Molecules and Cells

Minireview

SR Proteins: Binders, Regulators, and Connectors of RNA

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Serine and arginine-rich (SR) proteins are RNA-binding proteins (RBPs) known as constitutive and alternative splicing regulators. As splicing is linked to transcriptional and post-transcriptional steps, SR proteins are implicated in the regulation of multiple aspects of the gene expression program. Recent global analyses of SR-RNA interaction maps have advanced our understanding of SR-regulated gene expression. Diverse SR proteins play partially overlapping but distinct roles in transcription-coupled splicing and mRNA processing in the nucleus. In addition, shuttling SR proteins act as adaptors for mRNA export and as regulators for translation in the cytoplasm. This mini-review will summarize the roles of SR proteins as RNA binders, regulators, and connectors from transcription in the nucleus to translation in the cytoplasm.

Keywords: export, RNA-binding proteins, SR proteins, splicing, transcription, translation

INTRODUCTION

Gene expression regulation is highly connected process. Transcriptional regulation is interdependent on post-transcriptional processes both in the nucleus and cytoplasm (Maniatis and Reed, 2002). Regulatory proteins, such as RNA-binding proteins (RBPs), play important roles throughout the gene expression program, from the transcription to translation (Glisovic et al., 2008). One such important family of regulatory RBPs is the Serine-Arginine (SR) family of proteins. In this mini-review, the essential functions of SR proteins in splicing regulation, with a focus on their roles as RNA binders in the ribonucleoprotein complex (RNP) will be summarized. I will also discuss their emerging regulatory roles in mediating and connecting post-transcriptional processes from the nucleus to the cytoplasm. More extensive reviews on SR proteins are suggested for a comprehensive understanding of these multifunctional regulators of RNA metabolism (Anko, 2014; Howard and Sanford, 2015).

SR protein family members

SR proteins are RBPs mainly functioning in RNA splicing (Shepard and Hertel, 2009). The first SR proteins identified were SRSF1 (previously called SF2/ASF) and SRSF2 (previously called SC35) (Manley and Krainer, 2010). SR proteins are characterized by the presence of a C-terminal domain enriched with the Arginine (R) and Serine (S) amino acid sequences (RS domain) and an N-terminal RNA recognition domain (RRM domain) (Fig. 1). Additional SR proteins, RNA-binding SR-related proteins and other RS domain containing proteins have been identified (Long and Caceres, 2009). In general, RRM domains recognize RNA, whereas RS domains participate in diverse protein-protein and protein-RNA interactions. Most SR proteins are located exclusively in the nucleus, but some SR proteins (mainly SRSF1, SRSF3, and SRSF7) can shuttle between the nucleus and the cytoplasm. Having many such SR proteins with differential expression and regulatory patterns indicates possible non-redundant and distinct roles in pre-mRNA splicing (Zahler et al., 1993) as well as in other steps of gene expression, as will be discussed below.

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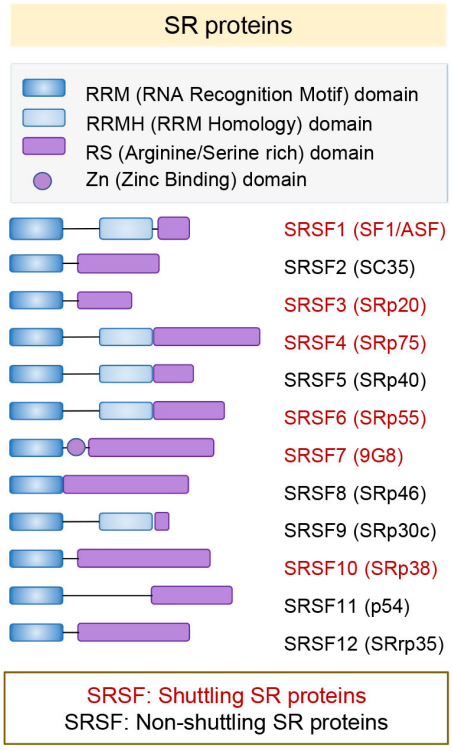

Fig. 1. List and domains of SR proteins. The domain structures (RRM, RRMH, RS, and Zn) are denoted as shown in the lower box. Current names for SR proteins are SRSFs, but aliases are also indicated in the parenthesis. Among 12 core SR proteins, 6 (red letters) are reported to shuttle between nucleus and cytoplasm (shuttling SR proteins), whereas the others (black letters) have not been shown to have shuttling activity (non-shuttling SR proteins), as indicated in the upper box.

SPLICING REGULATION

Importance of alternative splicing

Pre-mRNA splicing is an essential process, which occurs in the nucleus of eukaryotic cells. Splicing removes intervening introns from primary transcripts and joins exons to create mature mRNA by the spliceosome, a dynamic RNA-protein enzyme complex (Papasaikas and Valcarcel, 2016; Wahl et al., 2009). In addition to constitutive splicing, regulated splicing occurs to generate a large number of mRNA isoforms from given pre-mRNA by alternative splicing. In fact, large-scale transcriptome analysis revealed that up to 90% of human genes undergo alternative splicing (Pan et al., 2008). Moreover, splicing regulation is dynamically integrated to gene regulatory pathways (Braunschweig et al., 2013). Thus, alternative splicing greatly expands transcriptome as well as proteome diversities from a limited number of genes in a genome (Maniatis and Tasik, 2002; Weatheritt et al., 2016).

Splicing regulatory RNA elements

Splicing regulatory RBPs are thought to recognize distinct RNA sequences (splicing regulatory elements, SREs) and regulate splicing by "splicing code" (Fu, 2004; Wang and Burge, 2008). SREs include exonic splicing enhancers (ESE), exonic splicing suppressors (ESS), intronic splicing enhancers (ISE), and intronic splicing suppressors (ISS). In general, SR proteins bind ESE and enhance the splicing by recruiting the spliceosome. On the contrary, heterogeneous nuclear ribonucleoprotein (hnRNP) family proteins can antagonize the positive effect of SR proteins by binding to ESS or ISS and repressing splicing (Geuens et al., 2016).

Essential but complex role of SR proteins in alternative splicing

SR proteins are important alternative splicing regulators. In contrast to the robust splicing enhancing effect in constitutive splicing, SR-mediated alternative splicing regulation is more complex and subtle. Alternative exons generally hold shorter length and weaker 5′ spliced sites (5′ss), so SR proteins induce the inclusion of the alternative exon through increasing the recognition of weak splice sites by splicing machinery. Since the regulatory roles of RBPs in alternative splicing are position- and context-dependent (Fu and Ares, 2014), locations of SR-RNA interaction influence splicing outcome. For example, exon-bound SR proteins act as enhancers, but intron-bound SR proteins may function as suppressors (Shen and Mattox, 2012). So the location of SR-RNA interactions affect spliceosome assembly and splice site selection (Erkelenz et al., 2013). In addition, SR proteins can act as activators or repressors in a context-dependent manner with other RBPs (Fu and Ares, 2014; Han et al., 2011a).

SR PROTEINS AS RNA BINDERS

Selection of SR-binding RNA sequences *in vitro*

Since individual SR proteins are not functionally equivalent (Zahler et al., 1993), SR proteins are believed to bind RNA with unique or preferential specificity. Various analytical techniques for RNA-protein interaction have been utilized to determine the SR-binding RNA sequences. As an approach to identify RNA-binding sequences of SR proteins, *in vitro* SELEX (systematic evolution of ligands by exponential enrichment) experiments were performed (Fig. 2A). SELEX has the advantage of selecting high-affinity consensus RNA sequences (also called RNA aptamers) to target proteins among large pool of RNA library (at least 1015 different RNA sequences) (Tuerk and Gold, 1990).

SELEX approaches have been applied to determine specific SR-binding RNA sequences (Long and Caceres, 2009). SRSF1- and SRSF7-binding consensus RNA sequences are largely purine-rich elements with different RNA sequences, whereas pyrimidine-rich RNA sequence elements have been found for SRSF3. Selected RNA aptamers are useful for biochemical determination of protein-interacting RNA motifs and for inferring cellular target RNAs (Bunka and Stockley, 2006; Kim et al., 2012). Nonetheless, repeated selection procedures tend to amplify RNA aptamers with unique *in vitro* biochemical and biophysical interaction features to the target protein, so caution should be taken when interpreting the data (Bjerregaard et al., 2016).

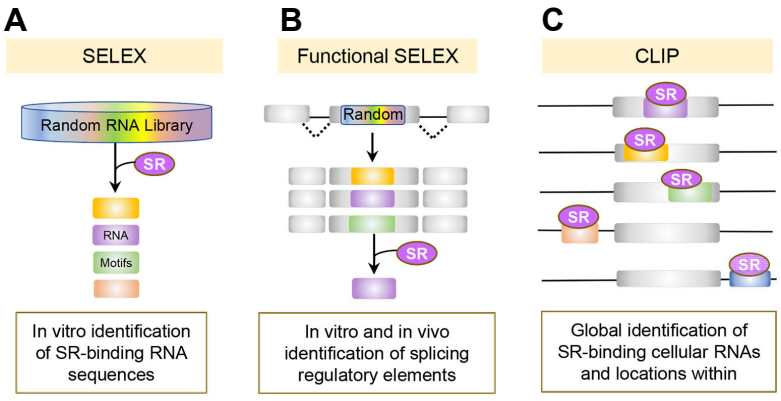

Fig. 2. Outline of techniques used for identification of SR protein-binding RNA elements. (A) SELEX (systematic evolution of ligands by exponential enrichment) for *in vitro* identification of SR-binding RNA motifs. A random RNA library was used for the selection of binding RNA sequences. (B) Functional SELEX. Reporter-based *in vitro* and *in vivo* identification of splicing regulatory elements. ESEs (Exonic Splicing Enhancers) can be selected in the reporter as shown here. (C) CLIP-Seq (Cross-linking and immunoprecipitation-sequencing) for global identification of SR-binding motifs in target RNAs.

Functional selection of splicing regulatory elements

Functional SELEX approaches have been developed to identify the splicing regulatory elements (SREs) in the cells (Fig. 2B). Randomized sequences are introduced in specific splicing reporters at the positions within or near an alternative exon and their splicing outcome are selected to identify ESEs in *in vitro* splicing reactions or in transfected cells (Cartegni, 2003; Coulter et al., 1997; Liu et al., 1998; Schaal and Maniatis, 1999).

Functional SELEX identified purine-rich ESEs, which resemble the *in vitro* selected SR-binding sequences. However, non-purine rich ESEs were also selected, suggesting other splicing proteins are also involved in enhancing alternative exon inclusion (Coulter et al., 1997). Degenerate and diverse ESEs have been recognized by SR proteins, implying a SR-RNA interactome with a broad sequence specificity (Liu et al., 1998). Subsequently, elaborate GFP-based splicing reporters and sorting of GFP-positive cells allowed the identification of ESSs (Wang et al., 2004) and intronic regulators (ISEs and ISSs) (Wang et al., 2012; 2013). Despite these new SRE sequences from functional SELEX analysis, it is still challenging to identify the types of RBPs and the direct contribution of SR proteins in the regulation of splicing.

Genome-wide identification of SR-binding cellular RNAs

Cellular RNP is formed by a complex interaction involving numerous RNAs and proteins, albeit with relatively low affinity (Jankowsky and Harris, 2015). Moreover, direct interaction between RBP and RNA is accompanied by indirect protein-protein interactions in RNP. Therefore, it is challenging to identify direct cellular RNA targets of RBPs and to map RBP binding RNA motifs. To identify cellular RBP-RNA interaction maps at a global scale, CLIP (cross-linking and immunoprecipitation) analysis has been developed. In the CLIP method, ultraviolet (UV) radiation is used to cross-link direct RBP-RNA interactions within a short distance (~1 Å) in the cells (Ule et al., 2005) (Fig. 2C). To gain insight into RBP-RNA interactions with precision, high throughput genomic techniques were combined with CLIP (CLIP-Seq or HITS-CLIP) (Konig et al., 2012). A couple of refinements were also made to the CLIP method to increase cross-linking efficiency (PAR-CLIP, Photoactivatable Ribonucleoside-Enhanced CLIP) or the binding site mapping precision (iCLIP, individual-nucleotide resolution CLIP).

CLIP methods have been applied to many SR proteins to identify SR-RNA interactions. In the case of SRSF1, CLIP-Seq revealed a functionally diverse landscape of RNA targets and identified purine-rich consensus motifs (Sanford et al., 2009; Wang et al., 2011). In contrast, CLIP analyses of SRSF3 and SRSF4 revealed their interactions to non-overlapping target genes, and identified distinct *in vivo* consensus binding motifs (Anko et al., 2010; 2012). However, in the case of SRSF1 and SRSF2, extensive overlap between two SR protein-binding targets has been observed (Pandit et al., 2013). These results indicate that SR-RNA interactions are generally degenerate and context-dependent. More refined genomic technologies and more detailed bioinformatics tools are required to map endogenous SR-RNA interactions and functional networks in the cells (Konig et al., 2012).

Large-scale identification of SR-regulated splicing

The SR-RNA interactome in the cell is likely to affect the SR-regulated transcriptome. Splicing-sensitive detection methods have been developed using known alternative splice junctions (Blencowe, 2006) or combined with deep sequencing and bioinformatics tools (Katz et al., 2010). RNA-Seq analysis has also been utilized to identify SR-regulated transcriptome and splicing outcome changes on a large scale. These analyses have been employed for SRSF1 (Anczukow et al., 2015), SRSF1/SRSF2 (Pandit et al., 2013) and SRSF3 (Ajiro et al., 2016). CLIP-analyzed SR-RNA interaction maps were compared with global SR-regulated splicing outcome. As expected, the splicing outcome of target RNA was shown to be dependent on multiple SR bindings onto target RNAs. In fact, cooperation and competition between the SRSF1 and SRSF2 proteins regulates alternative splicing events, which are related to synergistic and compensatory interactions to target RNA (Pandit et al., 2013). More extensive studies are required to understand the global splicing outcome from the collective contribution of many SR proteins.

REGULATORS OF NUCLEAR EVENTS

Regulating transcription-coupled splicing

Recent studies indicate that mRNA processing events are

functionally coupled to transcription (Bentley, 2014). Because splicing is a pivotal regulatory step in gene expression (Kornblihtt et al., 2013), splicing regulatory proteins could couple the transcription step to post-transcriptional steps. SR proteins were initially discovered as proteins associated with transcriptionally active chromatin in amphibian germinal vesicle and *Drosophila* embryo (Champlin et al., 1991; Roth and Gall, 1987). Since then, SR proteins have been proposed to be involved in chromatin-associated events, especially in co-transcriptional splicing (Fig. 3A). It should be also noted that post-transcriptional splicing takes place in the nucleus, as will be discussed below (Han et al., 2011b).

SR proteins directly or indirectly interact with the C-terminal domain of RNA polymerase II (Pol II-CTD) (Das et al., 2006; 2007; de la Mata and Kornblihtt, 2006; Sapra et al., 2009). In general, Pol II-CTD is the assembly site of various RNA processing factors on the transcription complex (Hsin and Manley, 2012; Munoz et al., 2010). However, SR proteins have been shown to associate with nascent RNA transcripts, rather than to be stably preassembled with Pol II-CTD (Sapra et al., 2009). Thus, co-transcriptional recruitment of SR proteins requires ongoing pre-mRNA synthesis, thereby facilitating the spliceosome assembly into pre-mRNA (Listerman et al., 2006; Sapra et al., 2009). Additionally, certain SR proteins (SRSF1 and SRSF3) bind to H3 tail and dynamically associate with chromatin (Loomis et al., 2009). Since some histone modifications regulate alternative splicing (Luco et al., 2010), the splicing regulatory function of SR proteins can also be regulated by histone modification and nucleosome occupancy (Luco et al., 2011).

It should be pointed out that nuclear SR proteins are also located in small nuclear bodies, called speckles (Fig. 3A). Speckles are enriched with many proteins required for the assembly and storage of splicing machinery, of which SR proteins are prominent components (Shepard and Hertel, 2009). Since co-transcriptional and post-transcriptional RNPs are found in speckles, how splicing is dynamically regulated in speckles remains to be understood (Han et al., 2011b). Also, relevant coupling factors for transcription and splicing need to be identified to elucidate the SR-mediated splicing mechanism in the nucleus.

Regulating transcription elongation and more

Once transcription is initiated at the transcription start site (TSS), Pol II pauses at the site just downstream of TSS and requires elongation factors to allow it to proceed. Switching of the RNA Pol II complex from the initiation to the elongation complexes is important for functional transcription, which is mediated by P-TEFb kinase phosphorylating Ser2 position in CTD (Fig. 3A) (Jonkers and Lis, 2015). As assumed, most of the mRNA processing complexes are assembled during the elongation step of transcription (Perales

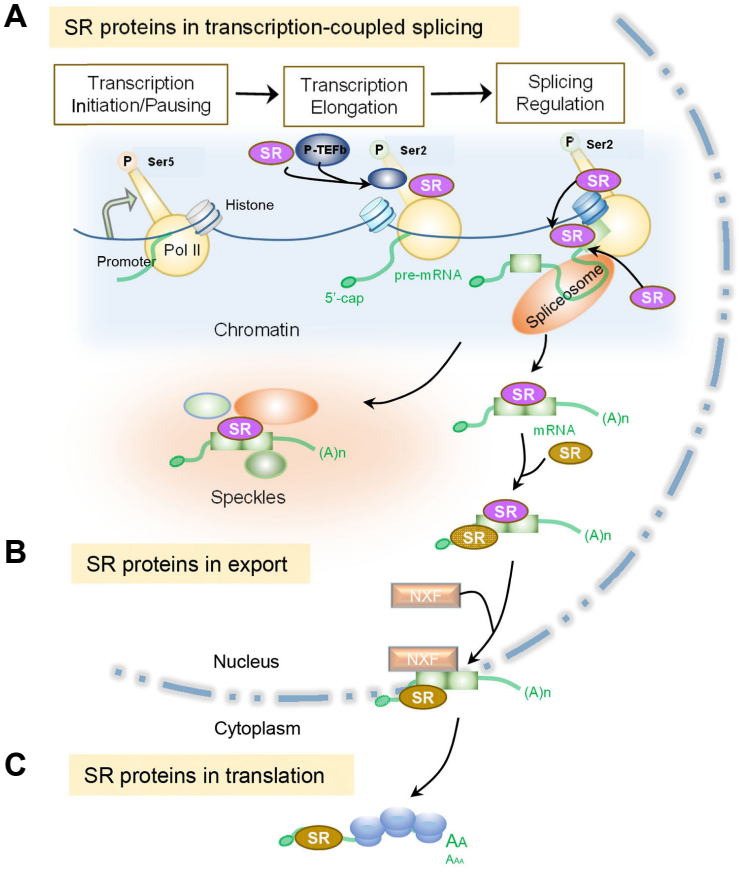

Fig. 3. Multiple roles of SR proteins during gene expression from the nucleus to the cytoplasm. (A) Transcription-coupled splicing in chromatin. Transcription elongation and splicing are regulated by Pol II phosphorylation, histone modification, and SR-protein interactions. More SR proteins (shown in different colors) are recruited, and spliceosome assembly occurs on nascent pre-mRNP. Storage and assembly of splicing machinery in speckles is also shown. (B) mRNA export from the nucleus to cytoplasm. Export receptor (NXF) is recruited to export adaptor SR-bound mRNA. (C) Translational regulation in the cytoplasm.

and Bentley, 2009) So chromatin-associated and pol II-interacting mRNA processing proteins are likely to function in regulating transcription elongation (Allemand et al., 2008).

A direct role for SR proteins in transcriptional regulation has been shown for SRSF2. In contrast to shuttling SR proteins (such as SRSF1, SRSF3, and SRSF7), SRSF2 is a non-shuttling protein located in the nucleus. Interestingly, SRSF2 associates with DNA only, but not with cytoplasmic mRNA, suggesting a role restricted to the nucleus (Sapra et al., 2009). Recently, SRSF2 has been shown to mediate the release of paused Pol II by switching p-TEFb from inhibitory 7SK RNP, which in turn activates transcriptional elongation in collaboration with promoter-associated nascent RNA with ESE (Ji et al., 2013). Such a transcription regulatory point would link the recruitment of the splicing machinery to the transcription complex, ensuring the proper assembly of transcriptional and co-transcriptional machineries (Jonkers and Lis, 2015).

SR proteins also play a role in many nuclear RNA processes, since nuclear mRNPs are dynamically assembled and function in transcription, splicing, export and nuclear surveillance (Muller-McNicoll and Neugebauer, 2013). In fact, some SR proteins have been reported to be involved in 3' end processing (Lou et al., 1998), mRNA packaging (Singh et al., 2012) and mRNA export (Huang and Steitz, 2005) as will be discussed later.

Regulation by RNA modifications

RNA modification is emerging as an important regulatory marks for RNA stability and translation in the cytoplasm (Zhao et al., 2017). In addition, RNA modification on N^6-methyadenosine (m^6A) was recently reported to be involved in alternative splicing in the nucleus. Reader protein for m^6A (nuclear reader YTHDC1) recruits or blocks SR proteins, SRSF3 or SRSF10, respectively. Therefore, RNA modification modulates SR protein access to the binding regions of target mRNA (Xiao et al., 2016). Considering the emerging importance of RNA modification during various steps of RNA metabolism, it will be interesting to decipher the mechanistic details of how m^6A can regulate SR protein functions.

Regulation by phosphorylation of SR proteins

As was initially identified with a monoclonal antibody detecting phosphorylated SR proteins (mAb104) (Roth et al., 1990), most of the nuclear SR proteins are phosphorylated. Since SR proteins contain a RS domain with repeated Arg/Ser sequences, they are subjected to phosphorylation by SR specific kinases. The phosphorylation status of SR proteins is related to their functions in spliceosome assembly and their localization inside the nucleus as well as in the cytoplasm (Zhou and Fu, 2013).

Two families of kinases are major regulators of SR phosphorylation: SR protein-specific kinases (SRPKs) (Gui et al., 1994) and Cdc-2 like kinases (CLKs) (Colwill et al., 1996b). The subcellular localizations and substrate specificities of these two kinase families are distinct. SRPKs are detected both in the cytoplasm and in the nucleus, whereas CLKs are constitutively located in the nucleus and co-localize with SR proteins in nuclear speckles (Colwill et al., 1996a; 1996b). SRPKs are retained in the cytoplasm by molecular chaperons; upon activation by EGF growth factor, they can be translocated to the nucleus and cause changes in the alternative splicing of many genes (Zhong et al., 2009; Zhou et al., 2012). In contrast, nuclear CLKs are activated by osmotic and heat-shock stresses, so SR proteins are re-phosphorylated by CLKs during the recovery phase of stress (Ninomiya et al., 2011). The two SR kinase systems appear to act symbiotically for proper phosphorylation of SR proteins and splicing regulation (Aubol et al., 2016). Thus, the roles of the dual SR kinases are interrelated and contribute in a coordinated fashion toward protein phosphorylation and localization in response to different stimuli (Corkery et al., 2015; Ghosh and Adams, 2011). Additionally, de-phosphorylation and re-phosphorylation of SR proteins seem to be important for cytoplasmic functions (Huang et al., 2004; Sanford et al., 2005).

CONNECTORS TO CYTOPLASMIC EVENTS

Regulating mRNA export

Since gene expression in cells is interconnected from the nucleus and to the cytoplasm (Moore and Proudfoot, 2009), nuclear SR proteins could be a connector for cytoplasmic events. In fact, most SR proteins dynamically localize to speckles and chromatin in the nucleus; but some SR proteins (shown as shuttling SR proteins in Fig. 1) can export out to the cytoplasm and shuttle continuously between the nucleus and the cytoplasm (Caceres et al., 1998). In general, export adaptors (such as TREX complex) link transcription to export of mRNA which is mediated by the export receptor, Nuclear export factor 1 (NXF1/TAP) (Wickramasinghe and Laskey, 2015). In addition, two shuttling SR proteins (SRSF3 and SRSF7) are export adaptors involved in specific mRNA export (Fig. 3B) (Huang, 2001; Huang et al., 2003). Structural analysis revealed that they interact with TAP export receptor via Arginine-rich peptide adjacent to RRM of SR proteins (Hargous et al., 2006).

Recent CLIP analyses of SR proteins (SRSF1 to SRSF7) and the NXF1 export receptor revealed co-binding of SR proteins to the export receptor. Among many SR proteins, SRSF3 emerges as the most potent adaptor for the NXF1 adaptor (Muller-McNicoll et al., 2016). As discussed above, the phosphorylation status of SR proteins is linked to the locations and functions of SR proteins. Dephosphorylated SR proteins act as export adaptors for specific mRNA (Huang et al., 2004; Sanford et al., 2005). Considering the critical role of mRNA export during gene expression regulation, the mechanisms underlying the role of SR proteins as molecular connectors from nuclear mRNA processing to cytoplasmic translation need to be elucidated.

Regulating mRNA decay and translation

Instead of acting as passengers in the mRNP journey from nucleus to cytoplasm, shuttling SR proteins actively engage in mRNA decay and translation thereby determining the ultimate fate of the bound mRNAs (Fig. 3C) (Huang and Steitz, 2005). Since spliced mRNP is assembled by EJC along

with SR proteins (Singh et al., 2012), mRNA decay process enhanced by EJC assembly on mRNA, such as non-sense mediated decay (NMD), can be regulated by SR proteins (Popp and Maquat, 2014). In fact, SRSF1 have shown to enhance NMD of the premature termination codon (PTC) containing model *β–globin* gene (Zhang and Krainer, 2004). SRSF1 have also reported to regulate the stability of *PKCI-γ* mRNA (Lemaire et al., 2002) and regulate translation (Sanford et al., 2004). Interestingly, it activates translation initiation by enhancing phosphorylation of 4E-BP1, a competitive inhibitor of cap-dependent translation (Michlewski et al., 2008), or represses translation of its own mRNA (Sun et al., 2010). Moreover, SRSF1 acts as an adaptor protein to recruit signaling molecules, such as mTORC1, during tumorigenesis (Karni et al., 2008) or forms an aberrant proteosomal complex to stabilize p53 protein during senescence (Fregoso et al., 2013). These studies highlight the important role of SRSF1 as a splicing and translation regulator, which is relevant to RNA-mediated pathology (Maslon et al., 2014; Sanford et al., 2008). Of note, SRSF1 is overexpressed in some cancers and regulates alternative splicing of many cancer-related genes (Jiang et al., 2016; Karni et al., 2007).

Other shuttling SR proteins, such as SRSF3 and SRSF7, can also function in the translation process. SRSF3 has been shown to regulate Internal Ribosomal Entry Site (IRES)-mediated translation initiation (Bedard et al., 2007), whereas SRSF7 plays a role in translation of un-spliced viral RNA containing Constitutive Transport Element (CTE) (Swartz et al., 2007). In the case of *pdcd4* mRNA, SRSF3 has been shown to regulate nuclear alternative splicing and RNA export as well as cytoplasmic translation (Kim et al., 2014; Park and Jeong, 2016). These data suggest that SR proteins could act as coordinators for post-transcriptional steps of mRNAs from the nucleus to the cytoplasm.

FUTURE PERSPECTIVE

Gene expression factory is formed by integration of transcription and RNA-processing machineries, which is mediated by mRNPs (Maniatis and Reed, 2002). Since mRNPs connect nuclear transcription and pre-mRNA processing to downstream cytoplasmic events (Moore and Proudfoot, 2009), SR proteins could be important parts of the gene expression machinery by forming various forms of mRNPs. Future research should be directed to identify diverse forms of SR-mRNPs in the nucleus as well as in the cytoplasm to understand multifunctionality of SR proteins.

Splicing regulation is performed by specific RBPs via their interactions with cis-acting regulatory elements on primary transcripts. Thus, SR-regulated splicing is likely to be modulated by many other RBPs. Recent proteomic analyses have indicated a large number of proteins that may be directly or indirectly linked to mRNA isoform production (Castello et al., 2016; Ray et al., 2013). Therefore, it will be important to identify known and novel RBPs relevant to SR-regulated splicing.

Master splicing regulators play important roles in generating the transcriptome (Jangi and Sharp, 2014); thus, SR proteins are involved in qualitative (splicing) and quantitative (transcription) regulations of gene expression program in the cells. Since alternative splicing contributes to cellular physiology in various environments (Kalsotra and Cooper, 2011), it will be important to identify signaling pathways and critical signaling molecules relevant to SR protein regulation. Especially, the extracellular signals or environmental cues required for the regulation of SR protein expression should be investigated. It will be interesting to dissect the signaling pathways involved in SR protein modifications, including phosphorylation.

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