Functional study of *Arabidopsis thaliana* ASF/SF2-like pre-mRNA SR splicing factors

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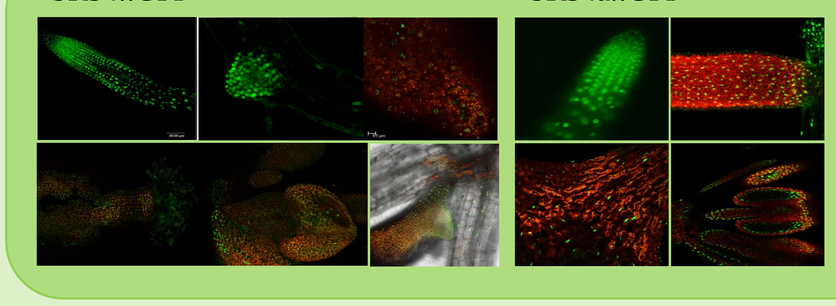
Introduction

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*.

Expression pattern using GFP fusion

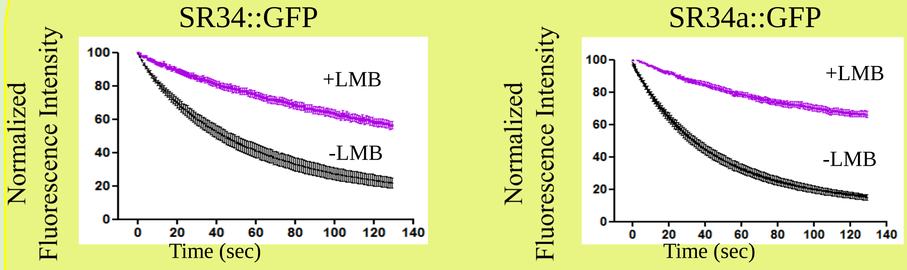
SR34::GFP SR34a::GFP



We have established the expression pattern of the SR34::GFP and SR34a::GFP fusion proteins controlled by there 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.

Nucleocytoplasmic shuttling by FLIP assay

SR34::GFP SR34a::GFP

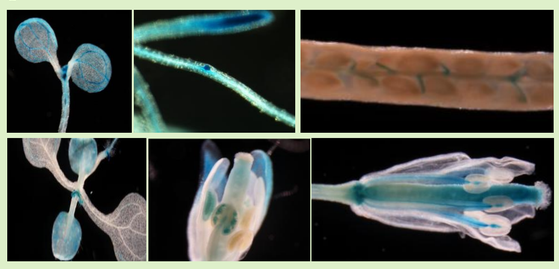


Nucleocytoplasmic shuttling of SR34 and SR34a was analysed in stably prom:SR::GFP Arabidopsis transgenics by confocal microscopy using cytoplasmic fluorescence loss in photobleaching (FLIP). FLIP-Shuttling monitoring in the absence (-LMB) and upon leptomicyn B (+LMB) treatment in root cells established that SR34 and SR34a are shuttling proteins. Indeed when a region of the nucleoplasm is repeatedly bleached, SR-GFP fluorescence gradually decreases in the nucleus. The nuclear inhibitor LMB strongly blocked the shuttling of these proteins, suggesting that they can exported through the atXPO1/CRM1 exportin (3,4).

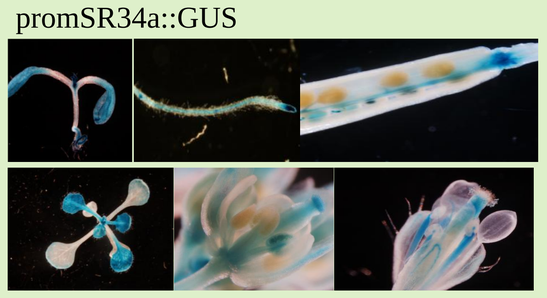
Expression pattern using promoter GUS reporter

GUS activity directed by SR34 and SR34a promoters was detected in root, leaves and floral tissues. GUS activity was observed in primary and lateral roots, in cotyledons (2 cotyledons stage only for SR34) and leaves. During floral developpment there expression was observed in sepals, and for SR34 in petals. SR34 and SR34a are expressed in stamen filaments, pollen sac, style and for SR34a also in stigma.

promSR34::GUS

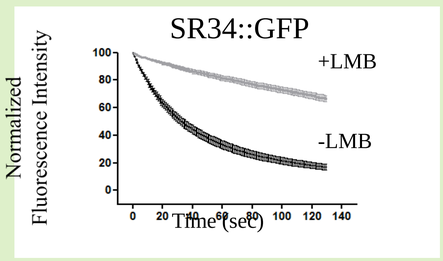


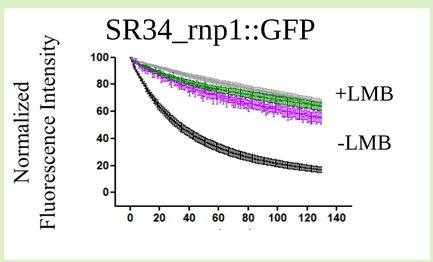
promSR34a::GUS

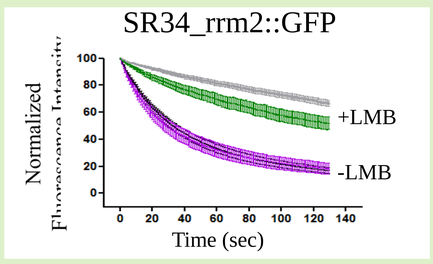


Dynamics and localization of native and mutant SR34 proteins

Dynamics



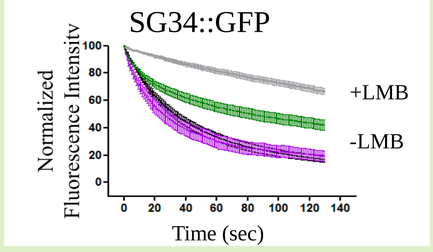


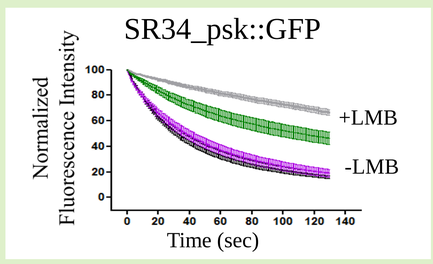


Structure

SR34 :	PG**Y**A**F**VEF	S**W**QDLKD... **F**	[RS-SR]	[PSK]
rnp1 :	PG**A**A**A**VEF	[RRM2]	[RS-SR]	[PSK]
rrm2 :	[RRM1]	S**A** QDLKD... **A**	[RS-SR]	[PSK]
SG34 :	[RRM1]	[RRM2]	**G**S-S**G**	[PSK]
TR34 :	[RRM1]	[RRM2]	R**T**-R**T**	[PSK]
TG34 :	[RRM1]	[RRM2]	**GT**-**TG**	[PSK]
psk :	[RRM1]	[RRM2]	[RS-SR]	[P**G**K]

SR34 contains two N-terminal RRM domains (blue). Two highly conserved motifs of six (RNP2) and eight (RNP1) amino acid residues are present in RRM1 domain. Conserved motif (SWQDLKD) is present in RRM2. The C-terminal RS domain (red) followed by PSK domain (orange). The mutagenized residues are indicated in bold.

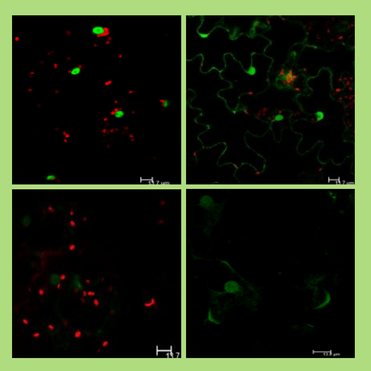




Cellular localisation

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 nucleus and/ or cytoplasm.

SR34::GFP SG34::GFP



TR34::GFP TG34::GFP

Comparison of nucleocytoplasmic shuttling between native and mutant SR34 proteins in tobacco leaf cells. FLIP-Shuttling was monitored in the absence (-LMB) and upon leptomicyn B (+LMB) treatment. Mutating the rnp1 motif (RRM1 domain) blocked the shuttling of SR34 in the absence of LMB treatment. The rrm2, 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.

FLIP SR34 wild-type
FLIP LMB SR34 wild-type
FLIP SR34 mutants
FLIP LMB SR34 mutants

Acknowledgements and references

(1) Kalyna M. and Barta A. (2004). *Biochemical Society*, 561-563.
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(3) Tillemans V. *et al* (2006) The Plant Cell, vol18. 3218-3234.
(4) Rausin G. *et al* (2010). Plant Physiology. Vol153. 273-284.

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