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Research Article

Co-localisation studies of *Arabidopsis* SR splicing factors reveal different types of speckles in plant cell nuclei

Zdravko J. Lorković[a,*], Julia Hilscher[a,b], Andrea Barta[a]

[a]Max F. Perutz Laboratories, Medical University of Vienna, Department of Medical Biochemistry, Dr. Bohrgasse 9/3, 1030 Vienna, Austria
[b]Institute of Applied Genetics and Cell Biology, University of Natural Resources and Applied Life Sciences, Muthgasse 18, A-1190 Vienna, Austria

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ABSTRACT

SR proteins are multidomain splicing factors which are important for spliceosome assembly and for regulation of alternative splicing. In mammalian nuclei these proteins localise to speckles from where they are recruited to transcription sites. By using fluorescent protein fusion technology and different experimental approaches it has been shown that *Arabidopsis* SR proteins, in addition to diffuse nucleoplasmic staining, localise into an irregular nucleoplasmic network resembling speckles in mammalian cells. As *Arabidopsis* SR proteins fall into seven conserved sub-families we investigated co-localisation of members of the different sub-families in transiently transformed tobacco protoplast. Here we demonstrate the new finding that members of different SR protein sub-families localise into distinct populations of nuclear speckles with no, partial or complete co-localisation. This is particularly interesting as we also show that these proteins do interact in a yeast two-hybrid assay as well as in pull-down and in co-immunopreciptiation assays. Our data raise the interesting possibility that SR proteins are partitioned into distinct populations of nuclear speckles to allow a more specific recruitment to the transcription/pre-mRNA processing sites of particular genes depending on cell type and developmental stage.

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Introduction

In both animal and plant cells the nucleoplasm contains numerous classes of "bodies" or compartments including the well characterized nucleolus, and those with less well defined function like Cajal bodies, gems, OPT domains, PML bodies and speckles [1-3]. Although the functional consequences of nuclear compartmentalization are not clear yet, emerging evidences suggest that it may have important roles in regulation of gene expression [4-8].

In mammalian and plant cells components of the pre-mRNA splicing machinery exhibit an irregular nuclear localization pattern, called speckled pattern or just speckles [2,9]. Speckles are diagnostic for proteins involved in pre-mRNA splicing; however, many other proteins like transcription factors, RNA polymerase II, and some proteins without ascribed function also localize to this sub-nuclear domain [9-11]. The function of speckles is still a matter of debate, although several lines of evidence support the idea that speckles are storage and/or assembly compartments from which splicing factors are recruited to sites of active transcription. Live-cell imaging showed that splicing factors are recruited from speckles to nearby transcription sites or that splicing factors accumulate in enlarged, rounded speckles upon inhibition of transcription or splicing. Transcription sites are often localised at the periphery of speckles and disassembly of speckles perturbs coordination between transcription and pre-mRNA splicing [4,5,9,12-14]. In addition, it has recently been

* *Corresponding author.* Fax: +43 1 4277 9616.
E-mail address: zdravko.lorkovic@meduniwien.ac.at (Z.J. Lorković).

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demonstrated that speckles in human cells consist of numerous sub-domains referred to as sub-speckles which are associated with sites of active transcription [15].

SR proteins are important splicing factors and have been shown to specifically regulate alternative splicing of certain transcripts [16-20]. In mammalian nuclei they have been found in speckles and were therefore extensively used to define this nuclear compartment [9]. The *Arabidopsis* genome encodes for 19 different SR proteins, a more complex set than found in any metazoan organism [18,21]. Depending on their domain organisation and evolutionary conservation *Arabidopsis* SR proteins can be classified into seven sub-families [18,21; see also Fig. 1A]. Compartmentalisation within the plant cell nucleus has only recently received more attention [2,3]. By using fluorescent protein [FP] fusion technology it has been shown that *Arabidopsis* SR proteins localise into an irregular nucleoplasmic network resembling speckles in mammalian cells. The same results have been obtained from (i) transgenic *Arabidopsis* plants expressing individual SR proteins under the control of the endogenous promoter [22], (ii) *Agrobacterium*-mediated transient expression in *Arabidopsis* or tobacco plants under CaMV 35S promoter [23,24], (iii) transgenic *Arabidopsis* plants expressing GFP-SR45 and RSp31-GFP under the control of CaMV 35S promoter [23-25], (iv) transiently transformed tobacco leaf mesophyll or *Arabidopsis* cell suspension protoplasts expressing SR proteins fused to GFP, RFP, YFP, and CFP under CaMV 35S promoter [26]. In addition, Docquier et al. [23] showed that in stably transformed *Arabidopsis* plants endogenous RSp31 co-localises with ectopically expressed GFP-tagged protein, indicating that irrespective of the system used the localisation of FP-tagged proteins likely corresponds to that of endogenous proteins. It has also been shown that speckles in plant cells are dynamic structures responding to treatments with phosphatase, kinase and transcription inhibitors [22-25,27]. By using FRAP and FLIP technology it has been demonstrated that SR proteins in plant cells, are continuously moving between speckles and surrounding nucleoplasm [22-24,27,28]. The number of speckles, their appearance (size and density), and the ratio of the signal in speckles compared to nucleoplasm were shown to vary between different cell types [22,23,25,26]. Meristematic cells or otherwise rapidly growing cells had the highest number of small speckles with most of the FP fusion proteins found in a diffuse nucleoplasmic pool. In contrast, highly differentiated cells (e.g. leaf mesophyll or epidermal cells) showed larger speckles and less FP fusion proteins in a diffuse nucleoplasmic pool. Therefore, it has been proposed that the distribution of

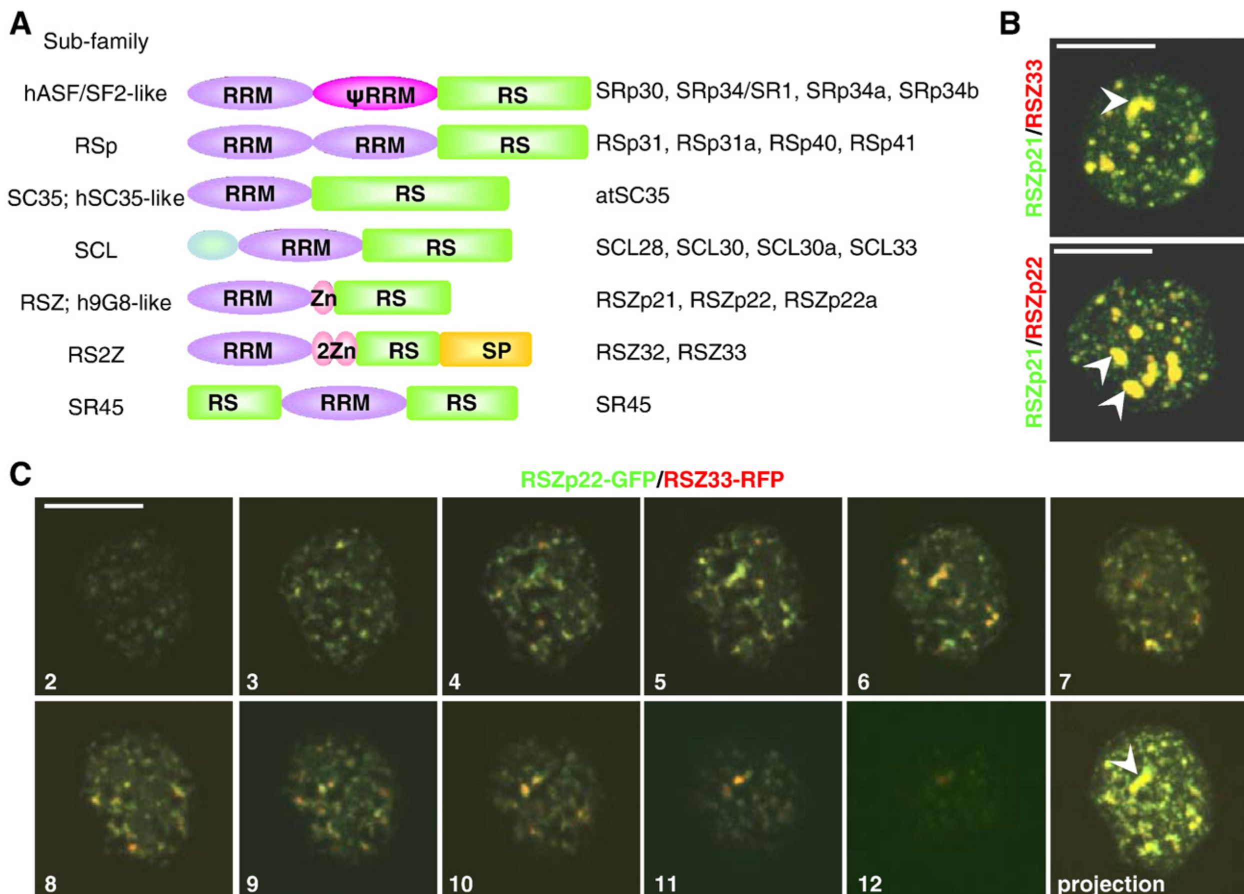

Fig. 1 – Co-localisation of zinc knuckle-containing *Arabidopsis* SR proteins in transiently transformed tobacco protoplasts. (A) Schematic representation of *Arabidopsis* SR proteins. RRM, RNA recognition motif. ψRRM, pseudo RRM. RS, arginine/serine-rich domains. Zn, zinc knuckles. SP, serine/proline-rich domain. (B) Maximum intensity projection images of nuclei from cells co-expressing RSZp21-GFP and RSZ33-RFP (upper panel) and RSZp21-GFP and RSZp22-RFP (lower panel). (C) Eleven out of fourteen merged serial confocal sections of the nucleus co-expressing RSZp22-GFP and RSZ33-RFP are shown. Last image (projection) is the maximum intensity projection of all fourteen images collected. Arrowheads point to larger speckles. Scale bars, 7 μm.

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