Division of Gene Expression Mechanism

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[Background and Significance]

In eukaryotes, most of gene transcripts or pre-mRNAs are interrupted by intervening sequences termed introns, which are precisely removed by the process called splicing. This process is essential since spliced mRNAs serve as templates of proteins. The higher eukaryotes have been evolved to gain more and more introns with larger size, which provides complexity and flexibility in splicing process, ends up generating alternative splicing. Alternative splicing is a successful major strategy for expressing a full proteome with at least 120,000 kinds of proteins from an unexpectedly small size genome with at most 20,500 genes. Recent studies have revealed that over 90% of human genes undergo alternative splicing, in which over 60% are tissue-specifically regulated.

Regulations in the splicing process are definitely crucial for a wide variety of biological and physiological phenomena. The process is therefore highly discriminatory and faithful, and misregulation in this process causes disorders in cell functions, often with severe clinical consequences. Our team in the section of Mechanism of Gene Expression challenges to elucidate unsolved fundamental problems in splicing and to advance our basic understanding of the aberrant splicing that causes serious diseases.

[Accomplishments of Research]

1. Oncogenic HMGA1a protein induces aberrant splicing in breast cancer

The *presenilin-2* (*PS2*) gene is one of the known Alzheimer's disease (AD)-associated genes. The aberrant splicing (skipping of exon 5) generates the deleterious protein PS2V that accumulates as visible PS2V bodies in the brains of sporadic AD patients, *i.e.*, one of the risk factors of neuronal cell death. Previously we purified and identified HMGA1a protein as a sequence-specific RNA-binding factor that causes specific exon 5 skipping. Recently, we have finally elucidated the definitive mechanism of this aberrant splicing [Ohe & Mayeda, 2010]; i.e., HMGA1a-induced aberrant exon skipping is caused by impaired dissociation of U1 snRNP from the 5' splice site, leading to a defect in exon definition. It is of considerable interest that analogous U1 snRNP-mediated systems are evolutionally conserved from yeast to human, as a strategy to modulate post-transcriptional events during gene expression, which ends up with a variety of physiological and medical phenotypes.

The *HMGA1* gene, coding HMGA1a protein, is well-known proto-oncogene that promotes tumor progression and metastasis when overexpressed in cells. We are trying to identify target genes of HMGA1a by its specific binding sequence.

2. Search for the potential implication of splicing silencers HMGA1a and hnRNP A1 in aberrant splicing found in Schizophrenia

Schizophrenia is a mental disorder caused by complex genetic and environmental factors. It is remarkable that aberrant splicing are often observed in the brains of mental and neurodegenerative diseases. Using peripheral lymphoblastoid cell lines prepared from 16 schizophrenia patients with age-matched controls, which were generously provided from Dr. N. Ozaki (School of Medicine, University of Nagoya), we are studying aberrant splicing associated with schizopherenia.

Interestingly, aberrant PS2V protein was also observed in the brains of schizophrenia. We thus examined the expression of HMGA1a, which is the inducer of PS2V, in the lymphoblastoid cells obtained from schizophrenia patients. We observed significantly higher HMGA1a mRNA and the increased HMGA1a protein in the nuclear fractions [Morikawa/Manabe et al., 2010]. The present study suggests the potential roles of HMGA1a in both transcription and splicing of the target genes linked with schizophrenia.

Defects in the development and maturation of oligodendrocytes appear to be a risk factor of schizopherenia. We are studying aberrant splicing involved in the abnormal oligodendrocyte maturation and myelinization that are observed in schizopherenia.

3. Pre-mRNA micro-introns: Are they spliced by the massive spliceosome?

Pre-mRNA introns in protozoan genomes are often extremely small (e.g., 20–33 nt, mean 25 nt in *Paramecium tetraurelia*), wherease those in higher metazoan are large (e.g., 43–4,500,000 nt, mean 5,430 nt in human). Such protozoan micro-introns yet possess highly conserved terminal dinucleotide (GT-AG), i.e., a hallmark of authentic major-class introns in metazoa. Genomes of higher eukaryotes have been evolved to gain much larger introns, which eventually demand sophisticated spliceosome for the splicing, e.g., an enormous ~3 MDa machinery including over 300 proteins in human. Human genome, however, still conserves micro-introns with less than 50 nt size, which could be a rudiment of protozoan micro-introns. These micro-introns are certainly spliced; nevertheless the mechanism of the splicing is poorly understood.

If human micro-introns are spliced in the authentic spliceosome, essential *cis*-acting elements, 5' splice site, branch site, and 3' splice site, must be simultaneously bound by the corresponding essential *trans*-acting factors in A complex, U1 snRNP (~247 kDa), U2 snRNP (~1850 kDa), and $U2AF^{65}$/$U2AF^{35}$ (~82 kDa), respectively. How are these essential splicing signals recognized without any steric hindrance? We predict a novel splicing mechanism that is not dependent on these essential splicing factors.

Using the annotated human genome database (H-InvDB), we selected *bona fide* micro-introns with 43–56 nt size whose splicing were verified *in vivo*. We found that splicing of these micro-inrons are inhibited by Spliceostatin A, suggesting that SF3b (U2 snRNP component) is necessary for their splicing. We are testing whether essential splicing factors, such as U snRNPs, $U2AF^{65}$/$U2AF^{35}$ and SR proteins, are required to splice these micro-introns *in vivo*. We are also investigating which of the factors contribute to the extent of mechanistic similarity and evolutional relationship between protozoan and metazoan introns.

[Papers]

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[Reviews]

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[Book Chapter]

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