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WHAT SPLICING BIOLOGY SUGGESTS TO MEDICINE

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Splicing is a highly regulated process which plays a significant role in eukaryotic genes expression, and is vital for cell function. Understanding splicing mechanisms, core components involved in this process will allow defining the reasons of various diseases. Splicing is performed by complex ribonucleoprotein particles termed spliceosomes. Mammals have two types of spliceosomes, each one comprised of five snRNPs and numerous proteins. Spliceosomes differ their compartments, recognition sites and recognition process, but they share a similar secondary structure. Although spliceosomes functions are equivalent, they are not permutable, so defects in major or minor one leads to various diseases. Recent studies have shown that mutations in splicing associated genes linked with such disorders as spinal muscular atrophy (SMA), autosomal dominant retinitis pigmentosum (adRP), microcephalic osteodysplastic primordial dwarfism type I (MOPD I). In addition, very exciting recent results of RNA deep-sequencing demonstrate splicing Abnormalities in Alzheimer's disease.

Keywords: splicing, ribonucleoproteins, spliceosome, retinitis pigmentosa, spinal muscular atrophy, Taybi-Linder Syndrome.

ТРАНСЛЯЦИЯ БИОЛОГИИ СПЛАЙСИНГА В МЕДИЦИНУ

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Большинство генов эукариот транскрибируются с образованием прематричной РНК, которая затем под воздействием процесса сплайсинга превращается в матричную РНК. Это жизненно важный процесс, и его нарушение может привести к различным патологиям.

Процесс сплайсинга осуществляется сложным рибонуклеопротеидным комплексом - сплайсосомой. У млекопитающих существует два типа сплайсосом, которые отличаются по рибонуклеопротеидному составы, сайтам сплайсинга и механизму связывания с сайтом сплайсинга. Каждая сплайсосома состоит из пяти основных рибонуклеопротеинов и более 80 белков. Механизм сплайсинга включает в себя несколько стадий: узнавание сайта сплайсинга, сборку сплайсосомного комплекса и двухфазный катализ (включающую реакции трансэстерификаци и гидролиза), при котором происходит выщипление интрона и сшивание экзонов.

Таким образом, сплайсинг пре-мРНК является сложным, высокорегулируемым процессом, значительные нарушения которого приводят к клеточной гибели. Тем не менее, в процессе сплайсинга и в регуляции этого процесса участвует значительное количество белков, мутации в генах которых могут стать причиной различных заболеваний. К таким заболеваниям можно отнести пигментный ретинит, спинальную мышечную атрофию, синдром Тауби–Линдера. Разные типы пигментного ретинита являются наследственными заболеваниями, связаны с изменениями в генах PRPF31, PRPF8, PRPF3, RP9, белки которых участвуют в формировании каталитического ядра сплайсосомы. Мутации в генах SMN1 и SMN2, ассоциированных с процессом сплайсинга, приводят к развитию спинальной мышечной атрофии. Довольно редкая разновидность карликовости (синдром Тауби–Линдера) на сегодняшний день единственное заболевание, при котором мутации происходят в гене одного из основных рибонуклеопротеинов малой сплайсосомы – RNU4ATAC, возможно, мутации в других генах рибонуклеопротеинов летальны для человека, еще в эмбриональном периоде.

Последние исследования указывают на роль сплайсинга при болезни Альцгеймера и церебральной амилоидной ангиопатии. Изменения в уровне U1 рибонуклеопротеине большой сплайсомы приводят к изменению в целом эффективности сплайсинга пре-мРНК у больных Альцгеймера и к увеличению прекурсоров амилоидных белков. Также некоторые данные указывают на взаимосвязь между некоторыми белками, участвующими в сплайсинге, и рядом онкологических заболеваний, хотя эти данные связаны с недостаточным пониманием самого процесса сплайсинга.

Ключевые слова: сплайсинг, рибонуклеопротеины, сплайсосома, пигментный ретинит, спинальная мышечная атрофия, синдром Тауби–Линдера.

Splicing-associated human diseases

Most eukaryotic genes are expressed as precursor mRNAs (pre-mRNAs) that are converted to mRNA by splicing, an essential step of gene expression in which noncoding sequences (introns) are removed and coding sequences (exons) are ligated together. In vertebrates, including humans, most genes have multiple introns and are spliced in more than one pattern to produce considerable mRNA variation. Deep sequencing has recently revealed that >95% of human genes are alternatively spliced, often in a developmental, tissue-specific, or signal transduction-dependent manner [Aaron et all, 2012]. Splicing process is performed by large, multi-megadalton, complex ribonucleoprotein particles called spliceosomes.

Both the conformation and the composition of the spliceosome are highly dynamic, thus providing the splicing machinery with its accuracy and flexibility simultaneously. Mammals have two types of spliceosomes that splice out different, exclusive types of introns: the U2-dependent spliceosome (the major spliceosome), the U12-dependent spliceosome (the minor spliceosome) [Padgett, 2012]. Although the structure and the functioning of the both spliceosome types are well know, the biological role of the less abundant U12-dependent spliceosome is more poorly studied.

The U2-dependent spliceosome is assembled from U1, U2, U5, and U4/U6 small nuclear ribonecpeoproteins [HybriQ collaboration, Khrenkova and Aleksandrova, 2013] (snRNPs) and numerous non-snRNP proteins [Will and Lührmann, 2011].

Although the sequences of the snRNAs with equivalent function are quite divergent in the two spliceosomes, they share a common overall secondary structure [Will and Lührmann, 2011]. Main subunits of the U12-dependent spliceosome are, mostly in contrast, U11, U12, U5, and U4atac/U6atac snRNPs [Will and Lührmann, 2011]. Thus, four of the U-snRNPs are unique to each spliceosome while the U5 snRNP is present in the both. U1, U2 snRNPs in the major and U11, U12 components in the minor spilceosome are responsible for intron recognition and initiation of spliceosome assembly. Major spliceosome U4, U5, U6 snRNPs are the main catalytic core components. In the minor spliceosome, U4atac, U6atac, and U5 similarly associate to form a catalytic core [Will and Lührmann, 2011].

Non-snRNP proteins are very diverse in the either spliceosome. Approximately 80 proteins are present in the human spliceosome that are reported to be essential for the process of spliceosome-dependent splicing. The total number of proteins associated with the human spliceosomes may reach over 200 proteins [Agafonov et all, 2011].

U2- and U12-dependent spliceosomes differ in their recognition sites. Introns are recognized by spliceosomes by two types of sites: those (splice sites) located at the exon-intron junctions, and branch sites found 18–40 nucleotides upstream the 3' splice site. [Alioto, 2006].

Typically, U2 introns have GU dinucleotide at the 5'-splice site, AG dinucleotide at the 3' -splice site, and CURACU sequence at the branch site, where the A is the branch point adenosine. There is also a pyrimidine rich region between the branch and 3' splice sites in U2-dependent introns [Dietrich et all, 2001].

Whereas the vast majority of U2 introns have GU and AG dinucleotides at the intron-exon junctions, U12 introns have RU and AS dinucleotides at the 5'- and 3'-positions, respectively. Thus intron-flanking dinucleotides are partially shared between U2- and U12-dependent spliceosomes. The minor (U12-) spliceosome branch site is UCCUURACU, where, similar to major spliceosome, the A is the branch point adenosine. U12-introns lack a pyrimidine rich region. [Bartschat and Samuelsson, 2010]. Splice sites and branch sites are highly conserved sequences although U12 branch sites are more conserved than their counterparts in U2 introns. Even if some mutation arise in branch site there is no data about branch point adenosine replacement [Rogozin et all, 2012]

Although U2- and U12-introns differ in the branch site sequences, the interactions between the spliceosome and branch site are similar in the both spliceosome types. Proteins of SF3b complex, which is characteristic for the both spliceosomes, probably promote branch site recognition [Turunen et all, 2013].

The two spliceosomes also differ in the order of the spliceosomal assembly, though share further pathways.

Minor (U12-dependent) spliceosome components U11 and U12 form a dimer, which then recognizes the splice sites and branch point simultaneously, while major spliceosome snRNPs U1 and U2