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Critical Review

Regulating the Regulators: Serine/Arginine-rich Proteins Under Scrutiny

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Summary

Serine/arginine-rich (SR) proteins are among the most studied splicing regulators. They constitute a family of evolutionarily conserved proteins that, apart from their initially identified and deeply studied role in splicing regulation, have been implicated in genome stability, chromatin binding, transcription elongation, mRNA stability, mRNA export and mRNA translation. Remarkably, this list of SR protein activities seems far from complete, as unexpected functions keep being unraveled. An intriguing aspect that awaits further investigation is how the multiple tasks of SR proteins are concertedly regulated within mammalian cells. In this article, we first discuss recent findings regarding the regulation of SR protein expression, activity and accessibility. We dive into recent studies describing SR protein auto-regulatory feedback loops involving different molecular mechanisms such as unproductive splicing, microRNA-mediated regulation and translational repression. In addition, we take into account another step of regulation of SR proteins, presenting new findings about a variety of post-translational modifications by proteomics approaches and how some of these modifications can regulate SR protein sub-cellular localization or stability. Towards the end, we focus in two recently revealed functions of SR proteins beyond mRNA biogenesis and metabolism, the regulation of micro-RNA processing and the regulation of small ubiquitin-like modifier (SUMO) conjugation.
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INTRODUCTION

Splicing and Alternative Splicing

The two-step process of pre-mRNA splicing is catalyzed by the spliceosome, a dynamic macromolecular machine composed of five small nuclear ribonucleoprotein particles termed U1, U2, U5 and U4/U6 snRNPs and many non-snRNP splicing factors. The assembly of the spliceosome is initiated with the recognition of the 5′ splice site (ss) by the U1 snRNP together with U2 snRNP binding to the branch point to form the A complex. The joining of a pre-assembled U4/U6-U5 snRNP (tri-snRNP) results in the formation of the B complex. After a series of macromolecular rearrangements, involving replacement of U1 by U6 small nuclear RNA at the 5′ ss and release of U1 and U4 snRNPs, the first step of splicing proceeds. Following further rearrangements, the second step takes place and the spliceosome dissociates, releasing the mRNA and the intron (*1*). Poorly conserved mammalian splice sites are typically not sufficient to recruit the spliceosome with high affinity. In addition, spliceosome assembly can be prevented by pre-mRNA secondary structure or by RNA-binding factors (*2*). This gives rise to alternative splicing, a process by which different sequence segments within the pre-mRNA can be included or excluded from the mature mRNA. Auxiliary factors bound to non-splice site sequences within exons or introns can influence the recognition of splice sites by the splicing apparatus. Sequences that promote spliceosomal recognition of an exon are called exonic or intronic splicing enhancers (ESE or ISE), whereas sequences that inhibit recognition of an exon are called exonic or intronic splicing silencers (ESS or ISS) (*3*).

Splicing Regulatory Factors: Two Well-known Families of Proteins

The most studied splicing regulators—acting at both constitutive and alternative splicing—are grouped into two well-characterized families of RNA-binding proteins: serine/arginine-rich

(SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) (*4*).

hnRNPs, which are among the most abundant nuclear proteins, stably associate with newly synthesized pre-mRNA, playing important roles in nuclear and cytoplasmic steps of mRNA metabolism (*5*). In particular, the mammalian hnRNP family consists of at least 24 structurally diverse polypeptides (*6*). SR proteins constitute a family of about twelve evolutionarily conserved polypeptides (*7*) that contain one or two N-terminal RNA-recognition motifs (RRMs) and a region of variable length that is enriched in Arg-Ser dipeptides (RS domain). Although this latter domain was initially characterized as a mediator of protein–protein interactions, it has been later demonstrated that it can directly contact the pre-mRNA. Furthermore, the RS domain undergoes extensive phosphorylation on Ser residues, which affects the sub-cellular distribution as well as the interactions of these proteins, consequently regulating their activity (*8*). Similarly, RRM domains, initially described as responsible for the RNA binding ability of SR proteins, are also capable of mediating protein-protein interactions (*9, 10*).

SR proteins and hnRNPs can either promote exon inclusion or exon exclusion through their binding to splicing enhancers or splicing silencers, respectively. Most transcripts studied so far contain multiple ISE/ESEs and ISS/ESSs and are bound by different combinations of regulatory proteins that can antagonize one another directly or indirectly. Therefore, subtle changes in the balance of expression or binding capacity of individual regulatory proteins can frequently alter the ratio of mRNA isoform expression.

It is well established that all steps of pre-mRNA processing can occur co-transcriptionally and indeed that is more a rule than an exception (*11*). In this respect, the interconnection between different steps of gene expression is even broader as mRNA export and translation are also coupled to transcription and processing (*12, 13*). SR proteins play a crucial role in assembling this complex mesh. They connect many stages of gene expression, ultimately contributing to the expansion of the proteomic diversity that allows the generation and maintenance of eukaryotic multicellular organisms. Apart from the initially identified and widely studied role in splicing regulation, SR proteins also participate in genome stability, chromatin binding, transcription elongation, mRNA stability, mRNA export and mRNA translation (*14*). This list of SR protein activities seems far from complete, as unexpected functions keep being unraveled by different laboratories.

How the multiple tasks of SR proteins are concertedly regulated within mammalian cells is not fully understood. In this article, we will discuss recent and intriguing findings regarding the regulation of SR protein expression and activity (Fig. 1, Table 1), the involvement of SR proteins at different tiers of gene expression regulation beyond splicing and even beyond mRNA metabolism, and whenever possible, regarding the cellular consequences of the connection between these two aspects.

REGULATION OF SR PROTEIN EXPRESSION: FROM GENE TO PROTEIN

Post-transcriptional Regulation of SR Protein-coding Genes

Cellular homeostasis requires a precise control of gene expression regulators. Transcription factors usually recognize their own as well as other transcription factors promoters, eliciting feedback loops. Similarly, splicing factors carry out regulatory feedback loops to maintain homeostatic expression levels.

An interesting case was revealed by studying ultra-conserved DNA elements within SR protein-coding genes along evolution (*17, 18*). These elements overlap with alternatively spliced regions. In particular, alternative splicing of these regions is involved in the generation of non-sense mediated decay (NMD)-sensitive isoforms, either through the incorporation of premature translation termination codons (PTC) or the generation of exon-exon junctions downstream the canonical stop codon. This phenomenon that couples alternative splicing to NMD (AS-NMD) was described for several genes, being particularly frequent and conserved in SR genes and providing a regulatory mechanism by which splicing factors could exercise their function as alternative splicing regulators to maintain their expression levels (*25*). Furthermore, the Brenner laboratory has demonstrated the existence of unproductive splicing in 11 human SR genes. They estimated that NMD-sensitive isoforms comprise 2–14% of the spliced mRNA population from each gene in untreated cultured cells, while this percentage rises to 40–70% when NMD is inhibited by Upf1 depletion. These results suggest that a large fraction of SR transcripts is being spliced into isoforms that are degraded by NMD (*17*).

In this line, the Biamonti laboratory has investigated the relevance of the regulation of SR protein expression by unproductive splicing in a physio-pathological context. They used a cell culture model for epithelial–mesenchymal transition (EMT) and its reversal mesenchymal-epithelial transition (MET). EMT is a highly regulated process in which epithelial cells lose their characteristics and acquire an invasive, motile, mesenchymal phenotype (*26*). Culturing colon adenocarcinoma cells at different densities, which allows the recapitulation of the EMT-MET process, lead to the finding that protein levels of the SR protein SRSF1 modulate the splicing pattern of Ron pre-mRNA, in turn determining the phenotype of these cells. Low levels of SRSF1 lead to the production of a full length, regulation-sensitive tyrosine kinase receptor Ron, responsible for an epithelial phenotype; while high levels of SRSF1 results in a truncated, constitutively active tyrosine kinase receptor isoform, named delta-Ron, that triggers malignant transformation upon overexpression (*27*). In this scenario, SRSF1 protein levels are controlled by AS-NMD. More precisely, epithelial-derived diffusible factors inhibit phosphorylation of the splicing regulator Sam68 by ERK1/2, consequently inhibiting Sam68 activity and leading to the generation of an NMD-sensitive SRSF1 splicing isoform (*28*). This regulatory pathway results in low protein levels of SRSF1 perpetuating the epithelial phenotype.

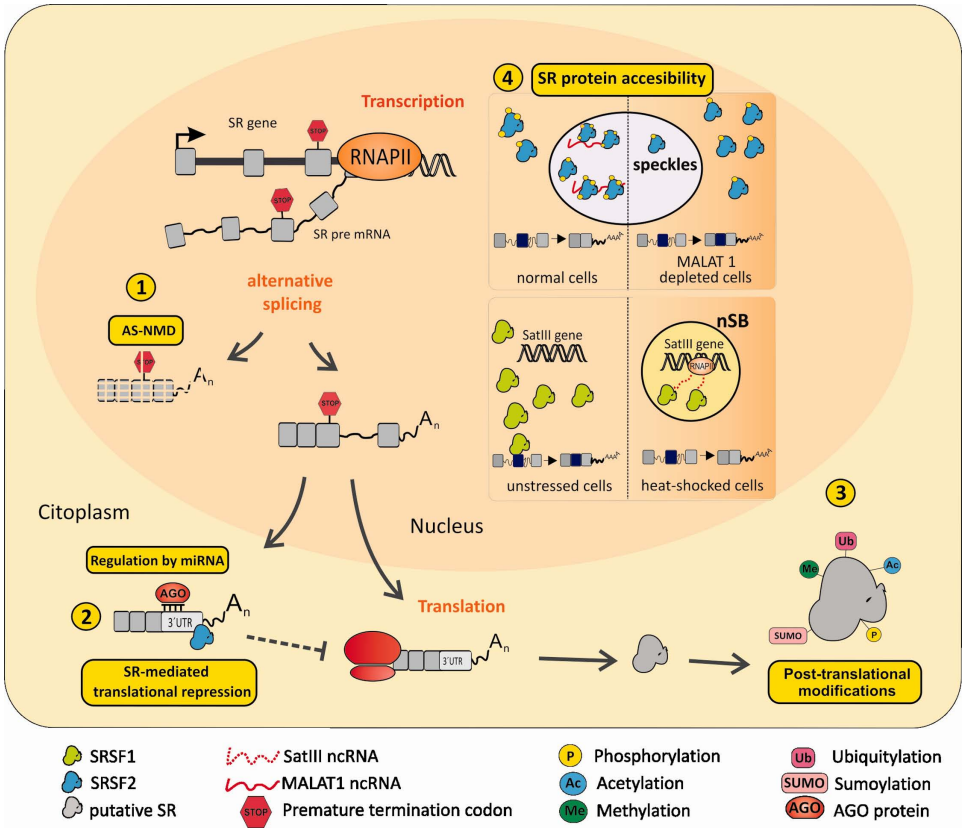

Figure 1. The scheme summarizes current knowledge about the regulation of SR protein expression and activity. (1) SR protein pre-mRNAs are subject to unproductive splicing or AS-enhanced NMD (AS-NMD), either as part of auto-regulatory feedback loops or cross-regulation among different splicing factors. (2) 3′UTR of SR protein mRNAs is involved in SR-mediated translational repression and is also a target for miRNA-dependent regulation. (3) SR proteins can be post-translational modified by phosphorylation, acetylation, methylation as well as Ub and SUMO conjugation. These PTMs can alter sub-cellular localization as well as SR protein activity. (4) SR protein accessibility and sub-nuclear localization can be regulated through the interaction of these proteins with different ncRNAs. See text for further details. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Moreover, it has been demonstrated by the Krainer laboratory that SRSF1 negatively regulates its own expression to maintain homeostatic levels. SRSF1 regulates alternative splicing of its own transcript, resulting in decreased production of the protein-coding isoform and increased production of unproductive mRNA isoforms that are either retained in the nucleus or degraded by NMD (*29*).

It is thus clear that changes in SR protein levels can alter the ratio between isoforms of diverse mRNAs, having profound consequences on cellular and organismal physiology. In particular, over-expression of SRSF1 has been shown to be sufficient to transform cells in culture. SRSF1-overexpressing murine immortal fibroblasts developed fibrosarcomas when injected into nude mice (*30*), and SRSF1-overexpressing mouse mammary epithelial cells COMMA1-D promoted invasive mammary tumors when engrafted into cleared mammary-gland fat pads of syngeneic mice. Furthermore, over-expression of SRSF1 enhanced proliferation and delayed apoptosis in a 3-D cell culture model that recapitulates different aspects of normal mammary gland morphogenesis, leading to larger epithelial acini (*31*). On the other hand, it has been reported that knockdown of SRSF1 leads cell cycle arrest and apoptosis in several cell lines (*32*). Exploring the mechanism by which SRSF1 depletion triggers cell death, the Manley laboratory underscored an important function of SRSF1 in cell physiology. This SR protein protects chromosomal DNA from the detrimental effects of R-loops, which are formed by hybridization between nascent transcripts and their corresponding template DNA during the process of transcription. Thus, SRSF1 depletion leads to DNA double-strand breaks and genomic instability (*33*).

To add complexity, SRSF1 auto-regulatory feedback results from the combination of multiple layers of control, including not only alternative splicing but also translation regulation: SRSF1 represses the translation initiation step of its own mRNA through the 3′UTR, which is also a putative target for diverse micro-RNAs (miRNAs) (*29*). Regarding the regulation of SRSF1 expression levels by miRNA, recent work from the Barettino laboratory (*19*) has shown that miR-10a

Table 1
The table details different stages of the regulation of SR protein expression

Gene symbol	Aliases	Type of AS NMD	miRNA regulator	Acetylation (*15*)	Methylation	Ubiquitylation (*16*)
SRSF1	ASF, SF2	3′UTR intron activation (*17, 18*)	miR-7, miR-10a, miR-10b (*19, 20*)	K38, K179	R93, R97, R109 (*21, 22*)	K30, K38, K138, K174, K179
SRSF2	SC35	3′UTR intron activation (*17*)	miR-193a-3p (*23*)	K36, K52	–	K36
SRSF3	SRp20	PTC exon cassette (*17, 18*)	–	K23	–	K23
SRSF4	SRp75	PTC exon cassette (*17*)	–	–	–	K27
SRSF5	SRp40	PTC exon cassette (*17, 18*)	–	K167	–	K25, K125
SRSF6	SRp55	PTC exon cassette (*17, 18*)	–	K101	–	K155
SRSF7	9G8	PTC exon cassette (*17, 18*)	–	K24, K185	–	K12, K24
SRSF8	SRp46	3′UTR intron activation (*17*)	–	K315	–	K36
SRSF9	SRp30c	PTC exon cassette (*17, 18*)	miR-1 (*24*)	–	–	K28, K36, K128, K169
SRSF10	SRp38	PTC 5′ASS (*17*)	–	–	–	–
SRSF11	p54	PTC exon cassette (*17, 18*)	–	–	–	K58, K197
SRSF12	SRrp35	–		–	–	–

Type of AS-NMD: *3′UTR intron activation*, the AS-NMD isoform is produced by an atypical splicing event in the 3′UTR of the pre-mRNA, which leaves an exon-exon junction more than 50 nucleotides downstream the canonical termination codon. *PTC exon cassette*, the AS-NMD isoform is generated by the inclusion of an alternative exon cassette that introduces a PTC. *PTC 5′ ASS*, the AS-NMD isoform is produced by the usage of an alternative 5′ss that extends the exon and introduces a PTC. *miRNA regulator:* miRNAs shown to regulate the expression of different SR genes are listed. *Acetylation, Methylation and Ubiquitylation*: the target residues for each different PTM are indicated. In every case, numbers within parenthesis indicate cited publications.

and 10b directly target SRSF1 3′ UTR, leading to SRSF1 mRNA destabilization during retinoic-acid induced differentiation of neuroblastoma cell line SH-SY5Y. In addition, changes in miR-10a and 10b levels alter SRSF1 regulatory activity both at alternative splicing and mRNA translation level, as evaluated by the analysis of tau exon 10 inclusion and translation of a reporter mRNA harboring an SRSF1-binding sequence, respectively. A proteomic analysis previously performed by the same laboratory had underscored that treatment of SH-SY5Y neuroblastoma cells with retinoic acid led to changes in the phosphorylation pattern of a great variety of splicing factors, among them different hnRNPs and SR proteins including SRSF1. These results clearly show that a specific extracellular signal, acting at different tiers of gene expression, can regulate both the level and the activity of a given SR protein (*34*).

Post-translational Modifications of SR Proteins: Above and Beyond Phosphorylation

A vast body of work has shown that the Ser residues within SR proteins' RS domains are extensively phosphorylated, and this post-translational modification has been implicated both in the regulation of the sub-cellular localization of these SR proteins as well as protein-protein interactions (*35*), thus influencing SR protein activities. Therefore, the search for signaling pathways and protein kinases affecting the phosphorylation status of these splicing factors has been the focus of different laboratories, and their discoveries have been extensively reviewed over the last decade (*36–38*). We will devote part of this review to the discussion of recent data about the regulation of SR proteins by post-translational modifications (PTMs) other than phosphorylation (Table 1).

Acetylation. Mass spectrometry analysis identified 1,750 proteins targeted by lysine acetylation. The classification of the identified proteins according to their cellular function revealed splicing factors as one of the predominant class of proteins within this acetyloma (*15*). Furthermore, when analyzed by protein domain structures, RRMs appeared over-represented. These results suggest that PTM by lysine acetylation could be involved in the regulation of pre-mRNA processing events. In agreement, Edmond and collaborators have recently found that the SR protein SRSF2 is modified by acetylation within its RNA binding domain at K52 (*39*). SRSF2 acetylation levels are regulated by the opposing action of the acetyl-transferase TIP60 and the deacetylase HDAC6 such that acetylation by TIP60 reduces SRSF2 protein levels through proteasome-dependent degradation, while deacetylation by HDAC6 exerts the opposite effect. Over-expression of TIP60 attenuates SRSF2 phosphorylation, revealing a cross-talk between acetylation and phosphorylation that regulates SRSF2 activity. In this line, TIP60 controls the nuclear localization of SRPK1 and SRPK2, two kinases that contribute to SRSF2 phosphorylation. Interestingly, cisplatin-induced genotoxic stress results in down-regulation of TIP60 protein levels, nuclear accumulation of SRPK1/2 and stabilization of hypoacetylated/phosphorylated SRSF2, in turn leading to caspase-8 pre-mRNA splicing switch and consequently to program cell death.

Methylation. RNA-binding proteins belonging to the hnRNP family are among the most abundant cellular targets for dimethylarginine modification mostly in the context of the RGG tripeptide. Accordingly, it was proposed many years ago that arginine methylation could have an important functional role in RNA metabolism (*40, 41*). Not so long ago, a global analysis of methylation sites by heavy methyl SILAC (stable isotope labeling by amino acids in cell culture) has revealed that many different hnRNPs and also the SR protein SRSF1 and other splicing factors such as Tra2β, are methylation substrates (*21*). Human SRSF1 contains three methylated Arg residues (R93, R97 and R109) in the linker between RRM1 and RRM2, and the functional relevance of these methylations has been recently investigated by the Krainer laboratory (*22*). By mutating these three Arg residues within SRSF1 to Ala, they found that the triple-Ala mutant was still able to shuttle between the nucleus and the cytoplasm. However, this mutant was predominantly cytoplasmic in contrast to the localization of wild-type SRSF1 to nuclear speckles. This increased cytoplasmic residence time of the triple-Ala mutant resulted in a diminished nuclear function of this protein both as a regulator of specific endogenous pre-mRNA alternative splicing and as a promoter of NMD; as well as an augmented cytoplasmic activity as measured by its ability to enhance translation of reporter mRNAs. Additional results suggested that the methylation state of SRSF1 linker region as well as the positive charge conferred by this modification could control protein-protein interactions, either directly or by cross-talking with other PTMs within SRSF1, finally contributing to the proper localization of this factor.

The enzymes that catalyze arginine methylation are known as protein arginine methyltransferases (PRMTs). SFRS9, a close paralogue of SRSF1, is a target for PRMT1-mediated arginine methylation in vitro, and this PTM is implicated in the sub-nuclear localization of SFRS9 (*42*). In this respect, it has been reported that the yeast protein arginine methyl transferase Hmt1, is crucial for nuclear transport of several hnRNPs and for the dynamic association between RNA-binding proteins and pre-mRNA during the biogenesis of mature messenger ribonucleoprotein particle (mRNP) (*43, 44*). Chen and collaborators have shown that Hmt1-catalyzed arginine methylation controls proper co-transcriptional recruitment of pre-mRNA splicing factors (*45*). PRMTs have been associated with alternative splicing regulation in a variety of organisms. By global analysis using tiling arrays as well as a high-resolution RT-PCR panel, it has been shown that the lack of PRMT5 modifies alternative splicing patterns in both *Arabidopsis thaliana* and *Drosophila melanogaster*. Alternative splicing changes were detected among others, in clock core and clock-related genes, affecting the circadian rhythm of the mutant organisms (*46*).

Conjugation by Ubiquitin and Ubiquitin-like Proteins. PTMs often consist of a small chemical substituent as is the case for phosphorylation, acetylation and methylation described above. Cellular proteins are also modified by the covalent attachment of other polypeptides such as ubiquitin (Ub) or members of the Ub family referred to as ubiquitin-like proteins (Ubls). These PTMs control a wide variety of cellular processes. It is well established that Ub chains can target proteins for degradation by the 26S proteasome, the major cytosolic proteolytic system in eukaryotes (*47*). However, it has become increasingly clear that conjugation of Ub or Ubls to proteins can alter their properties and function without labeling them for degradation. There are at least 12 members of the Ub family encoded by the human genome known to affect activity, structure, sub-cellular localization and repertoire of interactions of the target proteins. With respect to splicing, not only ubiquitylation substrates but also components of the ubiquitylation/de-ubiquitylation pathway coexist within the spliceosome. In particular, non-proteolytic ubiquitylation of the U4 component Prp3 promoted by the Prp19 complex is required for stabilization of tri-snRNP U4-U5/U6, while de-ubiquitylation of Prp3 by Usp4/Sart3 is required for further U4 dissociation and recycling (*48*).

High-throughput identification of specific ubiquitylation sites by mass spectrometry not only confirmed already known sites but also mapped more than 10,000 previously unidentified ones, including putative ubiquitylation sites in almost every member of the SR protein family (*16*). Site-specific ubiquitylation in response to proteasome inhibition by MG-132 was quantified by SILAC/mass spectrometry and, surprisingly, 40% of the quantified sites did not show an increase in ubiquitylation and even more, ubiquitylation in 15% of the sites was significantly reduced. Intriguingly, SR protein ubiquitylation sites were included in this latter category. These results indicate that a substantial fraction of the sites seems to be unrelated to proteasomal-mediated degradation and suggests that Ub conjugation to SR proteins may work as a regulatory signal instead of as a degradation labeling (*16, 49*). Likewise, proteomic approaches revealed that RNA-binding proteins are the predominant group among small Ubiquitin-like modifier (SUMO) conjugation substrates, including several hnRNPs, SR family members and spliceosome components (*50, 51*). Furthermore, SUMO conjugation has been found to regulate different aspects of mRNA metabolism such as pre-mRNA 3′end processing and RNA editing, by modifying the function of poly(A) polymerase, symplekin and CPSF-73 in the former case and ADAR1 in the latter (*52, 53*). It would be interesting to elucidate whether Ub or Ubl conjugation, in particular SUMO, could affect SR protein activities.

RNA-Dependent Regulation of SR Protein Accessibility. RNA molecules serve not only as SR protein substrates during mRNA metabolism but are also regulators of these protein levels and activities. The fact that the abundant, nuclear-retained, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1; a.k.a. Neat2) is highly conserved in mammals and localized to nuclear speckles led the Prasanth laboratory to investigate the involvement of this non-coding (nc) RNA in pre-mRNA metabolism (*54*). They found that MALAT1 does not play a role in the formation and maintenance of nuclear

speckles but instead, it interacts with a sub-set of SR proteins modulating their sub-nuclear distribution and even more intriguingly, influencing the cellular levels and the ratio of phosphorylated versus de-phosphorylated SR proteins. Consistent with these observations, MALAT1 plays a role in regulating alternative splicing patterns of certain endogenous pre-mRNAs. In particular, MALAT1 depletion exerts similar effects to SRSF1 over-expression in cultured cells. The authors proposed that MALAT1 could be acting as a "molecular sponge" to regulate localization, concentration and ultimately the activity of certain SR proteins at the splicing level. Interestingly, it has been shown that Pc2, an E3 ligase of the SUMO pathway, binds MALAT1 and in this context promotes SUMOylation of E2F1 transcription factor (*55*). In this scenario, it is tempting to speculate that MALAT1 could be functioning not only as a regulator of SR protein accessibility but also as an RNA scaffold involved in the regulation of SR protein PTMs.

The concept of the "molecular sponge" affecting splicing factor sub-cellular distribution and activity is reminiscent of the recruitment of SR proteins, including SRSF1, to nuclear stress bodies (nSBs). This recruitment is mediated by their interaction with stress-induced satellite III (SatIII) transcripts in heat-shocked human cultured cells, consequently altering alternative splicing profiles (*56, 57*).

Interestingly, CLIP-seq experiments had previously shown that SRSF1 binds functionally distinct transcripts such as miRNAs, snoRNAs and ncRNAs including MALAT1, as well as conserved intergenic transcripts of unknown function (*58*). The question that awaits further investigation is whether the MALAT1 or SatIII-dependent regulation is also affecting any of the other described activities of SR proteins, in particular those of SRSF1.

SR PROTEIN ACTIVITY BEYOND mRNA METABOLISM

Apart from their well-characterized role as splicing regulators, SR proteins exert a wide range of cytoplasmic and nuclear activities at different stages of mRNA metabolism. Readers are referred to numerous and detailed review articles on this matter (*14, 59*). Here, we will focus in two recently revealed functions of the SR protein SRSF1 beyond mRNA biogenesis and metabolism.

SR Protein-Dependent Regulation of miRNA Processing

Given that SRSF1 auto-regulation is greatly mediated by its 3′UTR and that this region is a putative target for several miRNAs (*29*), the Zhu laboratory tested the hypothesized that SRSF1 could increase the expression of one or more miRNAs, which in turn repress translation of its own mRNA (*20*).

By deep sequencing, miRNA expression profiles were monitored upon induced expression of SRSF1. Following this initial screening, miR-7 among others was validated as being consistently up-regulated by SRSF1 over-expression, and down-regulated by SRSF1 knock-down in HeLa cells. A variety of experimental approaches were used to demonstrate that the enhancement of miR-7 expression is mediated by a splicing-independent function of SRSF1 and by a direct interaction between SRSF1 and the primary miR-7 transcript. SRSF1 promotes the Drosha cleavage step of pri-miR-7 processing stimulating miR7 maturation. Notably, mature miR-7 in turn targets the 3′UTR of SRSF1 to repress translation of SRSF1 mRNA. This negative feedback loop may synergize with the already described SRSF1 auto-regulatory mechanisms to finely tune cellular levels of this protein. This previously unknown function of SRSF1 in miRNA processing is reminiscent of that one already described for the splicing factors hnRNP A1 and KSRP (*60*).

Several intriguing questions arise from this study, particularly due to the fact that one out of three distinct loci that code for human miR-7 is embedded in the last intron of the hnRNP K gene, which undergoes alternative splicing via alternative 3′ss. Therefore, it is possible to speculate about either a competitive or a cooperative action between the splicing and the miRNA processing machineries, being SRSF1 a common regulator of both gene expression regulatory processes. Also, as SRSF1 promotes the processing of miR-7 and other miRNAs (at least miR-221, miR222 and miR-29b1) and regulates the splicing of a wide variety of pre-mRNAs, it would be interesting to find out whether there are common targets of these two SRSF1 distinct functions.

SR Protein-Dependent Regulation of SUMO Conjugation

Our laboratory has recently reported a novel function for the prototypic member of the SR protein family, SRSF1, as a modulator of protein sumoylation (*10*). SRSF1 associates with the SUMO E2 conjugating enzyme Ubc9 and enhances sumoylation of specific substrates. In addition, SRSF1 interacts with PIAS1 (protein inhibitor of activated STAT-1), regulating PIAS1-induced overall protein sumoylation. SRSF1 plays a role in heat-shock-induced sumoylation and promotes SUMO conjugation to RNA-processing factors. This rather unexpected function of SRSF1 is dependent on its RRM2 domain and is also executed by SRSF9 (there is a high sequence identity between these two proteins' RRM2 domains) but it is neither carried out by SRSF5 (there is a low sequence identity between SRSF1 and SRSF5 RRM2 domains) nor SRSF3 which does not contain an RRM2. Deciphering the RRM2-mediated protein-protein interaction networks both within the spliceosome and between spliceosome and other cellular machinery components could provide some interesting insights into this complex regulation. Furthermore, the mechanism by which SFSR1 exerts its effect is yet to be determined.

Recently, work from the Melchior lab has uncovered an intricate mechanism of action of the SUMO E3 ligase RanBP2 (*61*). They have shown that the E3 activity relies in a multi-subunit complex composed of RanBP2, sumoylated RanGAP, and Ubc9. While multi-subunit E3 ligases have long been known in the Ub field, this work confirmed the existence of E3 complexes for the SUMO conjugation pathway. In this line, SRSF1 might function in conjunction with PIAS1 and/or other protein(s) as a "bridging factor" between mRNA metabolism-related proteins

and the sumoylation machinery. This is further supported by the fact that the two proteins co-exist within the spliceosome (*62*).

CONCLUDING REMARKS

It has become evident over the last decade that concepts such as RNA transcription, splicing or export "machineries" as separate entities are obsolete. The more we learn about the mechanisms operating at these various steps of gene expression, the more we realize the interconnectedness among them and therefore the need for an integrative view of gene expression regulation.

We now know that many mRNA metabolism-related processes are somehow co-regulated and also that PTMs provide a rapid and reversible way for these processes to be modulated in response to different cellular conditions. In this context, the search for molecules that concertedly regulate different stages, from transcription to protein function, turn out to be extremely relevant. SR proteins, originally identified as splicing regulators, have been involved in an increasing number of gene expression regulatory events. They appear as extremely versatile and controllable proteins, able to engage in a wide spectrum of interactions with proteins, RNA and even chromatin (*63*), suggesting they can fulfill this duty of nexus molecules.

Most of the studies describing the different functions of SR proteins apart from splicing regulation have been carried out with a small fraction of SR family members, and mainly with the prototypic SRSF1. It would be interesting to precisely define how this characteristic of "multitasking protein" applies to different members of this family and how different expression and/or activity patterns of these proteins could be orchestrating diverse physiological and pathological cellular responses.

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