

## Genetic Control of pre-mRNA Splicing and Diseases

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**Received Date:** 22 May 2014

**Accepted Date:** 25 May 2014

**Published Date:** 28 May 2014

**Citation:** Seino RA, Dongmo TI, Chifon RN, Shambo DN (2014) Possible Cytogenetic Effect of Capsicum frutescens (Solanaceae) Extracts on Meiosis in the Grasshopper Taphronota thaelephora Stal. (Orthoptera: Pyrgomorphidae). Enliven: J Genet Mol Cell Biol 1(1): 002.

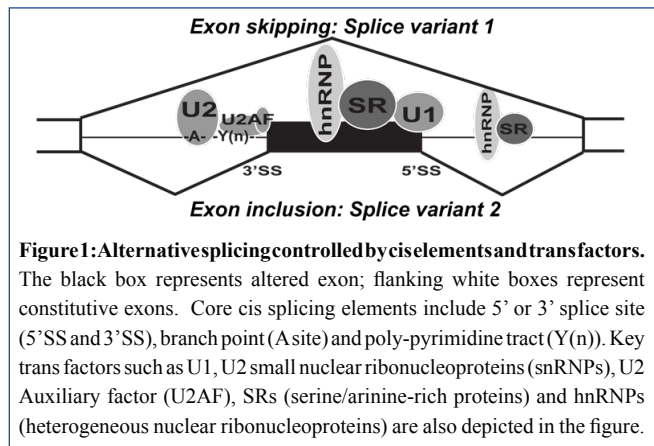
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### What Is Alternative Splicing?

Eukaryotic genes, primarily in multi-cellular organisms, have mRNA-encoding regions called exons that are interrupted by non-coding regions named introns. This split gene structure provides a fertile ground of post-transcriptional regulation which expands transcript repertoire through usage of various exon-exon combinations (i.e. alternative splicing) resulting in multiple mRNA isoforms (i.e. splice variants) produced from a single gene [1]. High-throughput RNA sequencing (RNA-seq) indicates that > 90% of multi-exon human genes undergo alternative splicing (AS) [2,3]. More importantly, AS can change a final protein's binding properties, modify enzymatic activity, and even reverse roles of its gene in cellular processes (e.g. two isoforms of Bcl-x through AS, the long form is anti-apoptotic, while the short one is pro-apoptotic) [4]. Besides the role of AS in normal development, evidence of pathogenesis and clinical relevance of aberrant splicing variants is growing exponentially [5], with an estimated 10% of splice site mutations (exon intron boundary) causing human inherited diseases [6-8]. Deciphering the pre-mRNA splicing code and functional characterization of splice variants will provide us new insights of pathogenesis of human diseases.

### How Is pre-mRNA Splicing Regulated?

The precision of pre-mRNA splicing relies on interactions between *cis* elements and *trans* regulators that recognize *cis* elements (Figure 1). The core *cis* splicing signals include two groups of elements: (1) the 5' or 3' splice site which determines the exon-intron boundary; (2) branch site and polypyrimidine tract that are initially recognized during intron cleavage steps. Other auxiliary exonic and intronic elements (i.e. ESE: exonic splicing enhancer, ISE: intronic splicing enhancer, ESS: exonic splicing silencer, ISS: intronic splicing silencer) also participate in the process, either promoting or inhibiting splicing. During the splicing process, the core splicing signals (e.g. 5' or 3' splice site) are recognized by spliceosome complex which is composed of five small nuclear RNAs (snRNAs: U1, U2, U4, U5, and U6) and associated protein factors, while auxiliary element region (e.g. ESE or ISS) can recruit SR (serine/arginine-rich)-proteins and hnRNPs (heterogeneous nuclear ribonucleoproteins). Interplay of RNA-protein and protein-protein interaction leads to final precise splicing in normal development [9].



**Figure 1: Alternative splicing controlled by cis elements and trans factors.**

The black box represents altered exon; flanking white boxes represent constitutive exons. Core cis splicing elements include 5' or 3' splice site (5'SS and 3'SS), branch point (A site) and poly-pyrimidine tract (Y(n)). Key trans factors such as U1, U2 small nuclear ribonucleoproteins (snRNPs), U2 Auxiliary factor (U2AF), SRs (serine/arginine-rich proteins) and hnRNPs (heterogeneous nuclear ribonucleoproteins) are also depicted in the figure.

### Pre-mRNA Splicing Defects in Diseases

Either mutation of *cis* elements or perturbation of *trans* factors could impair splicing which contributes to disease phenotypes [7]. For instance, the occurrence of congenital bilateral absence of vas deferens (CBAVD) and full brown cystic fibrosis disease is associated with loss of *CFTR* (Cystic fibrosis transmembrane conductance regulator) exon 9, an aberrant alternative splicing with production of an inactive CFTR protein. Further studies proved that the TG(m)T(n) polymorphic element in the vicinity of *CFTR* exon 9 affects the efficiency of its exon 9 splicing [10]. Another example of *cis* element mutation affecting splicing is in ataxia telangiectasia, a neurodegenerative disease. Baralle and his colleagues found that deletion of four nucleotides (GTAA) in intron 20 of *ATM* gene abolished accurate intron processing, caused activation of the cryptic exon of 65 bp [11]. The *ATM* allele with this 4nt deletion fails to produce a functional protein, which is vital for cell cycle checkpoint signalling in DNA damage response and genome instability. Among the diseases caused by splicing *trans* regulators, myotonic dystrophy (DM1) is a particularly well-studied example. An expanded CUG repeat in 3' UTR of *DMPK* gene leads to sequestration of MBNL protein and upregulation of the fetal splicing patterns controlled by CELF protein, which finally contributes to severe manifestations of disease [12].

Interestingly, disease severity can be modulated by the interplay of *cis* mutation and *trans* factor perturbation. One splicing genetic modifier of *SCN8A* gene has been characterized in mice. In C3H mice, a 4nt deletion (GTAA) close to 5' splice site of *SCN8A* exon 3 results in skipping of both exon 2 and exon 3 in most transcripts resulting in only 10% of the transcripts being correctly spliced. C57BL/6J mice, in addition to the 4nt deletion in *SCN8A* gene, have a stop mutation in *SCNMI* gene whose protein product acts as a splicing factor controlling *SCN8A* splicing patterns. With both the deletion in *SCN8A* and the mutation in *SCNMI*, only 5% of *SCN8A* will be correctly spliced in C57BL/6J mice. This small (10% vs. 5%) splicing difference however leads to dramatically distinct disease phenotypes. C3H mice only have a disorder of chronic movement while C57BL/6J mice develop a severe lethal neurological disease [13]. Such above evidence underscores the roles of AS in inherited diseases. In addition, aberrant splicing events observed in cancers through alteration of splicing factor concentration or localization are reviewed extensively elsewhere [14,15].

### Perspectives and Challenges of Studying Defective Splicing

Despite widespread use of AS in both major physiological and pathological aspects of cell biology, we still do not understand the function of most splice variants. Without functional data, we cannot decipher if these aberrant splicing events play a role in initiation and/or development of diseases or whether they are just "passenger" isoforms. In fact, functional characterization of certain transcript on a splicing level involves more technical difficulties than the gene level.

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