct sequencing of bands extracted from agarose gels or by clone sequencing.

**Results:** In clones from CD4+CD25+ lymphocytes, 8 different HIV-1 1-kb RNAs were detected in 7 (37%) of 19 individuals. Notably, in 6 (32%) individuals, clones from 1-kb RNAs were more abundant than clones from doubly spliced 2-kb RNAs coamplified in the same reaction. In samples in which 1-kb RNAs were amplified from PBMCs and directly sequenced, 9 1-kb RNAs were detected in 7 (50%) of 14 individuals. 1-kb RNAs used 13 different 3'ss, five of them previously reported and 8 newly identified. One RNA contained one exon in the antisense orientation between two sense-oriented exons. All identified 3'ss had the usual elements of metazoan 3'ss: an AG immediately upstream and a pyrimidine-rich tract further upstream. A majority of 1 -kb RNAs were predicted to code for a 33-34 amino acid peptide at the C-terminus of Nef protein, but 6 lacked predicted coding capacity. Phylogenetic sequence analyses revealed that viruses in which 1 kb RNAs were detected belonged to 5 subtypes and 3 recombinant forms.

**Conclusions:** HIV-1 1-kb spliced RNAs using diverse 3'ss near the 3' end of the viral genome are frequently detected in vivo in viruses of different genetic forms. Although a majority potentially code for a C-terminal Nef peptide, they frequently lack coding potential. A hypothetical function of these RNAs mediated by sequestering cellular miRNAs targeted to the 3' untranslated region of HIV-1 mRNAs is proposed.

**Keywords:** HIV-1, splicing, 1 kb RNAs, subtypes.

**Acknowledgements:** This work was funded through grants SAF2010-20961 and SAF2013-48488-P, Plan Nacional de I+D+I, Ministry of Economy and Competitiveness, Spain. We thank the personnel at the Genomic Unit, Centro Nacional de Microbiología, Instituto de Salud Carlos III, for technical help in sequencing.

**Correspondence:** Miguel Thomson (mthomson@isciii.es).

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Within the family of MELOE antigens, IRES-dependent translation conditions exclusive expression in melanoma cells and immunogenicity

M. Charpentier[1], M. Croyal3, D. Carbonnelle[1], A. Fortun[1], L. Florenceau[1, 2], M. Krempf[3], N. Labarrière[1], F. Lang[1]

[1] CRCNA INSERM U892, CNRS 6299, University of Nantes – Labex IGO. Nantes, France. [2] Nantes Hospital. Nantes, France. [3] INRA, UMR 1280, CRNH. Nantes, France

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## Abstract

We have previously reported that 2 melanoma antigens, MELOE-1 and MELOE-2 involved in T cell immunosurveillance of melanoma were translated from the polycistronic mRNA meloe by IRES-dependent mechanisms. We now explored whether upstream ORFs from this mRNA could be translated by a classical cap-dependent mode and indeed found that the most upstream ORF, named MELOE-4 (54 aa), was very efficiently translated in melanoma cells. Using melanoma cell lines transfected with various eGFP-tagged MELOE constructs, we could evidence that expression of MELOE-4 was more than 100 times higher than that of MELOE-1 in melanoma cells. We have previously shown that melanocytes also express meloe mRNA but are not recognized by MELOE-1 or MELOE-2 specific T cell clones suggesting that IRES-dependent translation of MELOE-1 and MELOE-2 is not activated in these cells. In contrast, we documented the presence of MELOE-4 in 4 melanocyte cell lines by mass spectrometry. This presence argues in favor of MELOE-4 as the physiological product of meloe mRNA in melanocyte.

We then questioned the immunogenicity of MELOE-4 by exploring the CD8 and CD4 T cells repertoire against this antigen in healthy subjects in comparison to the frequent T cell repertoire against MELOE-1 that we described previously. Using an in vitro protocol of accelerated DC differentiation and maturation, we stimulated PBMC from 4 healthy donors with overlapping peptides from either MELOE-1 or MELOE-4 and tested CD4 and CD8 T cell reactivities upon restimulation by INFg intracellular staining. In marked contrast with the high frequencies of CD4 and CD8 T cell responses against MELOE-1 in all 4 healthy donors, we found no CD8 and very rare CD4 T cell responses against MELOE-4. This suggests an immune tolerance towards this antigen that would be consistent with its expression in normal melanocytes. In conclusion, despite its high expression in melanoma cells, the classically translated MELOE-4 antigen represents a poor target for T cell immunotherapy because of its expression profile and very low immunogenicity. On the other hand, IRES-dependent MELOE antigens represent the best T cell targets for immunotherapy due to their very specific expression by melanoma cells and high immunogenicity. This prompts us to explore other IRES-dependent antigens as target for immunotherapy in cancer.

**Keywords:** IRES, polycistronic RNA, melanoma antigen

**Correspondence:** francois.lang@univ-nantes.fr.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Spliceosome inhibition as novel strategy against diffuse malignant peritoneal mesothelioma

R. Sciarrillo [1,2,3], V.E. Gomez [3], M. Pennati [6], A. Wojtuszkiewicz [1,2], G.J.L. Kaspers [1,2], C. Molthoff [4], N. Zaffaroni [6], G.J. Peters [3], G. Jansen [5], J. Cloos [1,2], E. Giovannetti [3,7]

[1] Department of Hematology, CCA - VU University Medical Center, De Boelelaan 1117, 1081 HV, Amsterdam, Netherlands. [2] Department of Pediatric Oncology - CCA - VU University Medical Center, De Boelelaan 1117, 1081 HV, Amsterdam. [3]Department of Medical Oncology - CCA - VU University Medical Center, De Boelelaan 1117, 1081 HV, Amsterdam. [4]Department of Radiology & Nuclear Medicine - VU University Medical Center, Location Radionuclide Center, De Boelelaan 1085c, 1081 HV Amsterdam. [5] Department of Rheumatology - CCA - VU University Medical Center, De Boelelaan 1117, 1081 HV, Amsterdam. [6] Fondazione IRCCS - Istituto Nazionale dei Tumori, via Venezian 1, 20133, Milan, Italy. [7] Cancer Pharmacology Lab - AIRC Start-Up Unit - University of Pisa - Ospedale di Cisanello, Edificio 6, via Paradisa 2, 56124, Pisa, Italy.

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## ABSTRACT

Purpose: Diffuse malignant peritoneal mesothelioma (DMPM) is an aggressive tumor that affects the lining of the abdomen, characterized by late clinical symptoms and poor prognosis. Systemic chemotherapy together with cytoreductive surgery and intraperitoneal hyperthermic therapy has been introduced as the best treatment option over the last decade, resulting in an overall 5-year survival rate of approximately 50%. To further increase survival, novel drugs targeting key molecular factors in DMPM are warranted. Analogous to pleural mesothelioma, such a factor may be alternative splicing which can be modulated by inhibiting the SF3B subunit of the spliceosome. This strategy is an emerging therapeutic opportunity for a number of solid tumors and hematological malignancies. In particular, the spliceosome inhibitor Pladienolide B (PB) has low nanomolar IC50 values against a range of human cancer cell lines and leukemic cells, but no data are available regarding its antitumor efficacy in DMPM.

The present study investigates (1) the activity of PB (alone or in combination with standard chemotherapeutics) in primary peritoneal mesothelioma cells through in vitro and in vivo assays, and (2) the molecular mechanisms of splicing inhibition through whole-genome RNA-seq and PCR of selected genes involved in apoptosis and invasion.

Experimental description: The antiproliferative effect of PB was investigated using the SRB assay on two primary mesothelioma cell cultures (MESOII and STO), obtained from resected tumors with well-annotated clinical characteristics. Further in vitro studies were performed to evaluate the pro-apoptotic and anti-invasive activities, while splicing profiles of treated and untreated cells were determined with RNA-seq.

Results: PB impaired DMPM cell growth in a dose-dependent manner, with IC50 values of 1.57 ± 0.30 nM in MESOII and 1.18 ± 0.16 nM in STO (n=3, mean ± standard deviation). The specific activity on the spliceosome was demonstrated by PB induced time- and dose-dependent alterations of splicing patterns for several apoptotic genes, such as Mcl-1, Bcl-X, Fas, and for the pro-metastatic tyrosine kinase receptor RON, which was shifted to its un-spliced and non-functional variant. In addition, RNA-seq showed several differentially expressed alternatively spliced genes in PB treated samples. The DMPM cells have also been genetically engineered to express Firefly- and Gaussia- luciferases, enabling monitoring of tumor growth inhibition by PB in in vivo orthotopic models.

Conclusions: These data provide evidence that PB has a strong antitumor activity against relevant models of DPMP, associated with modulation of splicing, induction of apoptosis and inhibition of invasion. RNA-seq represents a powerful tool for the identification of alternatively spliced genes that could serve as useful diagnostic markers as well as potential therapeutic targets for DMPM.

**Keywords:** mesothelioma, pladienolide, spheroid, orthotropic, novel therapeutics

**Correspondence:** Rocco Sciarrillo, Department of Hematology/Medical Oncology, CCA - Cancer Center Amsterdam, room 4.04, VU University Medical Center, De Boelelaan 1117, 1081 HV, Amsterdam. Phone: +316 526337700. E-mail: r.sciarrillo@vumc.nl.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# The oxygenase Jmjd6 – a master regulator of SR-proteins?

J. Wesche[1], S. Khn[1], A. Wolf[1]

[1] Institute of Molecular Toxicology & Pharmacology. Helmholtz Zentrum Muenchen. Ingolstaedter Landstr. 1. 85764 Neuherberg. Germany

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## Abstract

The Jumonji domain-containing protein 6 (Jmjd6) is a member of the superfamily of iron(II) and 2-oxoglutarate (2OG) dependent oxygenases. In recent years a large number of these 2OG oxygenases has been characterized to be involved in regulation of gene expression at different levels in humans. This includes epigenetic regulation, regulation of transcription and translation. The 2OG oxygenase Jmjd6 has been shown to be a nuclear localized protein with a JmjC-domain and has been described as a modulator of pre-mRNA splicing. The Jmjd6 protein is essential for embryonic development in vertebrates, including mice and zebra fish and is up-regulated in several cancers.

2OG oxygenases in humans catalyse transfer of molecular oxygen onto a substrate, which could either be an amino acid in a protein or a nucleotide in RNA or DNA. The Jmjd6 protein catalyses 2OG-dependent C-5 hydroxylation of lysine residues in splicing regulatory proteins, including U2AF65 and other SR- or SR-like proteins.

**Keywords:** Jmjd6, JmjC, Fe(II) and 2-oxoglutarate dependent oxygenases, alternative splicing, lysinehydroxylase, SR-proteins, alternative splicing

**Correspondence:** Alexander Wolf, Institute of Molecular Toxicology & Pharmacology, Helmholtz Zentrum Muenchen, Germany. Email: alexander.wolf@helmholtz-muenchen.de.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# The Significance of Splicing and Specific Intronic Elements for Intron-Mediated Enhancement

T. Akua, O. Shaul

The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 5290002, Israel

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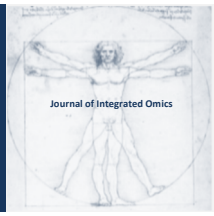
## Abstract

Introns stimulate gene expression in a wide range of organisms by increasing the levels of mature mRNA. Introns usually stimulate expression by a process termed intron-mediated enhancement (IME). This process has a great influence on gene expression in plants. The mechanism of IME is largely unknown. While splicing per se is not sufficient for IME, as evident from the fact that not all introns increase expression, it was not clear whether splicing of the enhancing introns is essential for enhancement. It is also not known why introns localized in the 5' untranslated region (5'UTR) are considerably longer than downstream introns. We showed that the leader intron (LI) of the Arabidopsis thaliana MHX gene (AtMHX) can substantially increase gene expression. This LI was utilized as a model to gain more knowledge on IME. We found that although AtMHX promoter showed almost no expression in the absence of this LI, this intron did not act as a transcriptional enhancer. This LI had different contributions to the expression mediated by different promoters. We also found that while splicing was essential for substantial IME, in the absence of splicing low-level enhancement could be obtained. The internal intron sequence played a significant role in mediating the low-level enhancement of the unspliced LI. Interestingly, we identified in this LI an internal element that considerably increased the efficiency of mRNA translation, without affecting splicing. Moreover, the ability of this intronic element to enhance translation was diminished by a minor downstream shift in the position of introns containing it from the 5'UTR into the coding sequence.T his raised the possibility that some of the extra length of 5'UTR introns results from the presence of elements that enhance translation, and, moreover, from the ability of 5'UTR introns to provide preferable platforms for such elements over downstream introns.

**Correspondence:** orsha@mail.biu.ac.il.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Blocking of iCLIP identified hnRNPA1 binding sites by splice switching oligonucleotides corrects aberrant splicing

Brage Storstein Andresen

Department of Biochemistry and Molecular Biology, University of Southern Denmark, Denmark

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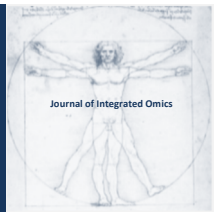
## Abstract

Correct splicing of exons with weak splice sites depends on a tight balance between positive and negative splicing regulatory elements (SREs). A large fraction of disease-causing mutations disrupt mRNA splicing by decreasing splice site strength or disrupting/creating SREs. We used iCLIP (individual-nucleotide resolution crosslink and immunoprecipitation) to create an in vivo binding map for the splicing regulatory protein hnRNP A1, which usually binds negative SREs to inhibit exon inclusion. HeLa cells with inducible expression of T7-tagged hnRNP A1 were UV irradiated generating irreversible crosslinks between RNA and RNA binding proteins allowing stringent purification of the bound RNA. The iCLIP libraries were subject to next-generation sequencing. We correlated the hnRNP A1 binding map with the hnRNP A1-regulated exons identified by hnRNP A1 knockdown and RNA-seq, and observed that the region immediately downstream of 5' splice sites is important for hnRNP A1-mediated exon repression.

We hypothesized that exons with weak splice sites, which are skipped due to a tipped balance between positive and negative SREs, could be reactivated by blocking iCLIP identified hnRNP A1 binding negative SREs using splice switching oligonucleotides (SSOs). We demonstrated the validity of this approach to activate a MTRR pseudoexon and the alternative exon 3 in SKA2 by SSO-mediated blocking of iCLIP-identified hnRNPA1 binding sites located immediately downstream of the 5' splice sites.

Interestingly, our iCLIP map revealed a hnRNP A1 binding site in intron 20 close to the 5' splice site of IKBKAP exon 20. Skipping of IKBKAP exon 20 causes familial dysautonomia (FD). FD is a recessive disease, affecting the sensory and autonomic nervous system and is frequent in Ashkenazi Jews. Almost all patients with FD are homozygous for a IVS20+6T>C mutation in IKBKAP, which decreases 5' splice site strength, causing exon 20 skipping. The iCLIP identified hnRNP A1 binding site was confirmed by in vitro studies. Therefore, we designed an SSO blocking the hnRNP A1 binding site in intron 20. Transfection of this SSO into FD patient fibroblasts fully restores IKBKAP exon 20 splicing.

Our study illustrates that the iCLIP generated hnRNP A1 binding map can be used to identify potential targets for SSO-based therapy.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Endogenized Transposable Elements Reshape the Human Transcriptome

Manvedra Singh[1], Laurence D. Hurst[2], Zsuzsanna Izsvák[1]

[1] Max-Delbrück-Center for Molecular Medicine (MDC), Robert-Rössle-Strasse 10, 13125 Berlin, Germany. [2] University of Bath, Department of Biology and Biochemistry, Bath, Somerset, UK, BA2 7AY.

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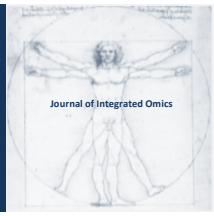
## Abstract

The evolutionary success of transposable elements (TEs) is powerfully underscored by the finding that about 45% of the human genome is TE-derived. While only a fraction of the human genome is actively mobile, TE-derived sequences can influence the human transrciptome in multiple ways. TEs, primarily endogenous retroviruses (ERVs), can provide alternative transcriptional regulatory sequences, including splice sites. ERVs can rewire the transcription of host genes, and contribute to novel chimeric transcripts. These alternative transcripts show spatio-temporal expression and also species-specific. Our data suggest that ERVs have massively reshaped the regulation of pluripotency during primate evolution.

In addition to retroelements, DNA transposons appear to modulate splicing by actively recruiting key factors of the splicing machinery. Surprisingly, our host-transposon interaction studies revealed a physical interaction between the piggyBac transposase protein and a host-encoded splicing factor. Curiously, certain domesticated piggyBac-like genes adopt this feature. This finding has important ramification for the presence of domesticated piggyBAC-like elements in the human genome.

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SPECIAL ISSUE: PROCEEDING ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN SPLICING (SPLICING 2016)

# Intergenic splicing in cancer and normal physiology

Hui Li

University of Virginia, Carter Harrison Bldg (MR6), Rm B524, Charlottesville, VA 22908, USA

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## Abstract

Gene fusions and their fusion products were thought to be unique features of cancer, and produced solely by chromosomal rearrangement. We have demonstrated that RNA trans-splicing can produce identical fusion RNAs and in some cases, fusion proteins in normal cells. In a proof of principle experiment, we found a cancer-signature fusion RNA and protein transiently produced during normal myogenesis. We are using such cancer-signature fusions to track down the cell-of-origin for mysterious cancers.

In addition to trans-splicing, cis-SAGe (cis-splicing between adjacent genes) is another mechanism to produce intergenic spliced chimeric RNAs. By manipulating CTCF level combined with RNA-seq,we are identifying other cis-SAGe fusions.

To investigate the scope of chimeric RNAs in normal physiology, we finished curating and analyzing 291 RNA-seq datasets of 30 non-cancer tissues and cell types. Over 10,000 fusion RNA events were found, involving over 10% of human genome. We coined the word “fusionome” to describe the phenomenon. In addition to tissue specific fusions, we found a group of fusions being ubiquitously expressed and silencing them resulted in significant reduction in cell growth and motility in normal cells. We call them “house-keeping fusions”.

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# Functionally distinct ryanodine receptor variants generated by alternative splicing using GG/AG intron

Hiroshi Okamoto[1, 2], Shin Takasawa[3], Yasuhiko Yamamoto[1]

[1] Kanazawa University, [2] Tohoku University, [3] Nara Medical University

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## ABSTRACT

Increases in the intracellular $Ca^{2+}$ concentration in pancreatic islets, resulting from the Ca2+ mobilization from the intracellular source through ryanodine receptor (RyR), are essential for the insulin secretion by glucose. Cyclic ADP-ribose (cADPR), a potent $Ca^{2+}$ mobilizing second messenger synthesized by CD38, regulates the opening of RyR. A novel *RyR* cDNA (the islet-type RyR) was found to be generated from the type 2 *RyR* gene by the alternative splicing of exons 4 and 75. The islet-type *RyR* mRNA was expressed in a variety of tissues such as in pancreatic islets, cerebrum, and cerebellum, whereas the authentic type 2 *RyR* mRNA generated using GG/AG splicing of intron 75, was expressed in heart and aorta. The islet-type RyR caused a further increase in the caffeine-induced Ca2+ release when expressed in HEK293 cells pre-treated with cADPR, thus suggesting that the novel RyR is an intracellular target for the CD38-cADPR signaling system in mammalian cells.

***Corresponding author:** Hisanori Kato, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Phone number: +81 358411607. Email Address: akatoq@mail.ecc.u-tokyo.ac.jp