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2001 Program
New Investigator (3-year project)

Project Title: Regulation of constitutive and alternative pre-mRNA splicing of human genes

Project Summary: The objective of this grant is to study the function of human splicing activator RNPS1, which is also an important factor to link splicing and the post-splicing process such as nonsense-mediated mRNA decay and translation. The study demonstrated that RNPS1 is incorporated in the active splicing complex, stimulates formation of the ATP-dependent splicing complex, and subsequently increases generation of spliced mRNA products. Interestingly, RNPS1 is phosphorylated at serine 53 by protein kinase CK2 *in vitro*. The point mutation to alanine 53 significantly reduced splicing stimulation activity *in vitro* and *in vivo*, suggesting the regulation of RNPS1 activity by phosphorylation at serine 53. The study performed a yeast two-hybrid screen to search for interacting factors with RNPS1; SR protein p54, human transformer-2 β , and pinin were identified and characterized *in vivo*. Over-expression of RNPS1 is prone to induce exon skipping in general, whereas co-expression of RNPS1 with p54 cooperatively stimulated exon inclusion in particular pre-mRNA. The study proposes that RNPS1 is a versatile factor that regulates alternative splicing of a variety of pre-mRNAs. Since the regulation of alternative splicing is responsible for generating malignant forms of many oncogenes, the project accomplishments will undoubtedly have a long-term impact on the treatment and prevention of cancer.

Project Successes: In 1999, a study first purified and identified human RNPS1 protein as a pre-mRNA splicing activator. Subsequent studies, supported by this grant, demonstrated that RNPS1 is a *bona fide* component of active spliceosomes and plays an important role in the regulation of alternative splicing both positively and negatively by interacting with its associated factors. If RNPS1 is a splicing regulator for a wide variety of alternatively spliced genes, it is physiologically important to control its activity in response to tissue specific and developmentally regulated signals. The project's discovery of site-specific phosphorylation of RNPS1 *in vitro* and *in vivo* supports a molecular signaling mechanism governing the activation of RNPS1, which enables modulation of splicing activity. It was concluded that phosphorylation of RNPS1 is important for modulating RNPS1 function as a splicing regulator. On the other hand, RNPS1 was also suggested to play a critical role in the post-splicing processes, such as nonsense-mediated mRNA decay (NMD), mRNA localization, and translation, as a component of spliced mRNA complex, or exon-junction complex (EJC). Together, these data indicate that RNPS1 is a key factor in linking splicing and post-splicing pathways to form 'gene expression network' that optimize the efficiency and specificity of each process.

Selected publications from BRP funded research in Peer Reviewed Journals:

Sakashita E, Tatsumi S, Werner D, Endo H & **Mayeda A**, Human RNPS1 and its associated factors: a versatile alternative pre-mRNA splicing regulator *in vivo*. *Mol. Cell. Biol.* 2004: 1174–1187.

Hu D, **Mayeda A**, Trembley JH, Lahti JM, Kidd VJ. CDK11 complexes promote pre-mRNA splicing. *J Biol Chem*. 2003;278:8623–8629.

Selected presentations from BRP funded research:

Mayeda A, Sakashita E, Tatsumi S & Endo H. Human RNPS1, as a versatile regulator of alternative pre-mRNA splicing. *The 8th Annual Meeting of the RNA Society*, The Congress Centre, Vienna, Austria, 2004

Hwang S, **Mayeda A**, Habara Y, Friendewey D, Ohshima Y & Tani T. A putative *S. pombe* homologue for RNPS1 is involved in pre-mRNA splicing and mRNA export from the nucleus. *The 2nd International Fission Yeast Meeting*, Kyoto International Conference Hall, Kyoto, Japan. 2002

Sakashita E, Endo H & **Mayeda A**. Functional analysis of human splicing activator RNPS1. *Annual Meeting of the RNA Society of Japan*, Kobe University, Kobe, Hyogo, Japan., 2001

Hwang S, **Mayeda A**, Habara Y, Friendewey D, Ohshima Y & Tani T. Characterization of the *S. pombe* homologue for RNPS1: A functional relationship between spRNPS1 and Prp3p in mRNA export. *Eukaryotic mRNA Processing Meeting*, Cold Spring Harbor Laboratory, New York, U.S.A. 2001

Sakashita E, Trembley JH, Kidd V & **Mayeda A**. Regulation of human RNPS1 activity by phosphorylation. *Eukaryotic mRNA Processing Meeting*, Cold Spring Harbor Laboratory, New York, U.S.A. 2001

New grants based in part on BRP-funded work:

ACS

Project Title: Regulation of pre-mRNA splicing by splicing activator RNPS1

Project Period: 01/02/01 – 02/02/02

Amount: \$20,000 (1 year, no indirect cost, unsalaried, 5% of effort)

NSF (Pending Support)

Project Title: Molecular mechanisms of RNPS1-mediated splicing activation and regulation

Project Period: 2004 - 2008

Amount: \$500,000