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THE RNAissance Family: SR proteins as multifaceted regulators of gene expression

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Abstract

Serine and Arginine-rich (SR) proteins play multiple roles in the eukaryotic gene expression pathway. Initially described as constitutive and alternative splicing factors, it is now clear that SR proteins are key determinants of exon identity and function as molecular adaptors, linking the pre-mRNA to the splicing machinery. In addition, SR proteins are now implicated in many aspects of mRNA and ncRNA processing well beyond splicing. These unexpected roles, including RNA transcription, export, translation and decay may prove to be the rule rather than the exception. To simply define this family of RNA binding proteins as splicing factors belies the broader roles of SR proteins in post-transcriptional gene expression.

Post-transcriptional regulation is critical to the accurate expression of human genes. This process is overseen in part by a large superfamily of RS-domain-containing proteins found throughout metazoans[1], which contain SR and "SR-related" proteins (reviewed in[2]). Although it is often precarious to separate the two groups on a functional level, for the purposes of this review we will focus on the subfamily of "classic" SR proteins. SR proteins are structurally defined as a family of RNA binding proteins with a modular domain structure consisting of one to two amino- terminal RNA recognition motifs (RRMs) and a carboxyl-terminal domain rich in serine and arginine dipeptide repeats[3]. There are twelve canonical members of the SR protein family that share this characteristic domain structure (see Table 1). SR proteins are intimately involved in the gene expression pathway, influencing both nuclear pre-messenger RNA (pre-mRNA) processing as well as the cytoplasmic fate of the mature RNA (mRNA) message. Prominently known for their requirement in spliceosome assembly and regulation of alternative splicing decisions, important distinctions in SR protein biology have emerged over the years. For example, some SR proteins have a life in the cytoplasm whereas others remain confined in the nucleus. Here we will provide a summary of the nuclear roles of SR proteins as well as their emergent post-splicing functions in gene expression.

From the beginning, the functional characterization of SR proteins alluded to both diverse and redundant activities. The founding member of the SR protein family, SRSF1, was

identified and characterized by concurrent studies using biochemical complementation assays. Not only was SRSF1 seen to preferentially enhance usage of the proximal authentic 5′ splice site of a β-globin splicing reporter [4], but it also altered splicing ratios of SV40 pre-mRNA, enhancing small T mRNA isoform production[5]. These data implicated the first "classic" SR protein as a regulator of both constitutive and alternative splicing. A second SR protein, SFRS6, was shown to complement splicing-deficient extracts to promote β-globin splicing, as well as alternative 5′ splice site usage in β-thalassemic pre-mRNA[6, 7]. At roughly the same time, a third SR protein, SRSF2 was shown to influence splice site selection. Using RNase T1 protection and immunoprecipitation assays, SRSF2 was seen to interact with both the 5′ and 3′ splice sites independently[8]. Furthermore, interactions between U1 and U2 snRNPs bound to 5′ and 3′ splice sites were shown to occur in an SRSF2-dependent fashion, implicating SR proteins in early spliceosome architecture. SRSF2 was also shown to have similar effects on splice site selection as SRSF1, alluding to functional redundancy during spliceosome assembly.

The SR protein family was rapidly expanded through clever biochemical fractionation by Zahler and colleagues, who co-purified a group of proteins, (including SRSF1, SRSF2, and SRSF6), by ammonium sulfate fractionation and precipitation with magnesium chloride[9]. This approach revealed five proteins of various molecular weights, which were selectively purified from both HeLa cell extract and calf thymus. These proteins presented reactivity to mAb104, an antibody previously shown to recognize phosphorylated SR proteins[6, 10, 11]. Furthermore, four of these proteins were shown to rescue splicing of β-globin and *ftz* splicing reporters in splicing-deficient extracts, providing evidence that, like SRSF1 and SRSF2, these proteins were splicing factors[9]. Finally, microsequencing of these proteins showed highly similar amino acid compositions, as well as an abundance of serine/arginine dipeptides, on which their family name is based[9].

Regulation of SR proteins by post-translational modification

Post-translational modification plays critical roles in regulation of SR protein activity and localization. Phosphorylation of SR proteins is regulated by the SR-specific protein kinase (SRPK) family and other CMGC kinase family members, such as Clk/Sty (cdc2-like kinase/serine, threonine, and tyrosine kinase)[12, 13]. These kinases share similar abilities to phosphorylate serine residues throughout the RS domain but differ in their specificity and mechanism of phosphorylation[14–17]. Dynamic phosphorylation of SR proteins is vital to the initiation and progression of spliceosome assembly to catalysis[18–22]. Mechanistically, it is thought that phosphorylation of the RS domain increases RNA binding specificity[23] and is also important for specific protein-protein interactions within the pre-spliceosome[24]. Structurally, phosphorylation results in entropic reduction of the intrinsically disordered RS domain to promote more ordered side chains for molecular recognition[25]. Together these data advocate that phosphorylation states of SR proteins act as "molecular switches" during spliceosome assembly.

RS domain phosphorylation also influences the dynamics of SR protein localization in the cell. Release of SR proteins from nuclear speckles requires phosphorylation by the RS domain kinase Clk/Sty[13]. Following spliceosome assembly, SR proteins encounter one of

two potential paths: for a subset of SR proteins, re-phosphorylation by Clk/Sty and nuclear SRPKs will target the protein for nuclear recycling for further rounds of pre-mRNA splicing, whereas other SR proteins remain dephosphorylated and associated with spliced mRNAs[9, 26–28]. These studies suggest that dephosphorylated SR proteins retained on spliced mRNA may be a signal that an mRNA is ready for nuclear export. Following mRNA export and translation, SR proteins can then be rephosphorylated by cytoplasmic SRPKs, which facilitates interactions with transportin-SR and their import back into the nucleus[29, 30].

SR protein phosphorylation is further modulated in response to a variety of different cellular conditions and signals. Changes in phosphorylation and subcellular distribution of SR proteins accompany the global regulation of RNA metabolism during early development[31,32], viral infection[33], and cell cycle progression[12]. One recent example demonstrates SR protein phosphorylation as a direct result of epidermal growth factor (EGF) signaling. EGF signaling is shown to increase AKT activation, which in turn activates SRPK and subsequent up-regulation of SR protein phosphorylation[34]. These data implicate SR proteins as integral players in propagating EGF signaling, which is linked to numerous human cancers.

SR proteins are modified by a variety of other marks including methylation and acetylation. The consequences of these modifications are less well-understood than phosphorylation but they appear to be functionally relevant. For example, acetylation of SRSF2 occurs in response to genotoxic stress. Acetylation within the RRM domain correlates with pre-mRNA alternative splicing regulation of caspase-8, a factor involved in apoptosis[35]. Arginine methylation also influences SR protein localization and activity[37]. Blocking methylation affects alternative splicing, translation, and mRNA decay, most likely due to mis-regulation of SR protein localization[36–38].

The complex roles of SR proteins in pre-mRNA splicing

The spliceosome is assembled *de novo* on each and every intronic substrate. This dynamic process involves the sequential recruitment and rearrangement of Uracil-rich small nuclear ribonucleoprotein particles (U snRNPs). SR proteins contribute to spliceosome assembly primarily through the recognition of exonic splicing enhancers (ESEs)[39–41]. These interactions are particularly important during formation and stabilization of the Early (E) Complex (Fig. 1)[8, 42–45]. E complex is defined by association of both the U1 snRNP and the heterodimeric splicing factor U2 snRNP auxiliary factor (U2AF) with the 5′ and 3′ splice sites, respectively. This step is mediated by phosphorylation-dependent interactions between the RS and RRM domains of ESE-bound SR proteins and the U1-70K at the 5′ splice site[21, 46] and the small subunit of U2AF (U2AF35) at the 3′ splice site [47–50]. E-complex is assumed to form on either end of an intron, however, the same interaction network can occur across exons, in a process called exon definition (see below).

E complex is converted to A complex by the addition of the U2 snRNP. During A complex formation, SR proteins are thought to promote interactions of U2 snRNP with the branch point sequence through non-specific interactions of the RS domain with the phosphodiester backbone, possibly neutralizing its negative charge and enhancing base pairing[51].

Additionally, SR proteins are implicated in recruitment of the U4/U6.U5 tri-snRNP[52], forming a cross-exon "B-like" complex, which can ultimately rearrange into cross-intron B complexes[53]. The RS domain of SR proteins (presumably not associated with ESEs) are also hypothesized to associate with the phosphodiester backbone near the 5′ ss to promote U6 binding[54]. Finally, extensive remodeling and rearrangement of RNA-RNA and RNA-protein interactions, coupled with dephosphorylation of SR proteins, results in formation of the catalytically active C complex[18, 55, 56]. In summary, SR proteins promote recruitment of multiple factors throughout spliceosome assembly, and are critical in formation of the final catalytic core.

SR proteins also play important roles in establishing exon-intron boundaries in large metazoan genes. The process of "exon definition" is hypothesized to solve a significant problem related to finding relatively short exons within the context of long intronic sequences[57]. Exon definition occurs through a complex interaction network that links the 3′ss at the 5′ end of the exon with the 5′ss at the 3′ end of the exon (reviewed in [58]). In metazoans exon definition precedes intron-definition in which 5′ and 3′ splice sites are paired during spliceosome assembly[53]. SR proteins also contribute to intron-definition through a series of protein-protein interactions, mediated by the RS domain linking U1 snRNP at the 5′ss to U2AF35 at the 3′ss[45]. Intron bridging has been alluded to through protein-protein interaction studies, and observed on splicing substrates using electron microscopy[59], but the precise role of SR proteins is not well understood.

The mechanisms described above not only contribute to the roles of SR proteins in constitutive splicing, but similarly in alternative splicing. The distinction between the two processes is simply the context in which SR proteins engage the pre-mRNA[50] (see Fig. 2A). A general theme emerging from both *in vitro* and *in vivo* assays is that SR proteins act as enhancers of splicing when associated with exonic sequences, but function as silencers while binding to intronic sequences downstream of the 5′ splice site[39, 60]. However, this simplistic perspective belies the complex cis-regulatory landscape of most regulated exons. Several features distinguishing alternative exons from constitutive exons, including their shorter length and weaker 5′ splice sites, respectively. Exonic regulatory sequences (ESRs) are also more strongly conserved in the context of alternative exons as compared to their counterparts in constitutive exons[61], reflecting requirements for ESRs in definition of sub-optimal exons[50]. Remarkably, the same ESR sequence can have opposing affects on splicing when placed into distinct positions within the same alternative exon[61]. These studies suggest that the regulatory roles of SR proteins in alternative splicing are most likely highly position- and context-dependent.

SR proteins can also have long-range effects on regulation of alternative exons. Several studies described a new mode for SR protein-mediated splicing regulation that occurs through their association with constitutive exons that are adjacent to alternative exons[62–64] (see Fig. 2B). For example, SRSF1 has been shown to promote skipping of exon 16 in CamKIIδ through its association with downstream constitutive exon 17[63]. A similar mechanism influences splicing of the receptor tyrosine kinase MET, a key driver of malignant breast cancer[65]. In this context, elevated SRSF1 levels lead to increased skipping of exon 11, an effect mediated by ESEs located within exon 12[66]. These data suggest an

intriguing model in which SR proteins may alter the competition between 3′ splice sites of adjacent exons with a common upstream 5′ splice site [61,62,81,82].

Another important aspect of the mechanisms through which SR proteins influence alternative splicing involves their interplay with members of the hnRNP family. The hnRNPs include several well-established splicing repressors, which mediate the repressive effects of exonic splicing silencers (ESSs). The functional antagonism of SR proteins and hnRNP proteins was first observed between SRSF1 and hnRNP A1 on several different alternative splicing modalities[67] (see Fig. 2D). Not surprisingly, the underlying molecular mechanisms can be quite distinct. In the case of competing splice site donors, SRSF1 promoted selection of the proximal 5′ splice site (closest to the 3′ss) whereas hnRNP A1 promoted usage of more distal sites[68] by reducing binding of U1 snRNP at the proximal site. This functional antagonism also extends to alternative cassette exons. In an elegant series of experiments, Zhu and Krainer demonstrated that binding of an SR protein to an ESE inhibits the repressive affects of hnRNPs bound to adjacent silencers[69]. Because the relative expression levels of SR proteins and hnRNPs can vary dramatically across tissues and during tumorigenesis[70, 71], the complex functional interplay between hnRNPs and SR proteins are likely to play important roles in regulating patterns of alternate splicing across a wide array of conditions.

While often thought of as general splicing enhancers, there are also instances where SR proteins can inhibit splicing (see Fig. 2C). For example SRSF9 promotes skipping of exon 7B in the hnRNP A1 pre-mRNA[72]. This activity requires an intronic splicing silencer element located upstream of the exon 7B 3′ ss. Likewise, the poorly characterized SRSF11 is reported to promote skipping of exon 10 of the Tau pre-mRNA by binding an exonic splicing silencer[73]. By contrast to these transcript specific affects, SRSF10 functions as an inducible, global repressor of splicing[74]. SRSF10 activity is inhibited by phosphorylation-dependent interactions with 14-3-3 proteins. Conditions that promote activation of protein phosphatase 1, including heat shock and mitosis, leads to dephosphorylation of SRSF10, liberation from 14-3-3 proteins and activation of splicing repressor activity[75]. Although the mechanisms of splicing inhibition are likely to be very different for each of theses SR proteins, it is nonetheless intriguing that SR proteins are capable of having potentially opposite affects on splicing depending on their phosphorylation state[76] or the context in which they engage the pre-mRNA.

Global analysis of SR protein RNA binding specificity

SR proteins are sequence-specific RNA binding proteins. For most SR proteins, a putative consensus motif has been identified (reviewed in [77]) but the challenge now is to determine how these elements function within different sequence contexts. Additionally, it is clear that there is significant functional redundancy in binding specificity[78–80]. These data imply that SR proteins may compete with each other for binding to closely related sites.

The first clues for understanding how this competition plays out on a global scale emerged from studies of SR proteins distribution on fixed insect polytene chromosomes and amphibian oocyte lampbrush chromosomes[10, 81]. Imaging of nascent transcripts on

Chironomus tentans polytene chromosomes revealed that SR proteins are distributed across the genome in a non-random pattern. Distinct SR protein staining patterns were observed at different loci, suggesting that different combinations of SR proteins associated with nascent transcripts. High-resolution analysis of the Balbiani Ring (BR) locus revealed that the BR mRNP is extensively remodeled as it is matured. Many of the SR proteins are replaced between the steps of mRNA export and translation, such that only SRSF1 is bound to polyribosome-associated mRNPs[82]. This observation is consistent with work from mammalian cells, which demonstrate that SR proteins are sorted on nascent transcripts through a phosphorylation-dependent mechanism[83].

The high throughout sequencing and crosslinking immunoprecipitation (HITS-CLIP) method allowed for global analysis of *in situ* protein-RNA interactions and provides key information such as consensus binding motifs, genome-wide binding site distribution, gene ontology of RNA targets, etc.[84]. HITS-CLIP analysis of SRSF1 revealed a diverse pool of RNA transcripts, including mRNA, miRNAs, snoRNAs, and intergenic transcripts of unknown function, advocating roles for SR proteins beyond pre-mRNA processing[62, 85]. Subsequent studies confirmed many of these hypotheses, including interactions with long non-coding RNAs MALAT1 and *Xist*, as well as pre-cursors of miRNA processing[86–89]. Furthermore, gene ontology analysis of SRSF1 mRNA targets showed an enrichment for RNA processing factors, suggesting a broad, highly integrated post-transcriptional network that governs splicing factor levels and ultimately global gene expression[85]. Like SRSF1, SRSF3 and SRSF4 engage a functionally diverse pool of RNA transcripts. However, their consensus binding sites and their CLIP tag distribution across transcripts is distinct[90]. Most intriguing was that CLIP tags for both SRSF3 and SRSF4 were enriched in ncRNAs, many of which have yet to be prescribed functions within the cell. Finally, SRSF3, but not SRSF4, was seen to regulate splicing of other splicing factors, further supporting the hypothesis of regulatory cascades that may extend the roles of SR proteins beyond their known targets[85, 90, 91].

Global studies of SR protein RNA target specificity demonstrate that most exons are bound by at least one SR protein[64, 82, 85, 90]. A major challenge now is to understand how different combinations of SR proteins influence splicing of specific exons. Recent work from Pandit et al. suggests that this is likely to be a complex problem[64]. An initial comparison of SRSF1 and -2 *in situ* binding sites in mouse embryo fibroblasts (MEFs) demonstrated that both proteins have considerable overlap in their binding specificity, suggesting that there may be competition for binding to related exon sequences. Interestingly, depletion of SRSF2 resulted in complex changes in SRSF1 binding site occupancy. In some cases SRSF1 binding increased in the absence of SRSF2, whereas the opposite pattern was observed at other locations. Together these data suggest that SR proteins can play both redundant roles and cooperative roles in exon recognition.

Emerging roles for SR proteins in gene expression

While it is generally accepted that the majority of splicing occurs in a co-transcriptional manner, only recently have these two processes been observed to directly regulate one another[92, 93]. Live cell imaging initially showed dynamic recruitment of various splicing

factors from nuclear speckles to sites of transcription activation[94]. Indeed, SR proteins co-localize with RNA polymerase II in nuclear speckles, an interaction mediated by the Pol II C-terminal domain (CTD)[95] in a serine phosphorylation-dependent manner[22, 96]. Truncation of the CTD prevents targeting of splicing factors to sites of transcription and markedly inhibits pre-mRNA splicing[97]. Also, selective mutations in the CTD cause diffusion of SR proteins away from nuclear speckles, and accumulation of unspliced β-globin transcripts[98]. These data indicate that interactions between SR proteins and Pol II are involved in splicing regulation. Using a minigene spicing reporter, de la Mata and colleagues showed that the CTD was required for SRSF3 recruitment and subsequent exon exclusion. Furthermore, the affect of the CTD on SRSF3-regualted alternative splicing was independent of transcription kinetics[99]. Together, these data imply that the CTD may play a direct role in spliceosome assembly through SR protein recruitment. Conversely, recent data imply that the association of SR proteins and RNA Pol II may only occur after initiation of transcription. In the context of nascent FOS RNA, association of various SR proteins with Pol II was seen to be RNA-dependent[100], suggesting that recruitment of SR proteins to actively transcribed genes may not occur during initiation in all contexts. Additional experiments are needed to determine the mechanisms through which SR proteins regulate co-transcriptional alternative splicing. Regardless, these data provide a functional link between the processes of Pol II transcription and alternative splicing decisions mediated by SR proteins.

SR proteins may also directly regulate elongation rates of RNAPII. In general, depletion of either SRSF1 or SRSF2 have global effects on Pol II transcription in cells, and SRSF2 levels have been shown to affect the accumulation of Pol II at gene loci[101]. Mechanistically, SRSF2 is thought to enhance the release of transcriptional regulator TEFb from 7SK RNA due to emergence of an SRSF2-recognized ESE following initial Pol II elongation. This may induce SRSF2 to switch from the 7SK RNA to nascent RNA, triggering the coordinated release of P-TEFb from the 7SK complex, and subsequent phosphorylation and un-pausing of Pol II[102]. This suggests that some SR proteins may have direct effects in recruitment of Pol II factors to initiated Pol II complexes to facilitate elongation.

Compartmentalization of genetic material in the nucleus allows for separation of mRNA transcription from its fate as a template for protein synthesis. The discovery that a subset of SR proteins shuttle between the nucleus and cytoplasm (reviewed in [103]) immediately suggested that SR proteins might remain bound with their mRNA targets beyond pre-mRNA splicing (Fig. 3). At least one non-canonical function for shuttling SR proteins appears to be in mRNA export pathways. Specific roles for the SR proteins SRSF3 and SRSF7 in intronless histone mRNA export provided the first direct evidence for SR protein moonlighting[104]. Shuttling SR proteins can interact with the canonical mRNA export factor nuclear RNA export factor 1 (NXF1; also known as TAP)[105, 106]. These data suggest that SR proteins may function broadly in the export of both spliced and unspliced mRNAs[104, 107, 108]. Interactions between NXF1 and SR proteins require dephosphorylation of the RS domain, suggesting an elegant mechanism for signaling the completion of an export-ready mRNA[106]. Surprisingly, depletion of specific SR proteins does not induce general defects in mRNA export[83], suggesting that SR proteins may function redundantly or play roles in nuclear export of specific mRNAs.

The nucleocytoplasmic shuttling SR protein SRSF1 is readily detectable in the polyribosome fraction of cultured human cells suggesting that SR proteins may be involved in mRNA translation[109]. This hypothesis was confirmed through three different functional assays. Perhaps most importantly, non-shuttling mutants of SRSF1 failed to enhance mRNA translation[28, 109]. Subsequent experiments demonstrated that SRSF1 stimulates translation initiation through a novel mechanism involving the mechanistic target of rapamycin complex (mTOR)[110]. These data support an intriguing model whereby SRSF1 functions as an adaptor protein linking specific mRNA transcripts to translational control by mTOR (see Fig. 4A). Recent work from the Caceres laboratory dramatically extends this model with the identification of >500 mRNAs that are likely to be translationally controlled by SRSF1 and mTOR[111].

Moreover, other shuttling SR proteins including SRSF3 and SRSF7 are implicated in translational control. SRSF3 and SRSF7 mediate the effects of two distinct cis-regulatory elements including a viral IRES and cellular constitutive transport elements (CTEs)[108, 112] (see Fig. 4B). Likewise, SRSF5 and SRSF6 enhance translation of *gag* protein from unspliced HIV-1 RNA, an activity that depends on their ability to shuttle from the nucleus to the cytoplasm[113]. SR proteins also have the potential to repress translation. During *Xenopus* development SRSF10 has been shown to interact with the peptidyltransferase center of 28S rRNA in undifferentiated neural cells[114]. Furthermore, this mechanism may help neuronal stem cells to maintain an undifferentiated state. These data paint a larger role for SR proteins in translation through regulating interactions with their respective RNA targets and translational machinery.

The roles of SRSF1 in mRNA translation suggest an intriguing hypothesis, that the fates of mRNA isoforms generated by alternative splicing may be subject to differential translation[109, 115, 116]. This idea is supported by the recent observation that >30% of alternative mRNA isoforms exhibit differential polyribosome association[117]. Shuttling SR proteins, such as SRSF1, which remain associated with its' targets throughout the gene expression pathway, are likely to contribute to this mechanism[62, 83]. Over-expression of SRSF1 results in isoform-specific recruitment of mRNAs to polyribosomes, suggesting a direct role in coordinating the alternative fates of mRNA isoforms[111].

In addition to splicing, export and translation, SR proteins also influence mRNA stability[118]. This activity can occur through several different mechanisms. First, SR proteins regulate alternative splicing and in many cases, such as post-transcriptional control of splicing factor levels, alternative splicing generates isoforms that are inherently unstable[91, 119]. Unstable isoforms contain pre-mature termination codons that trigger the nonsense mediated decay (NMD) RNA surveillance pathway. By contrast to this splicing based mode of gene regulation, SR proteins have also been shown to directly enhance NMD[120]. Intriguingly, this study showed the RS domain is required for augmentation of NMD, but not SR protein shuttling activity. These data suggest that SR proteins stimulate a rate-limiting step in the nucleus or that SR proteins may regulate the expression of NMD factors. Alternatively, SRSF1 may stimulate the pioneer round of mRNA translation leading to more efficient NMD[121]. Taken together there is little doubt that SR proteins have complex effects on transcript stability.

By contrast to their roles in pre-mRNA splicing, relatively little is known concerning the molecular mechanisms through which SR proteins influence post-splicing steps of gene expression. One hypothesis is that shuttling SR proteins work in concert with the exon junction complex (EJC) to influence mRNA export[105, 122], stability[118] and translation[109, 110, 123]. The EJC is deposited near exon-exon junctions as a result of pre-mRNA splicing and regulates post-transcriptional control of mature mRNAs. Proteomic analysis of EJC factors revealed numerous RNA-independent interactions with SR proteins. This observation is in good agreement with analysis of EJC RNA footprints, which revealed a myriad of non-canonical binding sites (i.e. those not centered 24 nucleotides upstream of an exon-exon junction). A significant proportion of these footprints overlapped with exonic splicing enhancers (ESEs), which are often occupied by SR proteins. EJC-SR protein interactions appear to be functionally significant as SRSF1 and -3 exhibit reduced mRNA binding activity when the EJC factor eIF4AIII is depleted from cells [124]. Together, these data reveal extensive, cooperative associations between SR proteins and the EJC in mRNP biogenesis and may explain their functional redundancy in regulation of mRNA export[105, 122], translation[109, 110, 123], and decay[121, 122], as well as maintenance of genomic stability[125, 126].

The roles of SR proteins in gene regulation extend beyond mRNA processing. SR proteins have recently been attributed functional roles in microRNA biogenesis. Specifically, SRSF1 has been found to associate with primary-miR-7 transcript thorough a putative SRSF1 binding site in the stem loop. This interaction promotes cleavage of the pre-miR by the microprocessor complex protein Drosha[89]. The role for SRSF1 in microRNA biogenesis appears to be direct, as it is independent of its role in splicing. HITS-CLIP analysis of SRSF1, -3 and -4 suggests that most SR proteins interact with a small but distinct group of miRNAs, suggesting that shuttling SR proteins are involved in miRNA biogenesis on a more general level[85, 90]. Overall, these data highlight a potential coordination between splicing regulation and miRNA-mediated transcriptome regulation.

Conclusion

The characteristics of SR proteins mirror another general regulator of nucleic acid structure and function: histones. Like histones, SR proteins control the accessibility of their nucleic acid targets to the gene expression machinery. The similarities extend to their biochemical properties as well. Both are highly alkaline, associate with and regulate the use of their respective bound nucleic acids, and can form homo- and heteroligomers to package DNA/RNA within the cell. Also, both histones and SR proteins are extensively post-translationally modified, which can control the functionality of the nucleic acid to which they are bound. Furthermore, both sets of proteins are used as the foundational basis for recruitment of additional factors to their respective nucleic acids to accomplish biochemical work, whether it be DNA-binding complexes that regulate and catalyze transcription or RNA-binding complexes that regulate pre-mRNA splicing, mRNA export, translation, and degradation. Similar comparisons have been made of hnRNP oligomers that have the ability to wrap up RNA species to form mRNPs that share some resemblance to nucleosomes[127, 128]. Clearly, this hypothesis warrants further investigation given the

intimate association of SR proteins with virtually every aspect of post-transcriptional gene regulation.

The coming years will undoubtedly see an explosion in data utilizing high-throughput assays (e.g. HITS-CLIP, iCLIP, etc.) to determine the transcriptome-wide RNA-interactions networks of SR proteins. These studies will provide a more general overview as to what RNAs SR proteins associate with and how they bind to them. This will certainly solidify the notion that SR proteins function in all aspects of RNA metabolism and gene expression rather than just splicing. The challenge for future work is to begin to determine how fluctuations in the levels of SR proteins influence the binding specificity of other SR proteins and splicing factors globally [64, 129–131]. These types of experiments will elucidate the context-specific interactions that determine how exon identity is established in living cells as well as other RBP "codes" as they function in downstream steps of gene expression. Overall, the near future holds a greater understanding of how SR proteins govern the RNA world.

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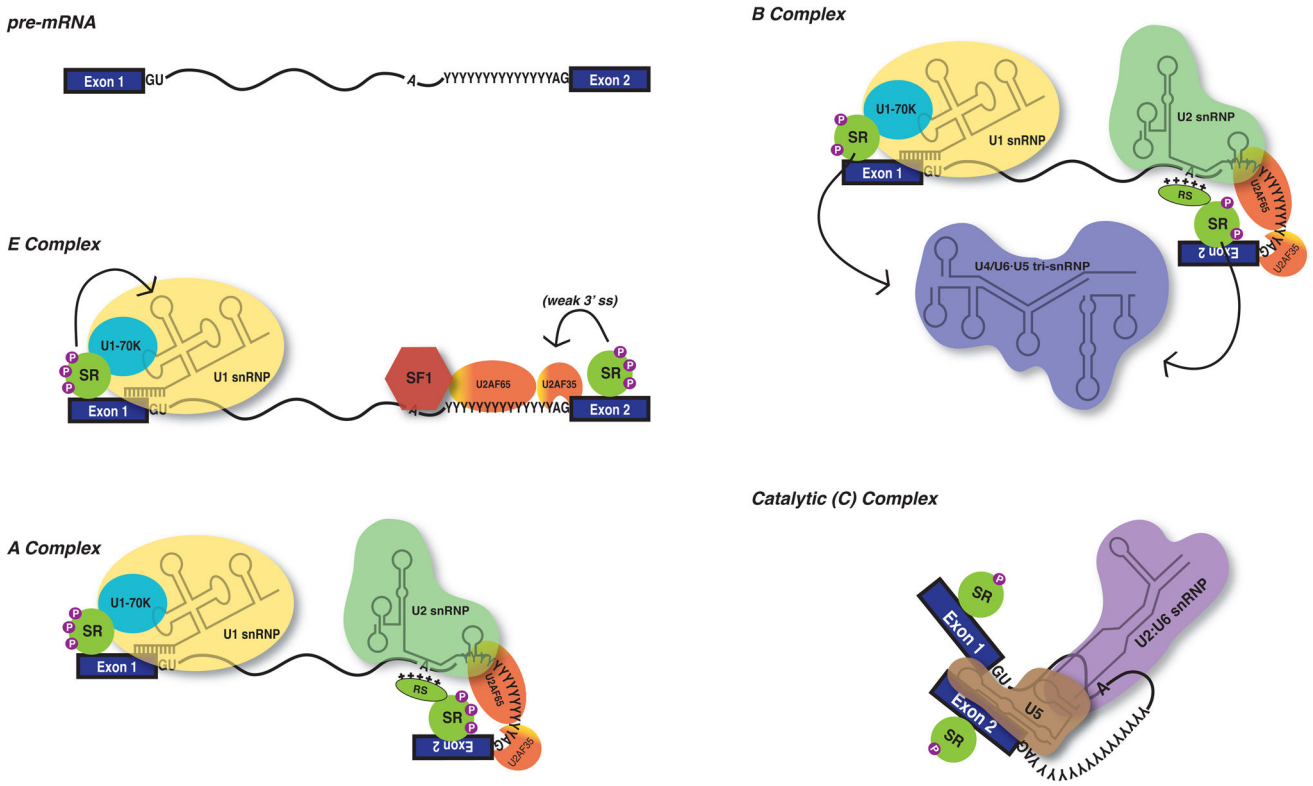

Figure 1. SR proteins regulate spliceosome assembly

Spliceosome assembly onto the pre-mRNA occurs in a coordinated, stepwise manner. In E complex, SR proteins regulate U1 snRNP recruitment to the 5′ splice site GU, and U2AF35/65 bound to the pyrimidine tract and 3′ splice site AG. In the A complex, SR proteins may facilitate U2 snRNP binding at the branchpoint by neutralizing the negative phosphodiester backbone charge. SR proteins can also recruit U4/U6•U5 tri-snRNP during B complex. Molecular rearrangements and dephosphorylation of SR proteins occurs to form the catalytically active C complex, in which U2 and U6 interact, and U6 replaces U1 snRNP, and U5 coordinates exons prior to splicing and ligation. SF1, splicing factor 1; snRNP, small nuclear ribonucleoprotein, SR, SR protein; RS, Arginine/Serine motif; 5′ and 3′ splice sites are indicated by GU and AG dinucleotides, respectively; (Y)n, polypyrimidine tract; P, phosphate moiety](References for presented data are included in text)

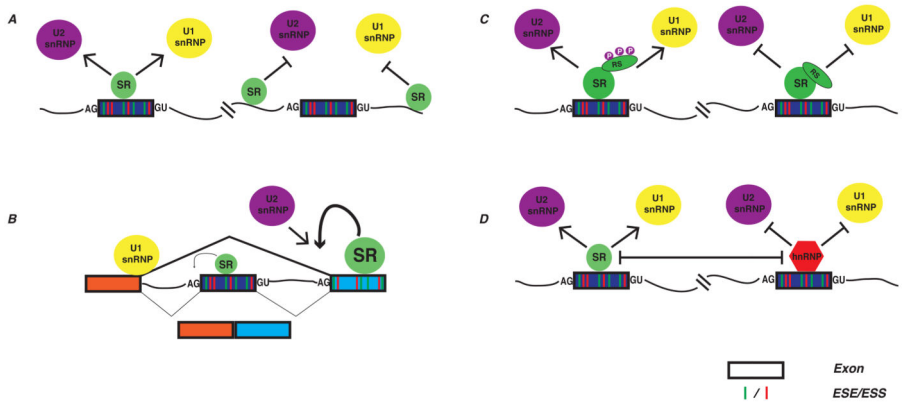

Figure 2. SR proteins regulate alternative splicing

A, SR proteins have been shown to promote or inhibit U1/U2 snRNP recruitment with respect to their orientation to 5′ and 3′ splice sites. **B**, SR proteins bound to adjacent exons can compete for U2 snRNP recruitment to their respective 3′ splice sites, likely depending on the “strength” of the SR protein to recruit spliceosomal factors. **C**, Phosphorylation states of the RS domain can influence SR protein-dependent recruitment of U1 and U2 snRNPs. **D**, Antagonistic relationships of SR proteins and hnRNP proteins often influence recruitment of spliceosomal factors. ESE/ESS, exonic splicing enhancers/exonic splicing silencers. (References for presented data are included in text).

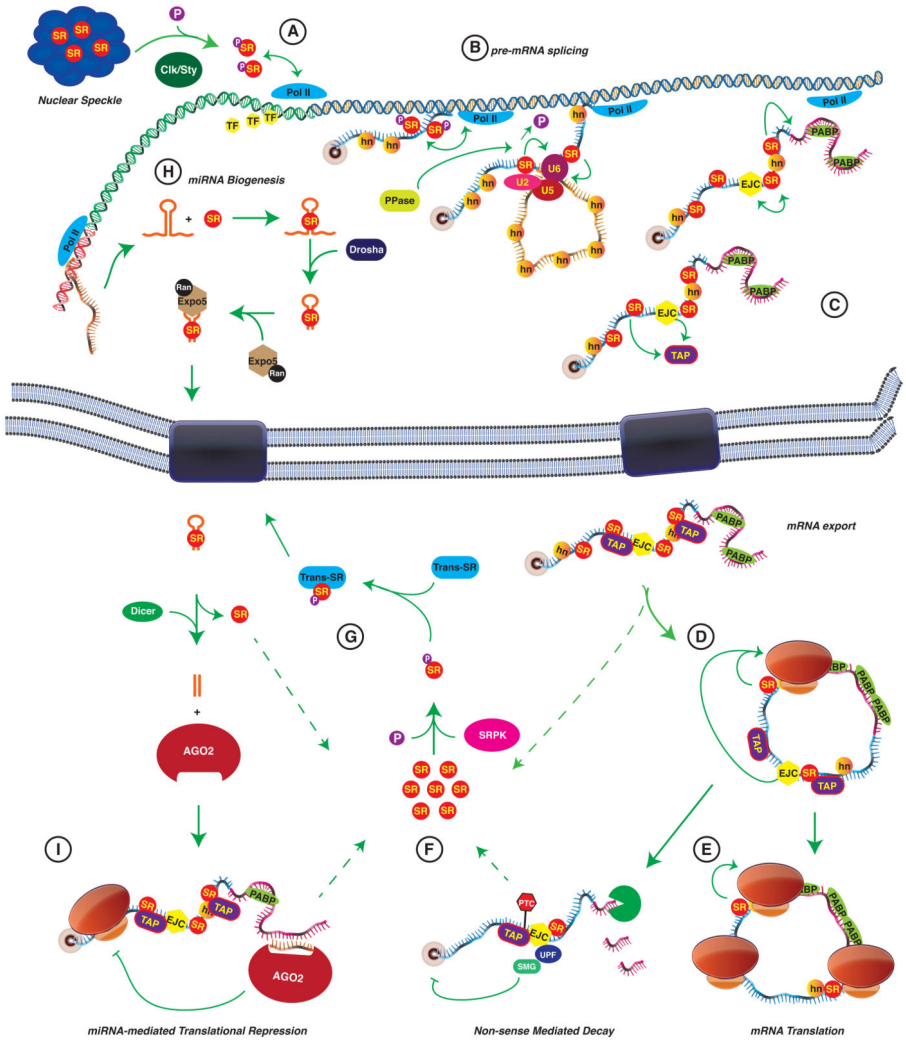

Figure 3. The life cycle of an SR protein

A, SR proteins remain localized to nuclear speckles until they are phosphorylated by Clk/Sty. At this point they can be recruited to areas of active transcription, possibly in Pol II-dependent manner. **B**, SR proteins can then bind to splicing enhancers in the nascent pre-mRNA transcript to facilitate spliceosome assembly co-transcriptionally in phosphorylation dependent manner. **C**, Following maturation of the mRNA transcript, SR proteins, along with other factors (e.g. EJC proteins) can facilitate TAP binding to the mRNP and subsequent nuclear export. **D**, After export, SR proteins can enhance the pioneering round of mRNA translation and send the transcript down one of two pathways: **E**, the ribosome encounters no pre-termination codons (PTC) and continues with steady-state translation, or **F**, a PTC is encountered and nonsense-mediated decay proceeds. **G,** Released SR proteins can then be phosphorylated by cytoplasmic SRPK, which triggers binding of Transportin-SRs and nuclear import of SR proteins for storage or further rounds of splicing. **H**, SR proteins may also play roles in miRNA biogenesis by facilitating export of pre-microRNAs to the cytoplasm for further processing and use in RNA-induced silencing **(I).** SR, SR protein. P, phosphate moiety. TF, transcription factor. Pol II, RNA polymerase II. hn,

hnRNP proteins. U2, U5, U6, U snRNPs. PPase, protein phosphatase. EJC, exon junction complex. PABP, poly-A binding protein. TAP, TAP/nuclear export factor 1. Exo, exosome. SRPK, SR protein Kinase. Trans-SR, Transportin-SR. Expo5, Exportin 5. AGO2, Argonaute 2. (References for presented data are included in text).

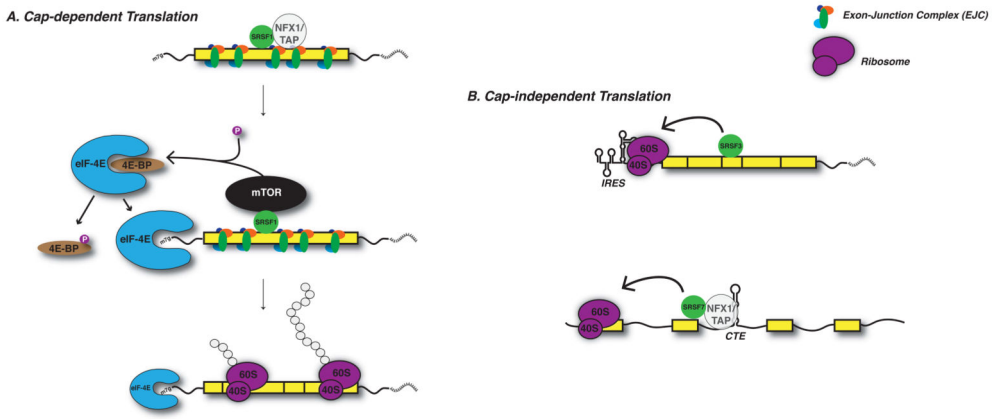

Figure 4. SR proteins function in translation initiation

SRSF1 bound to exported mRNAs can associate with mTOR kinase and recruit it to cytoplasmic mRNP complexes. This facilitates phosphorylation of 4E-BP, causing dissociation from eIF4E, and increasing efficiency of cap-dependent translation initiation. SR proteins have also been shown to enhance cap-independent translation initiation of viral RNAs that contain internal ribosome entry site (IRES) elements and constitutive transport elements (CTEs). (References for presented data are included in text).

Table 1

The SR Protein Family

Includes domain configuration of protein members, protein aliases, shuttling activities, reported molecular functions and biological processes.

Gene Symbol	Domain Structure	Protein Aliases	Shuttling	Molecular Functions	Biological Processes	References
SRSF1		**SF2, ASF, SRp30A**	**Yes**	pre-mRNA splicing; mRNA export; translation; miRNA biogenesis; mRNA stability; NMD; transcriptional elongation	apoptosis; cell-cycle; senescence; cell growth proliferation; SUMOylation; Genomic Stability; cytoskeleton organization; embryogenesis; retinal development; cardiac development; cancer	126, 132–141
SRSF2		**SC35, SRp30B**	**No**	pre-mRNA splicing; Genomic Stability; transcriptional elongation	cell survival; cell cycle; cancer; metastasis; senescence; apoptosis; development; neural plasticity; metabolism	37, 142–149
SRSF3		**SRp20**	**Yes**	pre-mRNA splicing; mRNA export; (viral) mRNA translation; transcriptional elongation	cell adhesion and migration; cell cycle; cell proliferation; cellular senescence; aerobic glycolysis; neuronal survival and growth; apoptosis; glucose and lipid metabolism; cholesterol homeostasis; LTM formation; development; neurological disorders; cancer	150–160
SRSF4		**SRp75**	**Yes**	pre-mRNA splicing;	neural differentiation	160
SRSF5		**SRp40**	**No**	pre-mRNA splicing; (viral) mRNA translation	insulin signaling; lipid transport; cell cycle; apoptosis; cancer; bipolar disorder	161–164
SRSF6		**SRp55**	**Yes**	pre-mRNA splicing; (viral) mRNA translation	drosophilia development; cardiac development; eye development; apoptosis; wound healing; cell cycle; cytoskeleton organization; genomic integrity; angiogenesis; lipid transport; muscle	163, 168–178

Gene Symbol	Domain Structure	Protein Aliases	Shuttling	Molecular Functions	Biological Processes	References
					development; calcium metabolism	
					development; calcium metabolism	
SRSF7		**9G8**	**Yes**	pre-mRNA splicing; mRNA export; (viral) mRNA processing	microtubules stabilization; viral infection	176, 177
SRSF8		**SRp46**	**ND**	pre-mRNA splicing	N/A	190
SRSF9		**SRp30c**	**ND**	pre-mRNA splicing mRNA translation	glucocorticoid signaling; apoptosis; cell-adhesion	178–181
SRSF10		**SRp38, SRrp40**	**Yes**	pre-mRNA splicing; mRNA translation	stress response; neuronal differentiation; cholesterol biosynthesis; cell cycle	73, 116, 182–186
SRSF11		**p54, NET2**	**ND**	pre-mRNA splicing; genomic stability	genomic integrity; ATP synthesis	187, 188
SRSF12		**SRrp35**	**ND**	pre-mRNA splicing	cell cycle	189

RRM, RNA recognition motif; RRMH, RNA recognition motif homology; RS, Arginine/Serine-rich motif; Zn, Zinc-binding domain. (References for presented data are included in table and text).