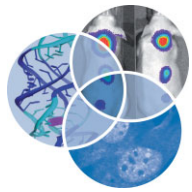
Advanced Review



Plant serine/arginine-rich proteins: roles in precursor messenger RNA splicing, plant development, and stress responses

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Global analyses of splicing of precursor messenger RNAs (pre-mRNAs) have revealed that alternative splicing (AS) is highly pervasive in plants. Despite the widespread occurrence of AS in plants, the mechanisms that control splicing and the roles of splice variants generated from a gene are poorly understood. Studies on plant serine/arginine-rich (SR) proteins, a family of highly conserved proteins, suggest their role in both constitutive splicing and AS of pre-mRNAs. SR proteins have a characteristic domain structure consisting of one or two RNA recognition motifs at the N-terminus and a C-terminal RS domain rich in arginine/serine dipeptides. Plants have many more SR proteins compared to animals including several plant-specific subfamilies. Pre-mRNAs of plant SR proteins are extensively alternatively spliced to increase the transcript complexity by about six-fold. Some of this AS is controlled in a tissue- and development-specific manner. Furthermore, AS of SR pre-mRNAs is altered by various stresses, raising the possibility of rapid reprogramming of the whole transcriptome by external signals through regulation of the splicing of these master regulators of splicing. Most *SR* splice variants contain a premature termination codon and are degraded by up-frameshift 3 (UPF3)-mediated nonsense-mediated decay (NMD), suggesting a link between NMD and regulation of expression of the functional transcripts of SR proteins. Limited functional studies with plant SRs suggest key roles in growth and development and plant responses to the environment. Here, we discuss the current status of research on plant SRs and some promising approaches to address many unanswered questions about plant SRs. *WIREs RNA* 2011 2 875–889 DOI: 10.1002/wrna.98

INTRODUCTION

A majority of protein-coding genes (up to 90%) in photosynthetic eukaryotic organisms contain noncoding introns.[1,2] As the transcriptional machinery synthesizes precursor messenger RNAs (pre-mRNAs), the introns are precisely removed from pre-mRNAs and the coding sequences (exons) are joined by two transesterification reactions. In constitutive splicing (CS), only one set of splice sites is used in all pre-mRNAs synthesized from a gene. Alternative splicing (AS), which generates two or more mature mRNAs from a single gene, uses different combinations of splice sites resulting in different pre-mRNAs produced from the same gene. Recent studies have shown that pre-mRNAs from about 95% of multiexon genes in humans undergo AS.[3,4] Similarly, in plants pre-mRNAs of over 40% of intron-containing genes produce more than one splice variant by AS.[5,6] AS is considered to play an important role in increasing transcriptome complexity and likely proteome diversity in multicellular eukaryotes.[1,7–9] Furthermore, AS is thought to play an

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important role in regulating the amount of functional protein by controlling the levels of functional and nonfunctional mRNAs through nonsense-mediated decay (NMD), recruitment of mRNA to ribosomes, and modulation of the translation efficiency of transcripts.[1,10,11] The proteins produced from splice variants may have an altered function (altered activity, altered interaction with its partners or ligands), stability, and/or cellular localization,[12] thereby generating functionally different proteins from the same gene.

Pre-mRNA splicing takes place in a large complex called the spliceosome that is made up of several uridine-rich small nuclear RNAs (UsnRNAs) and more than 300 proteins.[13,14] The core of the spliceosome consists of five small nuclear ribonucleoproteins (U1, U2, U4, U5, and U6 snRNPs) that are highly conserved between plants and metazoans.[15] In addition to snRNPs, numerous non-snRNP proteins are also involved in the spliceosome that play important roles in spliceosome assembly and regulated splicing. The spliceosome is a highly dynamic structure, which continually rearranges its components during various steps of splicing.[14] These rearrangements primarily occur in the five major snRNPs. Among the non-snRNP proteins, a family of proteins called serine/arginine-rich (SR) proteins are the most extensively studied in animals and to a lesser degree in plants.[1,9,16] SR proteins were originally identified in metazoans in 1990 as factors necessary for the splicing of pre-mRNA substrates in *in vitro* assays (reviewed in Ref 17). These proteins are evolutionarily conserved at the structural and functional level. All SR proteins are characterized by the presence of one or two RNA recognition motifs (RRMs) located in the N-terminus and a domain rich in serine and arginine dipeptides (SR) in the C-terminus.[18,19] In animal systems, it has been well established that SR proteins bind pre-mRNAs and function as activators or repressors of both CS and AS.[16] The RRMs bind specific RNA sequences (*cis*-acting elements that either enhance or suppress splicing) in pre-mRNA, whereas the arginine/serine-rich (RS) domain functions as a protein–protein interaction module to recruit other proteins and in some cases contacts the pre-mRNA branch point.[16,20] In addition, the RS domain harbors signals for nuclear and subnuclear localization and nucleocytoplasmic shuttling. Although the study of plant SRs is in its infancy, significant progress has been made in the last few years. Our goal here is to review the progress on various aspects of plant SRs, identify critical gaps in this area, and discuss some directions for future investigations.

PLANT SR PROTEINS

Plant SR proteins were first identified in 1996 using monoclonal antibodies to animal SR proteins, complementation assays with S100 splicing-deficient HeLa cell extract, and homology-based cloning.[21–23] Some SR proteins were isolated as interacting partners of U1 snRNP-70K, a U1 snRNP-specific protein.[24,25] The completion of the Arabidopsis genome sequence in 2000 and other plant genome sequences since then has led to the identification of a complete inventory of SR proteins in different plants using bioinformatic analyses.[26,27] Our recent analysis of SR proteins from 27 different sequenced organisms consisting of plants, animals, and unicellular eukaryotes revealed that the number of SR proteins varies between different groups of organisms. However, plant genomes in general encode many more SR proteins than animals (Richardson and Reddy, manuscript in preparation). For instance, Arabidopsis and rice have 18 and 22 SR proteins, respectively, whereas humans have 12 SR proteins. Soybean has the most SRs (25) of any sequenced organism. Phylogenetic and comparative analyses of plant SRs revealed six subfamilies (SR, SC, RSZ, SCL, RS2Z, and RS), and three of these subfamilies (SR, SC, and RSZ) have orthologs in mammalian systems while the other three (SCL, RS2Z, and RS) are specific to plants with some unique domains[19] (Figure 1). Ten of the Arabidopsis SRs fall into plant-specific subfamilies (Figure 1). The plant-specific SCL subfamily (SC35-like) has an RRM that is similar to SRSF2 (formerly called SC35) with a charged extension at the N-terminus. The plant-specific RS2Z subfamily members have two Zn-knuckles and an additional SP-rich region following the RS domain. The plant-specific RS subfamily members contain two RRMs but lack a phylogenetically conserved heptapeptide (SWQDLKD) motif in the second RRM, a characteristic feature of the SR subfamily. In addition, the RS domain of this subfamily is rich in RS dipeptides. The differences between plants and animals in intron/exon architecture and plant-specific *cis*-elements involved in pre-mRNA splicing could be the reason for plant-specific subfamilies of SRs.[28] Several SRs in plants have paralogs due to whole-genome and segmental duplications, a common feature in plants.[19,27] Whether the large number of SRs in flowering plants is simply a reflection of genome duplication events or if it points to the importance of AS regulation in plant growth, development, and responses to stresses remains to be seen. It is not known if the paralogs are functionally redundant or have evolved to perform nonredundant functions. Analysis of the expression of paralogous

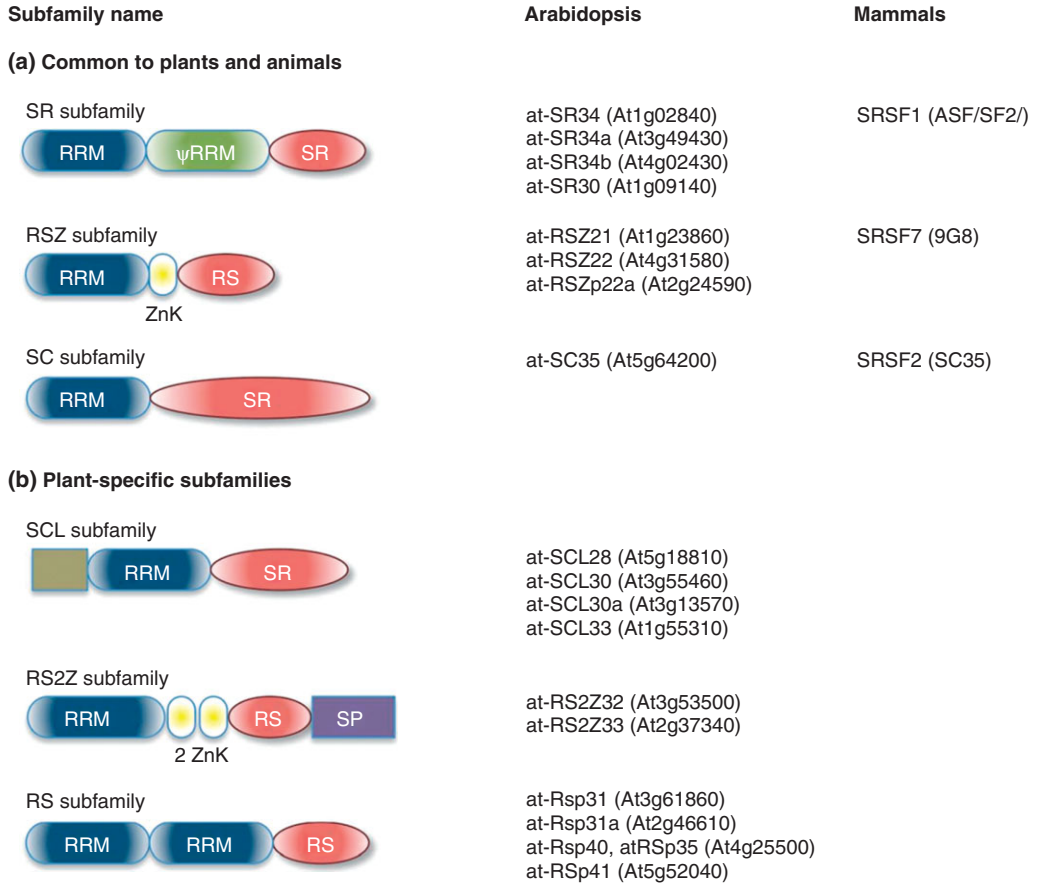

FIGURE 1 | Schematic diagram showing different domains and their organization in different subfamilies of plant serine/arginine-rich (SR) proteins. The Arabidopsis SR proteins in each subfamily and corresponding human orthologs are shown at the right of the schematic diagram. The old names of mammalian SRs are shown in parenthesis. In addition to the three mammalian SRs that are shown here, there are nine other SRs (SRSF3, 4, 5, 6, 9, 10, 11, and 12)[18] that have no orthologs in flowering plants. RRM, RNA recognition motif. (Reprinted with permission from Ref 19. Copyright 2010 American Society of Plant Biologists)

pairs in Arabidopsis and rice in different tissues and developmental stages indicates that one of the two paralogs in each pair is preferentially expressed, suggesting that one of the paralogs in each pair may be redundant (Richardson and Reddy, manuscript in preparation).

ALTERNATIVE SPLICING OF *SR* pre-mRNAs

Gene ontology analyses of alternatively spliced genes and targeted studies on genes encoding splicing regulators have shown that pre-mRNAs of splicing regulators, especially SR proteins, undergo extensive AS.[1,29–35] Analysis of AS of all Arabidopsis *SR* genes using RT-PCR has revealed that pre-mRNAs from 14 of the 18 genes produce more than 90 transcripts, suggesting a six-fold increase in transcripts by AS[30] (Figure 2). Only four SRs (*RSZ21, RSZ22, RSZ22a*, and *SCL28*) showed no AS, three of which belong to the RSZ family. Interestingly, the mammalian ortholog of the RSZ family (SRSF7/9G8) undergoes AS,[36,37] suggesting AS in this family is not conserved between plants and animals. Extensive AS in plant *SR* genes is not due to a high rate of missplicing of pre-mRNAs in plants as AS analysis in another gene family containing 26 genes with multiple introns with sizes similar to introns in *SR* genes revealed no AS.[38] In addition, AS of SR pre-mRNAs is regulated in a tissue- and development-specific manner. Pre-mRNAs from rice *SR* genes also undergo extensive AS.[31] Analysis of DNA methylation of *SR* genes and another gene family in which no AS is observed indicates that methylation is linked to AS (Richardson and Reddy, manuscript in preparation). All types of AS events are observed with intron retention as the most prevalent and exon skipping as the least prevalent AS event. AS due to alternative acceptor motif (NAGNAG) was also found to occur in Arabidopsis SRs and this appears to be

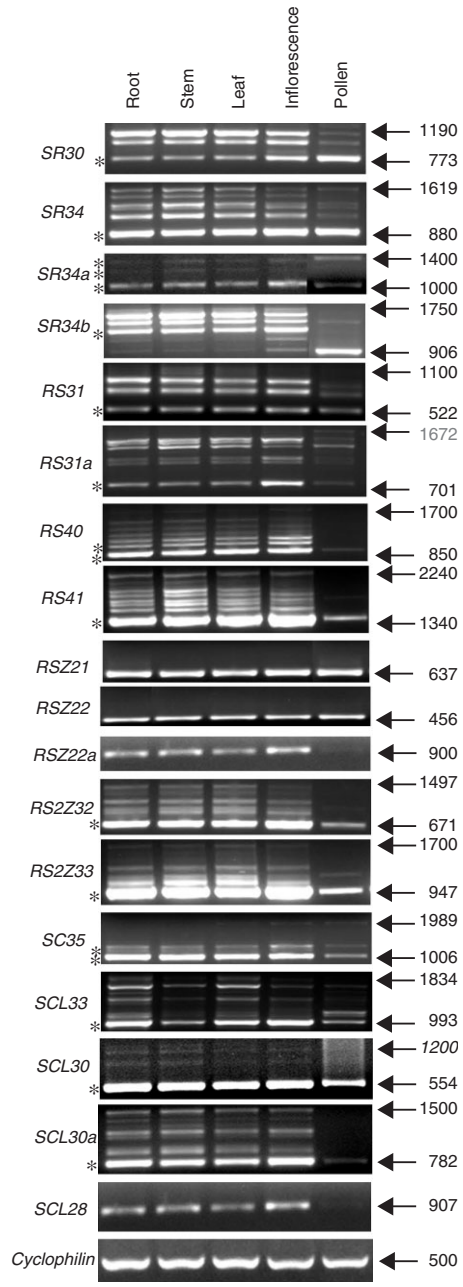

FIGURE 2 | Analysis of expression and splicing of Arabidopsis serine/arginine-rich (*SR*) genes in root, stem, leaf, inflorescence, and pollen. An equal amount of cDNA was used in polymerase chain reaction (PCR) with primers specific to each *SR* gene. An equal amount of template in each reaction was verified by amplifying a constitutively expressed cyclophilin. The name of the *SR* gene is shown on the left of each panel. Asterisks indicate the transcripts that encode full-length proteins. Arrows indicate DNA sizes in bp. (Reprinted with permission from Ref 30. Copyright 2007 Blackwell Publishing)

regulated by the developmental stage of plants as well as stresses.[39] Much of AS in *SR*s is confined to introns in the coding region. The extent of AS in plant SRs is much higher than in humans.[40]

Sequence analysis of all splice variants has revealed that more than 50 putative proteins, many of which lack one or more domains, can be produced by AS (Figure 3). As SRs have modular domains it is possible that proteins produced from splice variants may have different functions including potential dominant-negative roles of truncated proteins. However, currently there is little experimental evidence showing that splice variants produce proteins *in vivo*.[41] Analyses of recruitment of splice variants to ribosomes could provide some insights into this. However, the presence of a splice variant in polysomes does not provide conclusive evidence that they are translated as most of the NMD targets go through pioneering round of translation. Hence, studies with predicted isoform-specific antibodies or expression of individual splice variants in knockout background as fluorescently tagged proteins at the C-terminus of splice variants are needed. A large number of splice variants (about 55) contain a premature termination codon (PTC) at more than 50 nucleotides upstream of an exon–exon junction. In animals, transcripts with a PTC located >50 nucleotides upstream of an exon–exon junction are targeted for degradation through NMD, an mRNA surveillance and destruction system that detects aberrant mRNAs and degrades them,[42,43] suggesting that PTC-containing SRs are likely degraded by the NMD pathway. Furthermore, studies in mammalian systems suggest that PTC-containingtranscripts of SRs may regulate the level of functional transcripts through a mechanism called regulated unproductive splicing and translation (RUST).[40,44] Analysis of all splice variants for each alternatively spliced Arabidopsis *SR* pre-mRNA in a mutant that lacked up-frameshift 3 (UPF3), one of the core components of the NMD machinery, has revealed that about half of the 53 splice variants with a PTC produced in seedlings are the targets of degradation by NMD (Figure 4), suggesting that in plants, as in animals, RUST may play a role in regulating the level of functional transcripts of SRs.[40,45] Interestingly, the accumulation of PTC-containing transcripts resulted in concomitant reduction in the amount of functional transcript, suggesting a strong coupling between the level of PTC-containing transcripts and functional mRNA encoding the full-length protein. Wild-type seedlings treated with cycloheximide, a known inhibitor of NMD,[40,46,47] showed accumulation of the same PTC-containing transcripts that were increased in the *upf3* mutant,[45] indicating that the pioneering round of translation is necessary for degradation of these transcripts. The PTC-containing transcripts that were accumulated in the *upf3* mutant are detectable in the

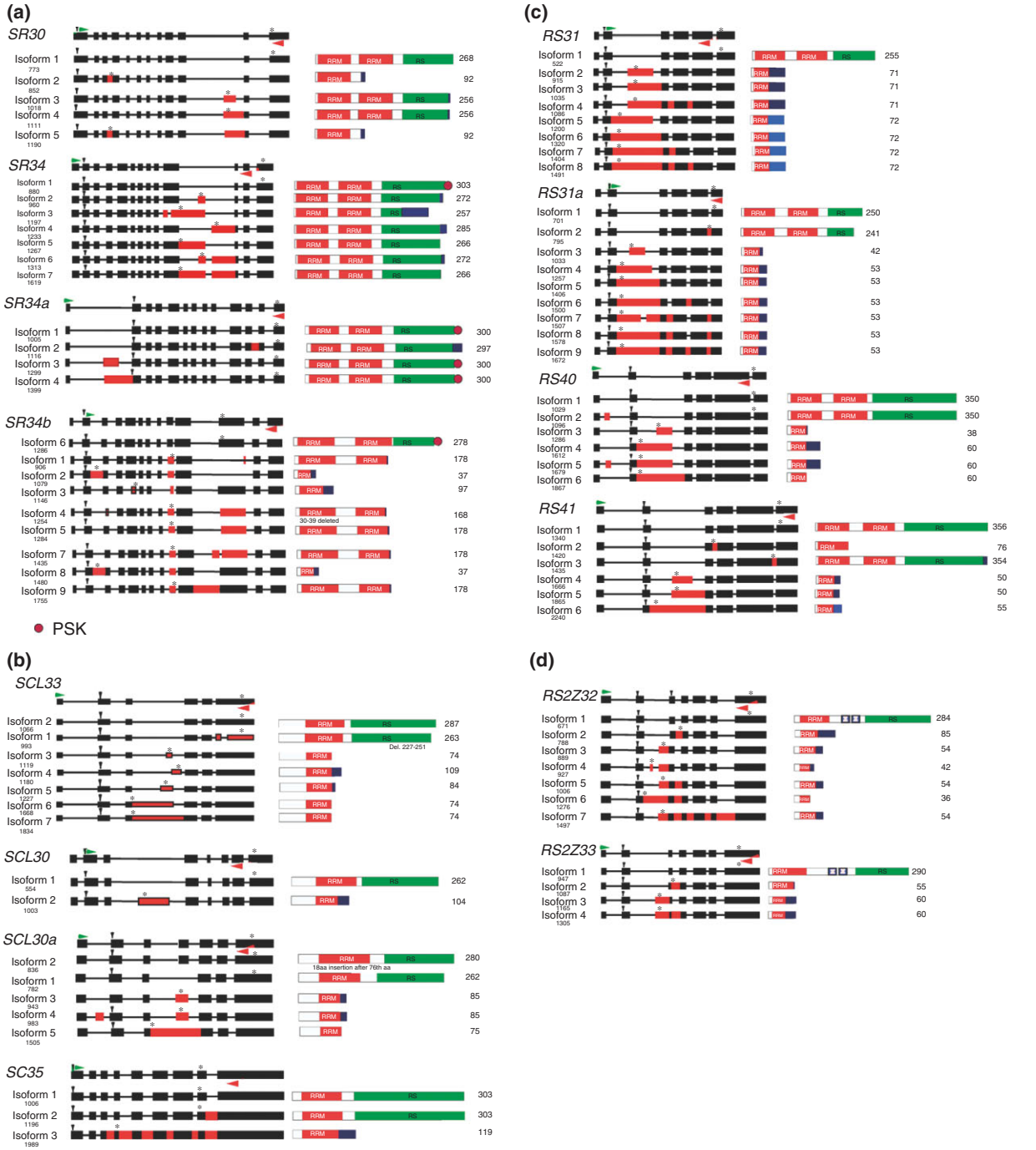

FIGURE 3 | Schematic diagrams of alternatively spliced transcripts and predicted proteins from splice variants of Arabidopsis serine/arginine-rich (*SR*) genes. Genes encoding SR subfamily (a), SC35 and SCL subfamilies (b), plant-specific arginine/serine-rich (RS) subfamily (c), and plant-specific RS2Z family SRs (d). The name of the *SR* gene is shown on the left of each panel. The schematic diagram for each gene shows the gene structure and its splice variants. (Numbers below each isoform indicate the length of transcript in nucleotides.) Isoforms are numbered in ascending order according to the size (isoform 1 represents the smallest transcript). In all cases, except *SR34b*, *SCL33*, and *SCL30a*, the smallest transcript (isoform 1) encodes a full-length protein. Predicted proteins from splice variants are shown to the right of each isoform. Exons are filled rectangles and introns are thin lines. Black rectangles represent constitutively spliced exons, whereas the red rectangles indicate the included regions in splice variants. Vertical arrowhead and '*' show start and stop codons, respectively. Horizontal green and red arrowheads above and below gene structures indicate the position of forward and reverse primers, respectively. In the schematics of predicted proteins, numbers to the right are the number of amino acids in the protein. PSK, a domain rich in proline, serine, and lysine; RRM, RNA recognition motif. Blue rectangle indicates a stretch of amino acids that are not present in functional SR proteins. (Reprinted with permission from Ref 30. Copyright 2007 Blackwell Publishing)

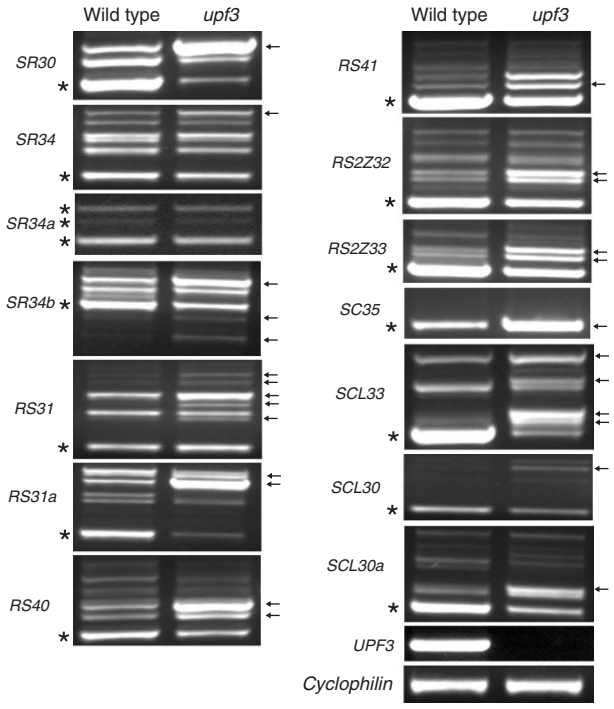

FIGURE 4 | Splice variants generated from serine/arginine-rich (*SR*) genes in Arabidopsis wild-type and *upf3* mutant plants. Forward and reverse primers corresponding to first and last exons, respectively, were used in RT-PCR. The name of the gene is shown on the left of each gel. A constitutively expressed cyclophilin was used to verify an equal amount of template. Asterisks indicate the transcript(s) for each gene that encodes a full-length protein. Splice variants with a premature termination codon (PTC) that are accumulated in the mutant are denoted with arrows. (Reprinted with permission from Ref 45. Copyright 2010 Blackwell Publishing)

wild type, suggesting that NMD does not remove the PTC-containing transcripts entirely. It may be that the PTC-containing transcripts perform some function, hence are not degraded completely. The fact that half of the PTC-containing transcripts are not accumulated in the UPF3 mutant suggests that not all transcripts with a PTC are degraded by the UPF3 pathway. There may be other pathways that degrade these transcripts. For example, in plants and yeast there are other mechanisms of degradation of PTC-containing transcripts that are independent of the exon–exon junction complex.[48–52] It is also possible that some PTC-containing transcripts evade NMD and produce proteins with altered functions. As SR proteins are highly modular with multiple functional domains, it may be that the truncated versions that lack one or more domains may have altered functions. Dominant-negative effects of truncated SR proteins on splice-site choice have been well documented.[53,54] It is interesting that AS location and events are quite conserved from algae to flowering plants in some *SR* genes, suggesting the functional significance of those events (reviewed by Barta et al.[55]).

REGULATION OF AS OF SR pre-mRNAs BY STRESSES

Abiotic and biotic stresses are some of the key determinants of plant growth and development and productivity. It is well established that plants adapt to various stresses by reprogramming their transcriptome by inducing specific genes and repressing others.[56–58] Most of the stress-regulated genes were analyzed for steady-state mRNA accumulation. It is likely that some of the effects of stresses on gene expression could be due to regulation of different splice variants. Gene ontology analysis of all alternatively spliced genes has shown that the genes involved in stress responses are overly represented.[5,29,59] Interestingly, AS of plant SR pre-mRNAs is dramatically altered in response to various abiotic stresses, whereas hormones affected splicing of a few genes.[30,34] New splice variants have appeared in response to some stresses. In addition, some splicing products are either increased or decreased by abiotic stresses. Figure 5 shows changes in splicing patterns of Arabidopsis *SR* genes in response to cold and heat stress. These observations suggest that altered ratios of splice variants in response to stresses may have a role in adaptation of plants to these stresses. The changes in the levels of master regulators of splicing in response to stresses may alter the splicing of many other pre-mRNAs, including auto- and cross-regulation of splicing of SR pre-mRNAs.[1,60] Pre-mRNAs of several genes involved in biotic and abiotic stresses are also alternatively spliced (reviewed by Reddy[1]; Ali and Reddy[60]). From the list of SRs affected by stresses, it is clear that AS of some SRs is affected specifically by one and not the other stresses, whereas AS of some SRs is affected by multiple stresses.[30] Cold and other stresses have been shown to affect AS profiles of Arabidopsis genes.[59] There is also evidence for auto- and cross-regulation of splicing of *SR* genes in plants.[32,33,41] How stresses regulate AS of SR pre-mRNAs is unknown. One possibility is that stress-regulated protein kinases and phosphatases may control the phosphorylation status of SRs and other spliceosomal proteins, which in turn regulate AS. In animals, heat shock represses splicing by dephosphorylating SRSF10 (formerly called SRp38).[61] Interestingly, the intranuclear distribution of several SR proteins is regulated by phosphorylation and stresses such as heat and cold (see section on *Spatiotemporal Organization of Plant SR Proteins*).

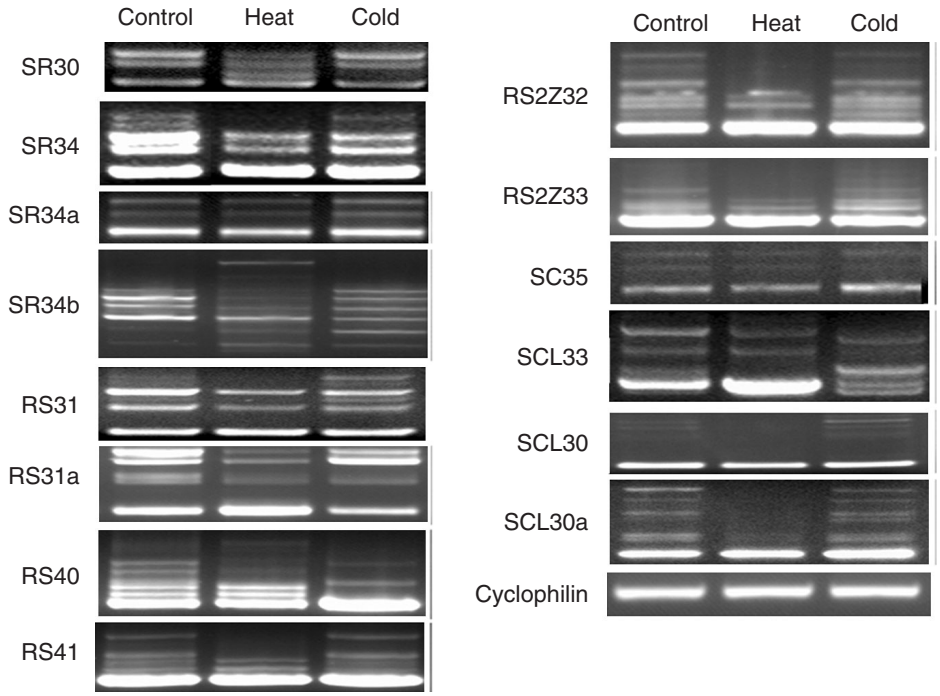

FIGURE 5 | Effect of heat and cold on serine/arginine-rich (*SR*) genes' expression and alternative splicing. Two-week-old seedlings were treated with cold at 4°C for 24 h and heat at 38°C for 6 h. RNA from control and treated samples was used for RT-PCR. (Reprinted with permission from Ref 30. Copyright 2007 Blackwell Publishing)

INTERACTIONS AMONG PLANT SRs AND BETWEEN SRs AND OTHER PROTEINS

Yeast two-hybrid (Y2H) screens/assays and *in vitro* pull-down studies have revealed extensive interaction among SR proteins and between SRs and many spliceosomal proteins involved in major and minor spliceosomes, proteins involved in transcription, as well as protein kinases that phosphorylate SR proteins,[1,24,25,55,62–65] suggesting that the functions of SR proteins depend on their interaction with numerous other proteins. U1-70K, a U1 snRNP-specific protein, interacts with several plant SRs (SR34, RSZ21, RSZ22, and SCL33) indicating that these SRs may bind to splicing regulatory *cis*-elements and recruit U1 snRNP to the 5′ splice site.[24,25,65] Some SRs interact with U2AF subunits suggesting that they can bind pre-mRNAs and recruit U2AF to the 3′ splice site.[62] The interaction of plant SRs with U11-35K, a U11 snRNP-specific protein, suggests that SRs can recruit U11 snRNP to the 5′ splice site during the assembly of the minor spliceosome.[62] Some of these interactions are confirmed in plants whereas others are yet to be confirmed. Also, the physiological significance of most of these interactions is not known.

FUNCTION OF PLANT SR PROTEINS IN SPLICING

On the basis of what is known in animals, it is thought that SR proteins bind to *cis*-acting regulatory sequences in exons (exonic splicing regulators, ESRs) and/or introns (intronic splicing regulators, ISRs) and recruit U1 snRNP to the 5′ splice site and U2AF to the 3′ splice site and/or bridge the interaction between components bound to the 5′ and 3′ splice sites (Figure 6). The biochemical characterization of plant SR proteins, especially the identification of *cis*-elements involved in splice-site choice using *in vitro* assays, is hindered by the lack of a plant-derived *in vitro* splicing system. Nevertheless, using the splicing-deficient HeLa cell S100 extract and model pre-mRNA substrates, it was shown that several plant SR proteins are functional in splicing alluding to their functional conservation across eukaryotes.[1,21,22,33,66,67] Using a variety of approaches, several reports have shown that plant SRs are involved in regulating splice-site choice[31,32,34,41] (reviewed by Reddy[1]). However, very little is known about the sequence elements in pre-mRNA that bind to specific SR proteins. Experimentally very few sequences that are responsible for AS were identified in plants.[68–70] Using gel shift assays with probes containing *cis*-elements for different animal SRs, it

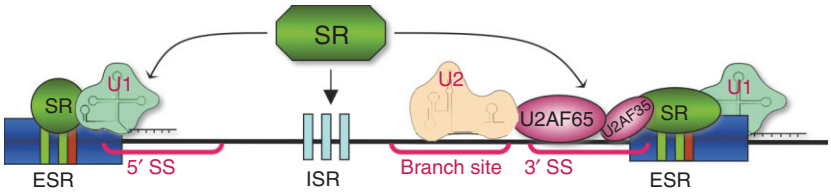

FIGURE 6 | Roles of plant serine/arginine-rich (SR) proteins in precursor messenger RNAs (pre-mRNAs) splicing. SR proteins bind to sequences in exons, called exonic splicing regulators (ESRs), and then recruit and stabilize U1 small nuclear ribonucleoprotein (snRNP) on the 5′ splice site (5′ ss) and the heterodimeric U2AF complex to the 3′ splice site (3′ ss) and U2 snRNP to the adjacent branch point. They also mediate interaction between the U2AF complex and U1 snRNP across an exon or by binding to RNA sequences called intronic splicing regulators (ISRs) in introns to mediate interaction between the U2AF complex and U1 snRNP across introns.

was shown that RSZ22 binds to sequences that are known to bind animals SRs 9G8 and SR20.[66] Using a computational approach to identify exonic splicing enhancers (ESEs) in Arabidopsis, Pertea et al.[71] identified 35 hexamers in exons, which have been shown to cause exon inclusion. However, it is not known if any of the Arabidopsis SRs bind to these ESEs. As described earlier, the protein–protein interaction data strongly suggest that SRs play an important role in recruiting U1 snRNP and U2AF to the 5′ and 3′ splice sites, respectively.

REGULATION OF SR PROTEIN ACTIVITY

The serine residues in the RS domain of animal SR proteins are extensively phosphorylated and the phosphorylation status of the SRs is highly regulated. This post-translational modification has an important regulatory role in subcellular localization of SRs and their interaction with other proteins and pre-mRNA targets.[16] Proteins kinases that belong to the SRPK (SR protein kinase) and the Clk/Sty family and long nuclear-retained regulatory RNA (nrRNA) have been shown to regulate the phosphorylation of animal SR proteins.[16,72] As plant SRs, like animal SRs, contain an RS domain with SR dipeptides and plants have homologs of protein kinases that phosphorylate SRs, it is thought phosphorylation of plant SRs is likely to regulate their activities. Using phosphoproteomic analysis, it was found that many Arabidopsis SR proteins are phosphorylated *in vivo*.[73,74] However, with the exception of a few biochemical analyses of some SR proteins,[25,75] the regulation of SR proteins by phosphorylation remains to be explored in plants. A Clk/Sty protein kinase, arabidopsis fus3-complementing gene 2 (AFC2), has been shown to interact with and phosphorylate several SR proteins and the interaction between SRs and AFC2 is modulated by phosphorylation.[25] Interestingly, one of the kinases, the tobacco LAMMER type PK12 that interacts with and phosphorylates SR34, has been shown to be induced by ethylene, suggesting that this hormone through PK12 might regulate splicing. Beyond showing that these kinases were localized to the nucleus,[75] nothing is known about their regulation. The mitogen-activated protein (MAP) kinases MPK6 and/or MPK3, which are activated by many stresses, also phosphorylate several SR proteins,[74] providing a possible link between stresses and the regulation of splicing. As several plant stresses are mediated through MAP kinases, it is likely that they fine tune specificity to stress responses.

SPATIOTEMPORAL ORGANIZATION OF PLANT SR PROTEINS

It is now widely accepted that animal and plant nuclei are compartmentalized into structurally and functionally distinct nonmembranous subnuclear domains.[76–78] Recently, *in vivo* approaches using fluorescent protein-tagged SR proteins have been used to investigate the subcellular localization and dynamics of SR proteins and the regulation of these processes in plant cells.[79–87] The availability of advanced fluorescence microscopes equipped with laser scanning modules that can monitor protein mobility in living cells together with the development of mathematical models to analyze the kinetics of mobility has provided insights into how plant SR proteins are functionally linked to transcription, splicing, and other cellular processes. Among various approaches used, fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) have been widely used in investigating the dynamics of plant SR proteins.[79] Collectively, these studies show that much like in animal systems all plant SR proteins, including the plant-specific ones, are distributed in a characteristic pattern of concentrated nuclear regions termed ‘speckles’, and in diffused nucleoplasmic regions. Consistent with observations in animal

cells, using transmission electron microscopy (TEM) some plant SR proteins were also shown to correspond to interchromatin granule clusters, reiterating conservation of structural–functional organization of SR proteins between plants and animals.[84] Nuclear speckles are thought to be storage, assembly, and/or modification sites of splicing factors. Surprisingly, coexpression of SRs that belong to different subfamilies showed distinct population of speckles with little or no colocalization, indicating the existence of different types of speckles that differ in the types of SRs they contain.[85] Furthermore, some SRs that are known to interact in the Y2H and pull-down assays did not colocalize in plant cells, suggesting that the results from Y2H and *in vitro* assays cannot be directly extrapolated to *in vivo* situation and that there are other regulatory mechanisms at play in *in planta* interactions.

The localization patterns of SR proteins also seem to depend on the metabolic/physiological states of cells. Using heat shock and pharmacological inhibitors of transcription, it has been shown that several SR proteins, RSZ22, RS31, SR34, SR30, and SCL33, accumulate in enlarged and irregularly shaped speckles.[80,81,86] Interestingly, heat also changed the AS pattern of several *SR* genes suggesting that subcellular localization might be one of the mechanisms that regulate AS.[30] As SRs activity is regulated by their reversible phosphorylation, these studies show that the inhibition of phosphorylation led to their accumulation in speckles. Furthermore using FRAP and FLIP analyses on phosphorylation-inhibited cells, it has been shown that the exchange of SR proteins between speckles and nucleoplasm requires phosphorylation.[80] Altogether, these studies reveal that the mobility of SR proteins is coupled to transcription and phosphorylation. These observations suggest that in the absence of transcription, and hence splicing, SR proteins stay in the speckles and that the recruitment of SR proteins to splicing sites, which are thought to be scattered throughout the nucleoplasm, requires their phosphorylation. Using these approaches, several groups have shown that a majority of plant SR proteins exchange constantly between speckles and nucleoplasm.[80,86] In addition, domain deletions and point mutations combined with pharmacological inhibitors are also providing clues as to how the distribution of SR proteins is regulated at the molecular levels.[87] These reports indicate that the speckle-targeting/retention signals and nuclear localization signals are most likely located in the RS domains. Some SRs are localized to the nucleolus and some shuttle between the nucleus and the cytoplasm.[83,86] Nucleolar localization of RSZ-22 was shown to be regulated by stress, ATP, and phosphorylation.[87] It is not known what roles the nucleolar-localized plant SRs play.

FUNCTIONS OF SR PROTEINS IN PLANT GROWTH AND DEVELOPMENT

Extensive genomic analyses in dicots and monocots have revealed that a majority of genes involved in almost all biotic and abiotic stress responses and developmental processes, including vegetative growth, flowering, fruit and seed development, senescence, and seed dormancy, possess multiple exons and several of them produce more than one isoform.[5,29] Given the essential role played by SR proteins in both CS and AS, it is likely that they will be instrumental in regulating various developmental processes. The use of loss-of-function mutants as well as overexpression of SRs in understanding their role in plant growth and development and stress responses should yield some useful information. So far, there are only a few reports on overexpression of SRs.[31,41] Analyses of overexpressor lines of two Arabidopsis SRs (*SR30* and *RSZ33*) have revealed pleiotropic phenotypes, which is consistent with their role as regulators of splicing.[32,41] Analysis of knockout mutants in plant SRs has not been performed yet. As many plant SRs have paralogs, it is likely that single mutants may not show phenotypes, hence it would be necessary to generate single/double/triple mutants to analyze SRs function.

In addition to studies on SR proteins, an SR-related protein (SR45) has been extensively characterized in plants. SR45 was isolated in a Y2H screen with U1 snRNP 70K protein.[25] Unlike SRs, it has two RS domains flanking the RRM. A loss-of-function *sr45* mutant displayed multiple phenotypes including delayed flowering (Figure 7A), reduced root growth, and abnormal floral organs.[88] The AS pattern of several other *SR* genes was also changed in the *sr45* mutant (Figure 7B),[88] suggesting that a change in the ratio of AS isoforms of other *SR* genes likely affects multiple phenotypes. The Arabidopsis *SR45* produces two alternatively spliced isoforms that differ in 21 nucleotides in the coding region, which are predicted to produce two distinct proteins that differ in 8 amino acids.[30] Interestingly, the long isoform complemented the defects in flower petals and not root growth, whereas the short isoform complemented the root growth phenotype but not petal defects, suggesting that each isoform has a specific function.[89] In addition to developmental defects, *sr45* mutant

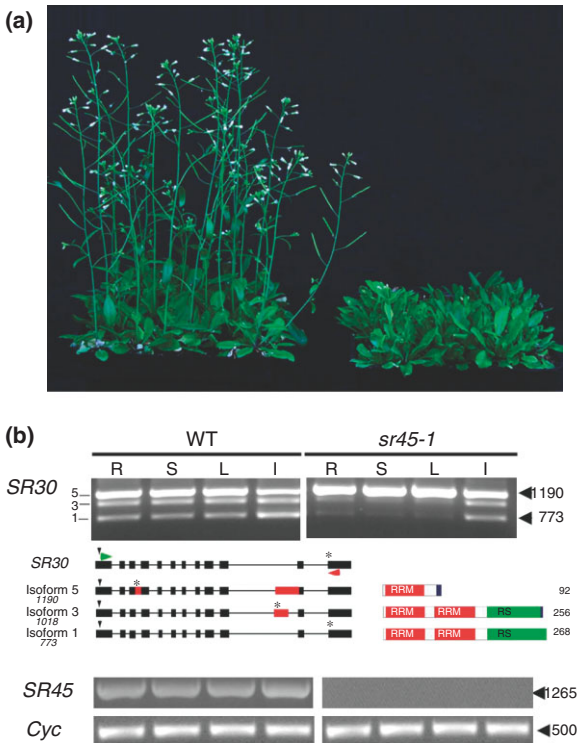

FIGURE 7 | Role of SR45 in flowering and alternative splicing. (a) *sr45* mutant is late flowering. Left, wild type; right, *sr45* mutant. (b) Expression and alternative splicing of precursor messenger RNA (pre-mRNA) of one of the serine/arginine-rich (*SR*) genes (*SR30*) in different organs in the mutant. An equal amount of template in each reaction was verified by amplifying a constitutively expressed cyclophilin (*Cyc*). DNA sizes are indicated on the right. Isoform number is indicated on the left side of the gel. I, inflorescence; L, leaf; R, root; S, stem. Schematic diagram in the bottom panel of *SR30* shows the gene structure and its alternatively spliced messenger RNA (mRNA) isoforms. (Numbers below each isoform indicate the number of nucleotides.) Predicted proteins from splice variants are shown to the right of each isoform. Exons are filled rectangles and introns are thin lines. Black rectangles represent constitutively spliced exons, whereas the red rectangles indicate the included regions in splice variants. Vertical arrowhead and '*' show start and stop codons, respectively. Horizontal green and red arrowheads above and below gene structures indicate the position of forward and reverse primers, respectively. In the schematics of predicted proteins, numbers to the right are the number of amino acids in the protein. RRM, RNA recognition motif; RS, arginine/serine-rich domain. Blue rectangle indicates a stretch of amino acids that are not present in functional SR proteins. (Reprinted with permission from Ref 88. Copyright 2007 PLoS ONE)

showed altered responses to glucose and abscisic acid (ABA).[90] These authors showed that SR45 negatively regulates glucose-induced growth arrest by repressing accumulation and signaling of ABA, which plays important roles in arresting seedling establishment under unfavorable conditions. In this case, both isoforms complemented the glucose overly-sensitive phenotype of the *sr45* mutant. These observations

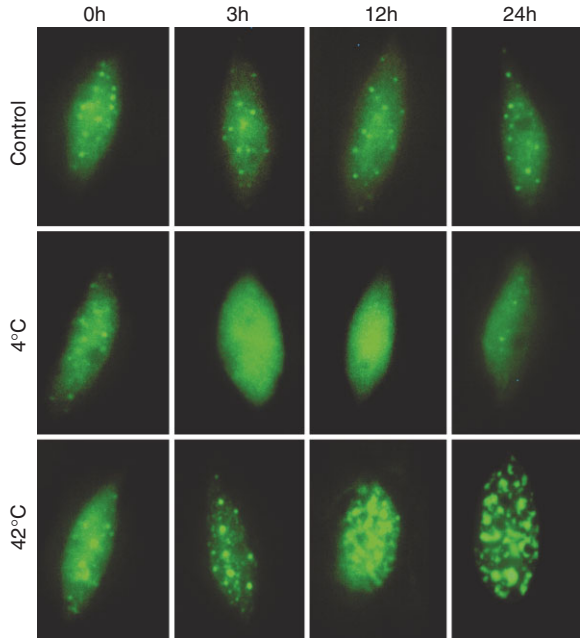

FIGURE 8 | Effect of stresses on SR45 localization in root epidermal cells of GFP-SR45 expressing Arabidopsis seedlings. Cold treatment relocalized GFP-SR45 mostly to the nucleoplasmic pool, whereas heat treatment induced redistribution of GFP-SR45 into irregularly shaped compartments. Controls were incubated at 22°C. (Reprinted with permission from Ref 91. Copyright 2003 Blackwell Publishing)

suggest that alternatively spliced isoforms will display specificity in affecting some processes and play a general role in others. Like SRs, it localizes to nuclear speckles and nucleoplasm, and the mobility of this protein in the nucleus is controlled by stresses (Figure 8), phosphorylation, and ATP.[80,91] It was shown that U1-70K and SR45 associate primarily in speckles and that this interaction is mediated by the RS1 or RS2 domain of SR45[79] (Figure 9). Together, these studies indicate that SR45 is an important splicing regulator and controls several developmental and stress responses.

CONCLUSIONS AND FUTURE PERSPECTIVES

In recent years, some progress has been made in understanding the roles of plant SR proteins in plant growth and development. However, much work still needs to be done to understand the roles of every plant SR protein. Also, of paramount importance is elucidation of functions of plant-specific SR proteins. Such investigations will require the development of tools and the use of novel approaches, which combine system-level analyses with conventional biochemical and genetic analyses. Because of multiple paralogs with high sequence

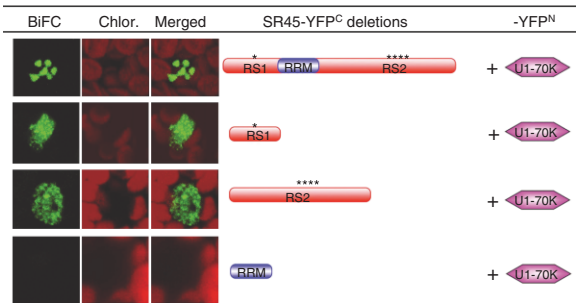

FIGURE 9 | Bimolecular fluorescence complementation (BiFC)-based mapping of SR45 domains involved in its association with U1-70K. Different domains of SR45 that are fused to YFP^c (middle) were introduced into protoplasts along with U1-70K fused to YFP^n (right) and visualized for reconstitution of yellow fluorescent protein (YFP) (right). RRM, RNA recognition motif; RS1 and RS2, arginine/serine-rich domains 1 and 2. (Reprinted with permission from Ref 79. Copyright 2008 PLoS ONE)

similarity, it is likely that mutations in single *SR* genes may not show phenotypes. Therefore, to understand the function of SR proteins, it is necessary to generate knockout mutants in single and multiple genes of an *SR* family (double or triple mutants) and then perform phenotypic analysis under different conditions. Important questions pertinent to splice variants of SRs are Do all splice variants produce proteins? If they do, what is the role of splice variants? To determine if they produce proteins, one can use isoform-specific antibodies or fuse cDNA of each variant to 5′ end of a fluorescent reporter and express them in a knockout background. Once the knockouts are analyzed for SR functions, then each splice variant can be expressed in the knockout background to assess the function of individual isoforms. Deep sequencing of transcriptomes using next-generation sequencing (RNA-seq)[3,5] in loss-of-function mutants under normal and stress conditions will permit global analysis of gene expression and AS affected by each SR. Data from such studies with each SR mutant will permit construction of splicing networks in plants and provide clues in designing phenotypic screens. Currently, we know nothing about the *cis*-elements in pre-mRNAs that bind to SRs and this is an area that can be addressed using global approaches. Recent high-throughput methods such as *R*NA *i*mmuno*p*recipitation followed by deep sequencing (RIP-Seq) and a variation of this (HITS-CLIP, high-throughput sequencing of UV cross-linked immunoprecipitated RNA)[92–94] offer opportunities for genome-wide identification of mRNA targets of a particular Sr. Analysis of splicing reporters *in vivo* using transient expression in protoplasts or *Agrobacterium*-infiltrated tissues of wild-type and SR mutants should also provide some mechanistic understanding of how SRs regulate splicing. The empirical data obtained from HITS-CLIP can be used for bioinformatic analyses to identify consensus sequences that bind to each SR protein, which can then be verified by gel-shift assays. The availability of a large database of alternatively spliced genes in plants makes it amenable to use computational tools for identification of *cis*-elements associated with different types of AS events and may pave the way for developing splicing code for plants as in animals.[95] RNA-seq data together with HITS-CLIP will help differentiate the splicing events that are regulated directly by SRs binding to pre-mRNAs and the secondary indirect effects. These studies should also help determine similarities and differences in the regulation of pre-mRNA splicing between plants and animals and identify plant-specific novel mechanisms that control CS and AS. In animals, SR proteins are involved in many other aspects of RNA metabolism and other processes including transcription elongation, translation regulation, genome stability, microRNA biogenesis, receptors of some carbohydrates, and NMD.[16,96,97] Whether plant SRs function in any of these other processes remains to be seen. The next decade is expected to provide many more new insights into SRs role in regulating CS and AS as well as their role in plant growth and development and stress responses.

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REFERENCES

1. Reddy ASN. Alternative splicing of pre-messenger RNAs in plants in the genomic era. *Annu Rev Plant Biol* 2007, 58:267–294.
2. Labadorf A, Link A, Rogers MF, Thomas J, Reddy AS, Ben-Hur A. Genome-wide analysis of alternative splicing in *Chlamydomonas reinhardtii*. *BMC Genomics* 2010, 11:114.
3. Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. Deep surveying of alternative splicing complexity in the

human transcriptome by high-throughput sequencing. *Nat Genet* 2008, 40:1413–1415.

4. Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. *Nature* 2008, 456:470–476.
5. Filichkin SA, Priest HD, Givan SA, Shen R, Bryant DW, Fox SE, Wong WK, Mockler TC. Genome-wide mapping of alternative splicing in *Arabidopsis thaliana*. *Genome Res* 2010, 20:45–58.
6. Zhang G, Guo G, Hu X, Zhang Y, Li Q, Li R, Zhuang R, Lu Z, He Z, Fang X, et al. Deep RNA sequencing at single base-pair resolution reveals high complexity of the rice transcriptome. *Genome Res* 2010, 20:646–654.
7. Barbazuk WB, Fu Y, McGinnis KM. Genome-wide analyses of alternative splicing in plants: opportunities and challenges. *Genome Res* 2008, 18:1381–1392.
8. Simpson CG, Lewandowska D, Fuller J, Maronova M, Kalyna M, Davidson D, McNicol J, Raczynska D, Jarmolowski A, Barta A, et al. Alternative splicing in plants. *Biochem Soc Trans* 2008, 36:508–510.
9. Simpson CG, Manthri S, Raczynska KD, Kalyna M, Lewandowska D, Kusenda B, Maronova M, Szweykowska-Kulinska Z, Jarmolowski A, Barta A, et al. Regulation of plant gene expression by alternative splicing. *Biochem Soc Trans* 2010, 38:667–671.
10. Muhlemann O, Lykke-Andersen J. How and where are nonsense mRNAs degraded in mammalian cells? *RNA Biol* 2010, 7:28–32.
11. Bhuvanagiri M, Schlitter AM, Hentze MW, Kulozik AE. NMD: RNA biology meets human genetic medicine. *Biochem J* 2010, 430:365–377.
12. Stamm S, Ben-Ari S, Rafalska I, Tang Y, Zhang Z, Toiber D, Thanaraj TA, Soreq H. Function of alternative splicing. *Gene* 2005, 344:1–20.
13. Jurica MS, Moore MJ. Pre-mRNA splicing: awash in a sea of proteins. *Mol Cell* 2003, 12:5–14.
14. Bessonov S, Anokhina M, Will CL, Urlaub H, Luhrmann R. Isolation of an active step I spliceosome and composition of its RNP core. *Nature* 2008, 452:846–850.
15. Wang BB, Brendel V. The ASRG database: identification and survey of *Arabidopsis thaliana* genes involved in pre-mRNA splicing. *Genome Biol* 2004, 5:R102.
16. Long JC, Caceres JF. The SR protein family of splicing factors: master regulators of gene expression. *Biochem J* 2009, 417:15–27.
17. Graveley BR. Sorting out the complexity of SR protein functions. *RNA* 2000, 6:1197–1211.
18. Manley JL, Krainer AR. A rational nomenclature for serine/arginine-rich protein splicing factors (SR proteins). *Genes Dev* 2010, 24:1073–1074.
19. Barta A, Kalyana M, Reddy AS. Implementing a rational and consistent nomenclature for serine/arginine-rich protein splicing factors (SR proteins) in plants. *Plant Cell* 2010, 22:2926–2929.
20. Shen H, Green MR. RS domains contact splicing signals and promote splicing by a common mechanism in yeast through humans. *Genes Dev* 2006, 20:1755–1765.
21. Lopato S, Mayeda A, Krainer AR, Barta A. Pre-mRNA splicing in plants: characterization of Ser/Arg splicing factors. *Proc Natl Acad Sci U S A* 1996, 93:3074–3079.
22. Lopato S, Waigmann E, Barta A. Characterization of a novel arginine/serine-rich splicing factor in Arabidopsis. *Plant Cell* 1996, 8:2255–2264.
23. Lazar G, Schaal T, Maniatis T, Goodman HM. Identification of a plant serine-arginine-rich protein similar to the mammalian splicing factor SF2/ASF. *Proc Natl Acad Sci U S A* 1995, 92:7672–7676.
24. Golovkin M, Reddy AS. The plant U1 small nuclear ribonucleoprotein particle 70K protein interacts with two novel serine/arginine-rich proteins. *Plant Cell* 1998, 10:1637–1648.
25. Golovkin M, Reddy ASN. An SC35-like protein and a novel serine/arginine-rich protein interact with Arabidopsis U1-70K protein. *J Biol Chem* 1999, 274:36428–36438.
26. Reddy AS. Plant serine/arginine-rich proteins and their role in pre-mRNA splicing. *Trends Plant Sci* 2004, 9:541–547.
27. Kalyna M, Barta A. A plethora of plant serine/arginine-rich proteins: redundancy or evolution of novel gene functions? *Biochem Soc Trans* 2004, 32:561–564.
28. Reddy ASN. Nuclear pre-mRNA splicing in plants. *Crit Rev Plant Sci* 2001, 20:523–571.
29. Wang BB, Brendel V. Genomewide comparative analysis of alternative splicing in plants. *Proc Natl Acad Sci U S A* 2006, 103:7175–7180.
30. Palusa SG, Ali GS, Reddy ASN. Alternative splicing of pre-mRNAs of Arabidopsis serine/arginine-rich proteins and its regulation by hormones and stresses. *Plant J* 2007, 49:1091–1107.
31. Isshiki M, Tsumoto A, Shimamoto K. The serine/arginine-rich protein family in rice plays important roles in constitutive and alternative splicing of pre-mRNA. *Plant Cell* 2006, 18:146–158.
32. Kalyna M, Lopato S, Barta A. Ectopic expression of atRSZ33 reveals its function in splicing and causes pleiotropic changes in development. *Mol Biol Cell* 2003, 14:3565–3577.
33. Kalyna M, Lopato S, Voronin V, Barta A. Evolutionary conservation and regulation of particular alternative splicing events in plant SR proteins. *Nucleic Acids Res* 2006, 34:4395–4405.
34. Lazar G, Goodman HM. The Arabidopsis splicing factor SR1 is regulated by alternative splicing. *Plant Mol Biol* 2000, 42:571–581.
35. Simpson CG, Fuller J, Maronova M, Kalyna M, Davidson D, McNicol J, Barta A, Brown JW. Monitoring

changes in alternative precursor messenger RNA splicing in multiple gene transcripts. *Plant J* 2008, 53:1035–1048.

36. Cavaloc Y, Popielarz M, Fuchs J-P, Gattoni R, Stevenin J. Characterization and cloning of the human splicing factor 9G8: a novel 35 kDa factor of the serine/arginine protein family. *EMBO J* 1994, 13:2639–2649.
37. Lareau LF, Brooks AN, Soergel DA, Meng Q, Brenner SE. The coupling of alternative splicing and nonsense-mediated mRNA decay. *Adv Exp Med Biol* 2007, 623:190–211.
38. Palusa SG, Golovkin M, Shin S-B, Richardson D, Reddy AS. Organ-specific, developmental, hormonal and stress regulation of expression of putative pectate lyase genes in Arabidopsis. *New Phytol* 2007: In press.
39. Schindler S, Szafranski K, Hiller M, Ali GS, Palusa SG, Backofen R, Platzer M, Reddy AS. Alternative splicing at NAGNAG acceptors in *Arabidopsis thaliana* SR and SR-related protein-coding genes. *BMC Genomics* 2008, 9:159.
40. Lareau LF, Inada M, Green RE, Wengrod JC, Brenner SE. Unproductive splicing of *SR* genes associated with highly conserved and ultraconserved DNA elements. *Nature* 2007, 446:926–929.
41. Lopato S, Kalyna M, Dorner S, Kobayashi R, Krainer AR, Barta A. atSRp30, one of two SF2/ASF-like proteins from *Arabidopsis thaliana*, regulates splicing of specific plant genes. *Genes Dev* 1999, 13:987–1001.
42. Maquat LE. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. *Nat Rev Mol Cell Biol* 2004, 5:89–99.
43. Chang YF, Imam JS, Wilkinson MF. The nonsense-mediated decay RNA surveillance pathway. *Annu Rev Biochem* 2007, 76:51–74.
44. Soergel DAW, Lareau LF, Brenner SE. Regulation of gene expression by coupling of alternative splicing and NMD. In: Maquat LE, ed. *Nonsense-mediated mRNA decay, Molecular Biology Interlligence Unit*. Landes Bioscience; 2006, 175–196.
45. Palusa SG, Reddy AS. Extensive coupling of alternative splicing of pre-mRNAs of serine/arginine (SR) genes with nonsense-mediated decay. *New Phytol* 2010, 185:83–89.
46. Green RE, Lewis BP, Hillman RT, Blanchette M, Lareau LF, Garnett AT, Rio DC, Brenner SE. Widespread predicted nonsense-mediated mRNA decay of alternatively-spliced transcripts of human normal and disease genes. *Bioinformatics* 2003, 19(Suppl 1):i118–i121.
47. Kurihara Y, Matsui A, Hanada K, Kawashima M, Ishida J, Morosawa T, Tanaka M, Kaminuma E, Mochizuki Y, Matsushima A, et al. Genome-wide suppression of aberrant mRNA-like noncoding RNAs by NMD in Arabidopsis. *Proc Natl Acad Sci U S A* 2009, 106:2453–2458.
48. Hori K, Watanabe Y. Context analysis of termination codons in mRNA that are recognized by plant NMD. *Plant Cell Physiol* 2007, 48:1072–1078.
49. Kerenyi Z, Merai Z, Hiripi L, Benkovics A, Gyula P, Lacomme C, Barta E, Nagy F, Silhavy D. Inter-kingdom conservation of mechanism of nonsense-mediated mRNA decay. *EMBO J* 2008, 27:1585–1595.
50. Kertesz S, Kerenyi Z, Merai Z, Bartos I, Palfy T, Barta E, Silhavy D. Both introns and long 3'-UTRs operate as *cis*-acting elements to trigger nonsense-mediated decay in plants. *Nucleic Acids Res* 2006, 34:6147–6157.
51. Rebbapragada I, Lykke-Andersen J. Execution of nonsense-mediated mRNA decay: what defines a substrate? *Curr Opin Cell Biol* 2009, 21:394–402.
52. Schwartz AM, Komarova TV, Skulachev MV, Zvereva AS, Dorokhov IuL, Atabekov JG. Stability of plant mRNAs depends on the length of the 3'-untranslated region. *Biochemistry (Mosc)* 2006, 71:1377–1384.
53. Caceres JF, Misteli T, Screaton GR, Spector DL, Krainer AR. Role of the modular domains of SR proteins in subnuclear localization and alternative splicing specificity. *J Cell Biol* 1997, 138:225–238.
54. Zhu J, Krainer AR. Pre-mRNA splicing in the absence of an SR protein RS domain. *Genes Dev* 2000, 14:3166–3178.
55. Barta A, Kalyana M, Lorković ZJ. Plant SR proteins and their functions. *Curr Top Microbiol Immunol* 2008, 326:83–102.
56. Thomashow MF. PLANT COLD ACCLIMATION: freezing tolerance genes and regulatory mechanisms. *Annu Rev Plant Physiol Plant Mol Biol* 1999, 50:571–599.
57. Ulm R, Baumann A, Oravecz A, Mate Z, Adam E, Oakeley EJ, Schafer E, Nagy F. Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of Arabidopsis. *Proc Natl Acad Sci U S A* 2004, 101:1397–1402.
58. Dangl JL, Jones JD. Plant pathogens and integrated defence responses to infection. *Nature* 2001, 411:826–833.
59. Iida K, Seki M, Sakurai T, Satou M, Akiyama K, Toyoda T, Konagaya A, Shinozaki K. Genome-wide analysis of alternative pre-mRNA splicing in *Arabidopsis thaliana* based on full-length cDNA sequences. *Nucleic Acids Res* 2004, 32:5096–5103.
60. Ali GS, Reddy AS. Regulation of alternative splicing of pre-mRNAs by stresses. *Curr Top Microbiol Immunol* 2008, 326:257–275.
61. Shin C, Feng Y, Manley JL. Dephosphorylated SRp38 acts as a splicing repressor in response to heat shock. *Nature* 2004, 427:553–558.
62. Lopato S, Borisjuk L, Milligan AS, Shirley N, Bazanova N, Parsley K, Langridge P. Systematic identification of factors involved in post-transcriptional

processes in wheat grain. *Plant Mol Biol* 2006, 62:637–653.

63. Goldberg RB, Barker SJ, Perez-Grau L. Regulation of gene expression during plant embryogenesis. *Cell* 1989, 56:149–160.
64. Lopato S, Forstner C, Kalyna M, Hilscher J, Langhammer U, Indrapichate K, Lorkovic ZJ, Barta A. Network of interactions of a novel plant-specific Arg/Ser-rich protein, atRSZ33, with atSC35-like splicing factors. *J Biol Chem* 2002, 277:39989–39998.
65. Lorkovic ZJ, Lopato S, Pexa M, Lehner R, Barta A. Interactions of Arabidopsis RS domain containing cyclophilins with SR proteins and U1 and U11 snRNP-specific proteins suggest their involvement in pre-mRNA splicing. *J Biol Chem* 2004, 279:33890–33898.
66. Lopato S, Gattoni R, Fabini G, Stevenin J, Barta A. A novel family of plant splicing factors with a Zn knuckle motif: examination of RNA binding and splicing activities. *Plant Mol Biol* 1999, 39:761–773.
67. Gao H, Gordon-Kamm WJ, Lyznik LA. ASF/SF2-like maize pre-mRNA splicing factors affect splice site utilization and their transcripts are alternatively spliced. *Gene* 2004, 339:25–37.
68. Lewandowska D, Simpson CG, Clark GP, Jennings NS, Barciszewska-Pacak M, Lin CF, Makalowski W, Brown JW, Jarmolowski A. Determinants of plant u12-dependent intron splicing efficiency. *Plant Cell* 2004, 16:1340–1352.
69. Simpson CG, Hedley PE, Watters JA, Clark GP, McQuade C, Machray GC, Brown JW. Requirements for mini-exon inclusion in potato invertase mRNAs provides evidence for exon-scanning interactions in plants. *RNA* 2000, 6:422–433.
70. Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S. Identification of a *cis* element for tissue-specific alternative splicing of chloroplast ascorbate peroxidase pre-mRNA in higher plants. *J Biol Chem* 2002, 277:40623–40632.
71. Pertea M, Mount SM, Salzberg SL. A computational survey of candidate exonic splicing enhancer motifs in the model plant *Arabidopsis thaliana*. *BMC Bioinformatics* 2007, 8:159.
72. Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, Freier SM, Bennett CF, Sharma A, Bubulya PA, et al. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol Cell* 2010, 39:925–938.
73. de la Fuente van Bentem S, Anrather D, Roitinger E, Djamei A, Hufnagl T, Barta A, Csaszar E, Dohnal I, Lecourieux D, Hirt H. Phosphoproteomics reveals extensive in vivo phosphorylation of Arabidopsis proteins involved in RNA metabolism. *Nucleic Acids Res* 2006, 34:3267–3278.
74. Feilner T, Hultschig C, Lee J, Meyer S, Immink RG, Koenig A, Possling A, Seitz H, Beveridge A, Scheel D, et al. High throughput identification of potential Arabidopsis mitogen-activated protein kinases substrates. *Mol Cell Proteomics* 2005, 4:1558–1568.
75. Savaldi-Goldstein S, Sessa G, Fluhr R. The ethylene-inducible PK12 kinase mediates the phosphorylation of SR splicing factors. *Plant J* 2000, 21:91–96.
76. Shaw PJ, Brown JWS. Plant nuclear bodies. *Curr Opin Plant Biol* 2004, 7:614–620.
77. Spector DL. SnapShot: cellular bodies. *Cell* 2006, 127:1071.
78. Brown JW, Shaw PJ. The role of the plant nucleolus in pre-mRNA processing. *Curr Top Microbiol Immunol* 2008, 326:291–311.
79. Ali GS, Prasad KV, Hanumappa M, Reddy AS. Analyses of in vivo interaction and mobility of two spliceosomal proteins using FRAP and BiFC. *PLoS ONE* 2008, 3:e1953.
80. Ali GS, Reddy AS. ATP phosphorylation and transcription regulate the mobility of plant splicing factors. *J Cell Sci* 2006, 119:3527–3538.
81. Ali GS, Reddy AS. Spatiotemporal organization of pre-mRNA splicing proteins in plants. *Curr Top Microbiol Immunol* 2008, 326:103–118.
82. Docquier S, Tillemans V, Deltour R, Motte P. Nuclear bodies and compartmentalization of pre-mRNA splicing factors in higher plants. *Chromosoma* 2004, 112:255–266.
83. Tillemans V, Dispa L, Remacle C, Collinge M, Motte P. Functional distribution and dynamics of Arabidopsis SR splicing factors in living plant cells. *Plant J* 2005, 41:567–582.
84. Fang Y, Hearn S, Spector DL. Tissue-specific expression and dynamic organization of SR splicing factors in Arabidopsis. *Mol Biol Cell* 2004, 15:2664–2673.
85. Lorkovic ZJ, Hilscher J, Barta A. Co-localisation studies of Arabidopsis SR splicing factors reveal different types of speckles in plant cell nuclei. *Exp Cell Res* 2008, 314:3175–3186.
86. Tillemans V, Leponce I, Rausin G, Dispa L, Motte P. Insights into nuclear organization in plants as revealed by the dynamic distribution of Arabidopsis SR splicing factors. *Plant Cell* 2006, 18:3218–3234.
87. Rausin G, Tillemans V, Stankovic N, Hanikenne M, Motte P. Dynamic nucleocytoplasmic shuttling of an Arabidopsis SR splicing factor: role of the RNA-binding domains. *Plant Physiol* 2010, 153:273–284.
88. Ali GS, Palusa SG, Golovkin M, Prasad J, Manley JL, Reddy ASN. Regulation of plant developmental processes by a novel splicing factor. *PLoS One* 2007, 2:e471.
89. Zhang XN, Mount SM. Two alternatively spliced isoforms of the Arabidopsis SR45 protein have distinct roles during normal plant development. *Plant Physiol* 2009, 150:1450–1458.

90. Carvalho RF, Carvalho SD, Duque P. The plant-specific SR45 protein negatively regulates glucose and ABA signaling during early seedling development in Arabidopsis. *Plant Physiol* 2010, 154:772–783.

91. Ali GS, Golovkin M, Reddy ASN. Nuclear localization and in vivo dynamics of a plant-specific serine/arginine-rich protein. *Plant J* 2003, 36:883–893.

92. Ule J, Jensen K, Mele A, Darnell RB. CLIP: a method for identifying protein-RNA interaction sites in living cells. *Methods* 2005, 37:376–386.

93. Licatalosi DD, Mele A, Fak JJ, Ule J, Kayikci M, Chi SW, Clark TA, Schweitzer AC, Blume JE, Wang X, et al. HITS-CLIP yields genome-wide insights into brain alternative RNA processing. *Nature* 2008, 456:464–469.

94. Wang Z, Tollervey J, Briese M, Turner D, Ule J. CLIP: construction of cDNA libraries for high-throughput sequencing from RNAs cross-linked to proteins in vivo. *Methods* 2009, 48:287–293.

95. Barash Y, Calarco JA, Gao W, Pan Q, Wang X, Shai O, Blencowe BJ, Frey BJ. Deciphering the splicing code. *Nature* 2010, 465:53–59.

96. Wu H, Sun S, Tu K, Gao Y, Xie B, Krainer AR, Zhu J. A splicing-independent function of SF2/ASF in microRNA processing. *Mol Cell* 2010, 38:67–77.

97. Hatakeyama S, Sugihara K, Nakayama J, Akama TO, Wong SM, Kawashima H, Zhang J, Smith DF, Ohyama C, Fukuda M, et al. Identification of mRNA splicing factors as the endothelial receptor for carbohydrate-dependent lung colonization of cancer cells. *Proc Natl Acad Sci U S A* 2009, 106:3095–3100.

