

Final Report

Proteins of a serine/arginine-rich (SR) family are part of the spliceosome and are implicated in both constitutive and alternative splicing of pre-mRNAs. With the funding from DOE we have been studying alternative of splicing of genes encoding serine/arginine-rich (SR) proteins and the roles of SR proteins that interact with U1-70K in regulating basic and alternative splicing.

Alternative splicing of pre-mRNAs of Arabidopsis serine/arginine-rich proteins and its regulation by hormones and stresses: We analyzed the splicing of all 19 Arabidopsis genes in different tissues, during different seedling stages and in response to various hormonal and stress treatments. Remarkably, about 90 different transcripts are produced from 15 SR genes, thereby increasing the transcriptome complexity of SR genes by about five fold. Using the RNA isolated from polysomes we have shown that most of the splice variants are recruited for translation. Alternative splicing of some SR genes is controlled in a developmental and tissue-specific manner (Palusa et al., 2007). Interestingly, among the various hormones and abiotic stresses tested, temperature stress (cold and heat) and ultraviolet light dramatically altered alternative splicing of pre-mRNAs of several SR genes whereas hormones altered the splicing of only two SR genes (Palusa et al., 2007).

Localization and dynamics of a novel serine/arginine-rich protein that interacts with U1-70K: We analyzed the intranuclear movement of SR45 fused to GFP by fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP). We demonstrate that the movement of GFP-SR45 is ATP-dependent. Interestingly, inhibition of transcription or phosphorylation slowed the mobility of GFP-SR45 (Ali et al., 2006). Our studies have revealed that the nuclear localization signals are located in arg/ser-rich domains (RS) 1 and 2, whereas the speckle targeting signals are exclusively present in RS2 (Ali et al., 2006). The regulation of SR45 mobility by ATP and a transcriptional inhibitor is in contrast to the mobility of SR family splicing factors in animals and suggests fundamental differences in the movement of plant and animals splicing factors.

In vivo interaction of U170K with SR45: To analyze the interaction of U170K with SR45, we expressed these proteins fused to RFP and GFP respectively, in protoplasts. Both the reporters co-localized to the same subnuclear domains. To determine direct interaction of these proteins, we fused full-length U170K to one part of split YFP and full-length or truncated version of SR45 to the second half of split YFP. Coexpression of these split YFP constructs resulted in

reconstitution of YFP in speckles, suggesting direct interaction of these proteins *in vivo* (Ali et al., 2008).

SR45 is a Novel Plant-Specific Splicing Factor and is Involved in Regulating Multiple

Developmental Processes: Using an *in vitro* splicing complementation assay, we showed that SR45 is an essential splicing factor. The *sr45-1* mutant exhibited a number of developmental abnormalities. Further analysis of flowering time has shown that the autonomous pathway of flowering is affected in the mutant. Expression analysis of several flowering genes has revealed that FLC, a key flowering repressor, is up-regulated in the SR45 mutant. Further, alternative splicing pattern of several other SR genes was altered in the *sr45-1* mutant in a tissue-specific manner. Hence, the observed pleiotropic effects on various aspects of development are likely due to altered level of SR protein isoforms, which in turn regulate the splicing of other pre-mRNAs. Expression of wild-type SR45 in the mutant complemented the phenotypic defects and changes in alternative splicing of SR genes. SR45 thus is a novel plant-specific splicing factor and plays a crucial role in multiple developmental processes.

Publications resulting from the DOE support:

1. Schindler et al., (2008) BMC Genomics 10: 9:159; pages 1-11.
2. Ali, et al., (2008). PLoS ONE 6;3(4):e1953; pages 1-13.
3. Ali, G.S. and Reddy, A.S.N. (2008) In Nuclear pre-mRNA processing (Eds. Reddy and Golovkin). Springer-Verlag, Berlin. Pages 103-118.
4. Ali, G.S. and Reddy, A.S.N. (2008) Regulation of alternative splicing of pre-mRNAs by stresses. Springer-Verlag, Berlin. Pages: 257-276.
5. Reddy, A.S.N (2007) Ann. Rev. Plant Biology 58: 267-294.
6. Palusa et al., (2007) Plant J. 49: 1091-1107.
7. Ali et al., (2007). PLoS ONE, 5: e471, Pages 1-13.
8. Ali, G. S. and Reddy, A.S. N. (2006) J. Cell Sci. 119: 3527-3538.
9. Reddy, A.S.N. (2004) Trends in Plant Sci. 9: 541-547.
10. Reddy, et al., (2004) In: "The Nuclear Envelope" Editors: David Evans and John Bryant, Bios Scientific Publishers Ltd, Oxford, UK 56: 279-95.