RNA metabolism and ubiquitin/ubiquitin-like modifications collide

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Abstract

Alternative splicing and post-translational modifications are key events for the generation of proteome diversity in eukaryotes. The study of the molecular mechanisms governing these processes, and every other step of gene expression, has underscored the existing interconnectedness among them. Therefore, molecules that could concertedly regulate different stages from transcription to pre-mRNA processing, translation and even protein activity have called our attention. Serine/arginine-rich proteins, initially identified as splicing regulators, are involved in diverse aspects of gene expression. Although most of the roles exerted by members of this family are related to mRNA biogenesis and metabolism, few recently uncovered ones link these proteins to other regulatory steps along gene expression, particularly the regulation of post-translational modification by conjugation of the small ubiquitin-related modifier. This along with the established link between ubiquitin, transcription and pre-mRNA processing points to a general mechanism of interaction between different cellular machineries, such as ubiquitin/ubiquitin-like conjugation pathways, transcription apparatus and the spliceosome.

Keywords: *SUMO conjugation; SR proteins; alternative splicing; post-translational modifications*

INTRODUCTION

After the sequencing of the human genome, it became apparent that the unexpectedly low number of genes could not account for the complexity of our organism. Thereafter, the general interest in the processes involved in generating transcriptome as well as proteome diversity has increased considerably. Although many processes contribute to this diversity, including alternative transcription start sites, alternative splicing, alternative polyadenylation and alternative translation among others, alternative splicing at the pre-mRNA level and post-translational modifications (PTMs) at the protein level account for most of the proteome variability generated [1, 2]. Currently, we not only know that the aforementioned processes generate an enormous proteomic expansion but also that there exists an intricate connection between them. In this review, we summarize different findings from our laboratory and others regarding the involvement of serine–arginine-rich (SR) proteins, in particular SRSF1, at different steps of gene expression and present a provocative and emerging connection between the machineries involved in mRNA metabolism and small ubiquitin-related modifier (SUMO) conjugation.

Alternative splicing

Pre-mRNA splicing is an essential step for gene expression in mammalian cells as most protein-coding genes contain intervening sequences known as

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introns. Intron removal is efficient and precise; however, most transcripts in higher eukaryotic cells also contain regions that are subjected to alternative selection, resulting in the production of different mature mRNA isoforms [3]. This process, known as alternative splicing, has been recognized as a key mechanism to increase the functional diversity of the proteome and to introduce additional layers for regulated gene expression. Different mRNA isoforms from a given gene are often produced in different cell types, tissues or developmental stages. Thus, alternative splicing has been increasingly linked to important biological pathways both in physiological as well as pathological situations [4–7].

Pre-mRNA splicing, whether constitutive or alternative, is catalyzed by the macromolecular machinery known as the spliceosome consisting of U1, U2, U4/U6 and U5 small nuclear ribonucleoprotein particles (snRNPs) and numerous protein factors [8].

Crucial auxiliary factors for constitutive and alternative splicing are two well-characterized families of RNA-binding proteins: SR proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs) [9]. These proteins are capable of either promoting or inhibiting the inclusion of alternative regions into mature mRNA through their binding to RNA sequence elements termed splicing enhancers or splicing silencers, respectively, and which are found in a combinatorial architecture along most transcripts studied so far.

SR and hnRNP proteins not only participate in splicing regulation but also play important roles in nuclear and cytoplasmic steps of mRNA metabolism. In particular, members of the SR protein family have been implicated in different cellular processes such as genome stability, chromatin binding, transcription elongation, mRNA stability, mRNA export and mRNA translation [10]. Intriguingly, the list of SR protein activities keeps growing constantly.

Post-translational modifications

Reversible PTMs are a versatile way to regulate protein activity. The most-studied PTM is phosphorylation, largely because of the current relative ease of detecting protein phosphorylation *in vivo* and *in vitro* [11]. However, many other types of reversible PTMs exist: acetylation, methylation, *O*-GlcNacylation, etc. Not only may small molecules be covalently attached to target proteins but also small peptides. Thus, 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 [12]. There are at least 12 members of the Ubl family encoded by the human genome (the most studied being SUMO and NEDD8) known to affect activity, structure, sub-cellular localization and repertoire of interactions of the target proteins, without labelling them for degradation.

SUMO conjugation is a reversible, ATP-dependent process that involves an activating enzyme (E1), a conjugating enzyme (E2) and different ligases (E3) [13]. In the Ub pathway, substrate specificity is usually provided by E3 ligases, which typically contain substrate-binding sites [14, 15]. In the SUMO pathway, the sole E2 enzyme (Ubc9 in mammals) usually binds the substrate directly, but the SUMO E3 ligases seem to contribute to substrate specificity. The best-characterized SUMO E3s are the *protein inhibitor of activated STAT1*, PIAS1 [16], Topors [17] and the polycomb protein Pc2, also known as CBX4 [18]. SUMO E3 ligases vary in their mechanism of action. Catalytic activity (SUMO transfer in sub-stoichiometric amounts) has been proven for RanBP2 [19, 20], Topors [17, 21] and PIAS [22].

Connection between mRNA metabolism and PTMs

SR protein phosphorylation

Sub-cellular localization and activity of SR and hnRNP proteins is regulated by phosphorylation, thus the search for different kinases responsible for phosphorylating them and signaling pathways connecting extracellular cues with the splicing machinery has been the goal of several laboratories, including ours [23]. Studying the regulation of fibronectin and Rac1 pre-mRNA alternative splicing in the context of cell–cell and cell–extracellular matrix (ECM) interactions as well as during epithelia–mesenchymal transitions, our group found that the activation of the PI3kinase-Akt pathway by mesenchymal soluble factors or certain growth factors stimulates inclusion of two alternative regions within fibronectin mRNA in mammary epithelial cells and other cell lines. This effect is mediated by the SR proteins SRSF1 and SRSF7, which were revealed as Akt substrates [24, 25]. Several reports have also implicated Akt in alternative splicing regulation, reinforcing and expanding these findings [26–29]. Furthermore, in

collaboration with the Cáceres laboratory, we demonstrated that this 'PI3kinase-Akt-SR protein' axis also regulates translation in a splicing enhancer-dependent manner [25], concluding that the activation of a given signal transduction cascade regulates the activity of at least two SR proteins, SRSF1 and SRSF7, simultaneously altering both nuclear and cytoplasmic steps of RNA metabolism: alternative splicing and translation. These results suggest that this concerted effort could increase both the speed and strength of the cellular response to a given extracellular stimulation [30]. Further studies from our laboratory have also implicated SRSF1 in regulating the balance between CAP- and IRES-dependent translation [31]. These functions exerted by SRSF1 are summarized in Figure 1, left panel.

Ubls and mRNA metabolism

High-throughput identification of specific ubiquitylation sites by mass spectrometry not only confirmed already known ubiquitylation sites but also mapped more than 10 000 previously unidentified ones, including putative sites in almost every member of the SR protein family [32]. Site-specific ubiquitylation in response to proteasome inhibition by MG-132 was quantified by SILAC/mass spectrometry revealing not only that 40% of the quantified sites did not show an increase in ubiquitylation but also that ubiquitylation in 15% of the sites was significantly reduced under this condition. Interestingly, SR protein ubiquitylation sites were included in this latter category. These results clearly show that a significant fraction of ubiquitylation sites seems to be unrelated to proteasomal-mediated degradation and suggests that Ub conjugation to certain substrates, such as SR proteins, may work instead as a regulatory signal [32, 33]. Also by proteomic approaches, RNA-binding proteins have been revealed as the predominant group among SUMO conjugation substrates, including several hnRNPs [34, 35], SR family members and spliceosome components [36–38].

It is worth mentioning that a close connection between splicing and Ub conjugation has already been proposed as ubiquitylation substrates together with components of the ubiquitylation/de-ubiquitylation pathway have been shown to co-exist within the spliceosome. In particular, the Prp19 complex promotes a non-proteolytic ubiquitylation of the U4 component Prp3, which is required for stabilization of tri-snRNP U4–U5/U6. Moreover, de-ubiquitylation of Prp3 by Usp4/Sart3 is required for further U4 dissociation and recycling [39].

With respect to SUMO conjugation, this particular PTM has been found to regulate at least two events along mRNA metabolism: 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 [40–42]. Whether Ub or Ubl conjugation, in particular SUMO, could affect SR protein activities awaits further investigation.

Taking the connection further

While studying different PTMs that could regulate the activity of splicing factors modulating Rac1 alternative splicing in the context of a mammary epithelial–mesenchymal transition [43], we came into a completely unexpected finding: certain SR proteins, in particular the prototypic member of this family SRSF1, function as modulators of the SUMO conjugation pathway [44].

We found that SRSF1 interacts with the SUMO E2 conjugating enzyme Ubc9 and enhances sumoylation of specific substrates, including RNA-processing factors such as Sam68 (Figure 1, left panel). Moreover, SRSF1 interacts with the SUMO E3 ligase PIAS1 (reported to be a component of the human spliceosome, [45]), regulating PIAS1-induced global protein sumoylation. This rather unexpected activity of SRSF1 is dependent on its RNA recognition motif (RRM) 2, as it is its re-localization to nuclear stress bodies (nSBs) upon heat shock [46]. nSBs are sub-nuclear domains form in human and other primate cells exposed to thermal stress and known to recruit not only heat shock transcription factors but also a subset of pre-mRNA processing factors (Sam68, hnRNP A1-related protein and SRSF1 among others) [47]. Even though SRSF1 involvement in the heat shock response has been studied [46–48], the precise role it plays under this condition is not completely clear. We have shown that SRSF1 is required for the increase in the levels of protein sumoylation observed upon heat shock, and furthermore that nSBs co-localize with SUMO-conjugated proteins. Based on these results, we speculate that SRSF1 could be part of a regulatory network accounting not only for splicing regulation but also for the regulation of sumoylation-dependent protein activity required for cell recovery upon hyperthermic stress.

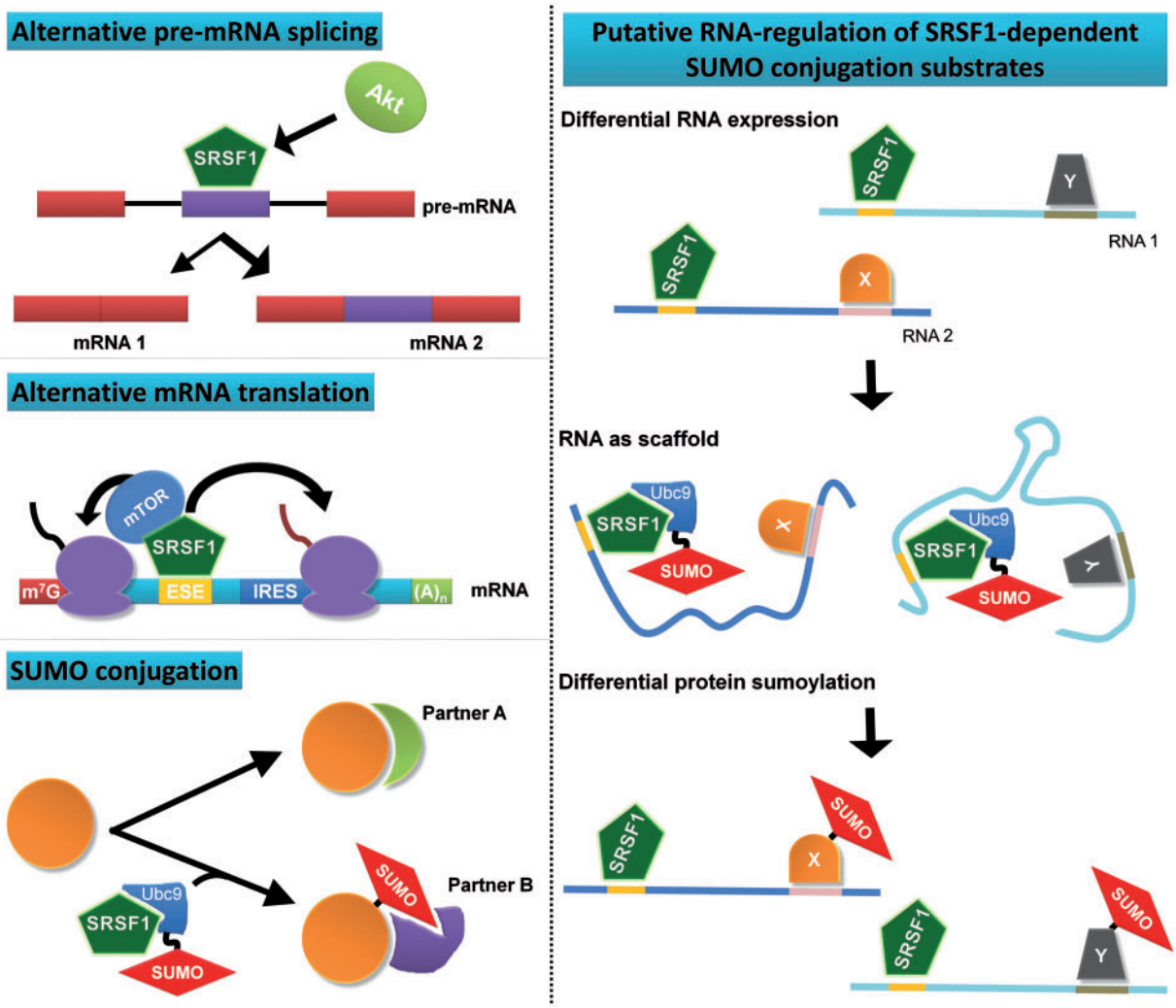

Figure 1: Left panel, the schemes summarize different activities described for the SR protein SRSFI at the level of alternative splicing (top), translation (middle) and SUMO conjugation (bottom), reported by our laboratory and others [24, 25, 31, 44, 50, 51]. Right panel, the scheme depicts our current hypothesis involving the regulation of SRSFI-dependent SUMO conjugation to specific substrates by different RNA molecules.

The SUMO conjugation-stimulatory activity displayed by SRSF1 is also performed by SRSF9 but is neither carried out by SRSF5 nor by SRSF3. It is worth mentioning that SRSF1 and SRSF9 RRM2 domains share 72% sequence identity, this drops to 37% between SRSF1 and SRSF5 RRM2 domains, while SRSF3 does not contain an RRM2. Thus, deciphering the RRM2-mediated protein–protein interaction networks both within the spliceosome and between the components of the spliceosome and other cellular machineries could provide some interesting insights into this complex regulation. Certainly, the mechanism by which SRSF1 exerts its effect on SUMO conjugation is yet to be determined. A provocative possibility, currently under investigation, is that different RNA molecules known to interact with SRSF1 may serve as scaffolds that mediate SRSF1-regulated SUMO conjugation to specific target proteins (Figure 1, right panel).

Perspectives

A large body of work has demonstrated that the delineation of RNA transcription, splicing or export 'machineries' as independent entities is outdated. The more we learn about the mechanisms and the molecules engaged in different steps of gene expression, the more we realize about their interconnection and thus the requirement for an integrative vision of gene expression regulation.

Multi-subunit protein complexes and RNA–protein complexes are responsible for a great variety of cellular functions. Thus, unraveling the interactions

among the different components and the dynamic of their assembly in response to different stimuli would certainly improve our understanding of living cells, tissues and organisms, both under normal and pathological circumstances.

We are currently aware of the co-regulation that exists among different processes during mRNA biogenesis and metabolism. We also know that PTMs represent a fast and reversible way of modulating these processes in response to a wide variety of extra- and intra-cellular cues. Under this perspective, it becomes appealing to look for molecules able to regulate different stages, from transcription to protein function, in a concerted manner. Several members of the SR family of proteins have been defined as multitasking proteins due to their involvement in different events along gene expression regulation. Their ability to engage in a broad range of molecular interactions with proteins, RNA and chromatin [49], place them as attractive candidates for this duty of linking molecules. We envision a scenario in which SRSF1-mediated sumoylation might be regulated by the different RNA molecules it encounters during transcription/splicing/export. In this model, different RNA molecules would drive SRSF1 target selectivity (Figure 1, right panel). Noteworthy, this could also apply to all other functions performed by SRSF1 as well as to other SR proteins.

Key points

- Alternative splicing and PTMs are a great source of transcriptome and proteome variability.
- Different stages of gene expression are regulated in a concerted manner.
- The search for nexus molecules that can account for the co-regulation of those stages is an attractive and growing field.
- Members of the SR family of proteins, in particular SRSFI, have been lately defined as multitasking proteins participating at a wide variety of gene expression regulatory steps.

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