**Functional study of Arabidopsis thaliana**

**ASF/SF2-like pre-mRNA SR splicing factors**

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**Introduction**

SR proteins constitute a highly conserved family of non-snRNP splicing factors (1). They have many functions in splicing as they participate in spliceosome assembly and in alternative splicing by influencing the splice site selection. In addition, they also have a role in post-splicing events such as mRNA export or translation efficiency. SR proteins have at least one N-terminal RNA-binding domain (RRM) and a C-terminal RS domain enriched in serine/arginine dipeptides (2). We have used different approaches to study gene expression patterns and dynamic localizations of ASF/SF2-like proteins in Arabidopsis thaliana.

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**Expression pattern using GFP fusion**

We have established the expression pattern of the SR34::GFP and SR34a::GFP fusion proteins controlled by their endogenous promoters in stably transformed A. thaliana plants. SR34 and SR34a are localized in the nucleus of primary and secondary roots and at the onset of root buds. There are also found in epidermal cells of leaves and anthers. In addition SR34 is localized in style and SR34a is found in the hypocotyl.

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**Dynamics and localization of native and mutant SR34 proteins**

**Cellular localization**

We focused in mutants of the RS domain. SR34 has a nuclear localization. The SG34 mutant (Arg → Gly) displays a cytoplasmic localization. The TR34 (Ser → Thr) and TG (Arg → Gly/ Ser → Thr) mutants showed a lower fluorescence than the native SR34 protein, and localized in nuclei and/or cytoplasm.

Comparison of nucleocytoplasmic shuttling between native and mutant SR34 proteins in tobacco leaf cells. FLIP-Shuttling was monitored in the absence (-LMB) and upon leptomycin B (+LMB) treatment. Mutating the rnp1 motif (RRM1 domain) blocked the shuttling of SR34 in the absence of LMB treatment. The rnp2, SG34 and psk mutants are shuttling proteins similar to native SR34. All mutants (excepted rnp1) shuttled upon LMB treatment but at a slower rate than in the absence of inhibitor.

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