CHAPTER 4

POST-TRANSLATIONAL REGULATION OF STAR PROTEINS AND EFFECTS ON THEIR BIOLOGICAL FUNCTIONS

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Abstract STAR (Signal Transduction and Activation of RNA) proteins owed their name to the presence in their structure of a RNA-binding domain and several hallmarks of their involvement in signal transduction pathways. In many members of the family, the STAR RNA-binding domain (also named GSG, an acronym for GRP33/Sam68/GLD-1) is flanked by regulatory regions containing proline-rich sequences, which serve as docking sites for proteins containing SH3 and WW domains and also a tyrosine-rich region at the C-terminus, which can mediate protein-protein interactions with partners through SH2 domains. These regulatory regions contain consensus sequences for additional modifications, including serine/threonine phosphorylation, methylation, acetylation and sumoylation. Since their initial description, evidence has been gathered in different cell types and model organisms that STAR proteins can indeed integrate signals from external and internal cues with changes in transcription and processing of target RNAs. The most striking example of the high versatility of STAR proteins is provided by Sam68 (KHDRBS1), whose function, subcellular localization and affinity for RNA are strongly modulated by several signaling pathways through specific modifications. Moreover, the recent development of genetic knockout models has unveiled the physiological function of some STAR proteins, pointing to a crucial role of their post-translational modifications in the biological processes regulated by these RNA-binding proteins. This chapter offers an overview of the most updated literature on the regulation of STAR proteins by post-translational modifications and illustrates examples of how signal transduction pathways can modulate their activity and affect biological processes.

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INTRODUCTION

The STAR family comprises a class of RNA-binding proteins (RBPs) that are evolutionarily conserved from yeast to humans.[1] The two common features of STAR proteins are the presence of a STAR RNA-binding domain (see below) and of several motifs that confer the ability to form protein-protein interactions and to be modified post-translationally. Several STAR proteins, including Sam68, GLD-1, QKI and GRP33, are capable of homodimerizing in the cell[2] and this feature is required for RNA binding and for many of their functions.[2,3] The ability to homodimerize relies on sequences in the STAR domain,[2] indicating that this region mediates protein-protein interactions in addition to RNA binding. On the other hand, motifs disseminated along the whole structure of different STAR proteins allow heteromeric complexes with numerous proteins involved in signal transduction events and RNA processing (Fig. 1A).[3-5] The only exception to this latter feature of STAR proteins is GLD-1, which lacks obvious hallmarks of motifs involved in signaling.[1] Remarkably, the protein-protein interactions engaged in by some STAR family members have been shown to play a role in propagation of signaling events,[3,4] but they also modulate RNA metabolism, as indicated by the reduced affinity of Sam68 for RNA when bound to SH3 domains.[6] An additional layer of plasticity is provided by the many post-translational modifications of STAR proteins in response to activation of various signal transduction pathways. For instance, tyrosine phosphorylation in the C-terminal tail of many STAR proteins reduces their affinity for RNA and impairs homodimerization (Fig. 1A).[1-3] In line with the impairment of RNA binding,[7,8] tyrosine phosphorylation suppresses the effect of the STAR proteins Sam68 and SLM-1 on alternative splicing.[5,9] Similarly, serine/threonine phosphorylation of Sam68 improves its affinity for specific RNAs, thereby modulating alternative splicing of a target pre-mRNAs (Fig. 1A,B).[10] Moreover, recruitment of Sam68 onto the polysomes and its function in translation appears to depend on phosphorylation (Fig. 1A,B).[11,12] Additional post-translational modifications, such as methylation, acetylation and sumoylation, have been shown to affect specific functions of at least one STAR protein, even though a link to a specific biological process has been less firmly established (Fig. 1A).

Thus, several observations indicate that STAR proteins are crucial integrators of signaling events with regulation of RNA metabolism. Without doubts, the most characterized member of the family in this sense is the mammalian Sam68 protein. For this reason, this chapter will begin with reviewing the information available on post-translational modifications affecting Sam68 functions. Next, examples of other STAR proteins regulated by signal transduction pathways will also be discussed.

Sam68: A BRIEF OVERVIEW

Sam68 is a prototypic STAR protein, with a STAR domain of ~200 amino acids, including the maxi-KH domain embedded in the conserved N-terminal QUA1 and C-terminal QUA2 regions that confer homodimerization and RNA-binding specificity properties.[3] Up-stream and downstream of the STAR domain, Sam68 contains three proline-rich sequences (P0-P5) on each side. In addition, this RBP has RG motifs and a C-terminal tail enriched in tyrosine residues that flank a bipartite nuclear localization signal (Fig. 2A).[3] These features allow Sam68 to interact with multiple proteins,[13] leading to the hypothesis that it might function as a scaffold to cluster signaling proteins in

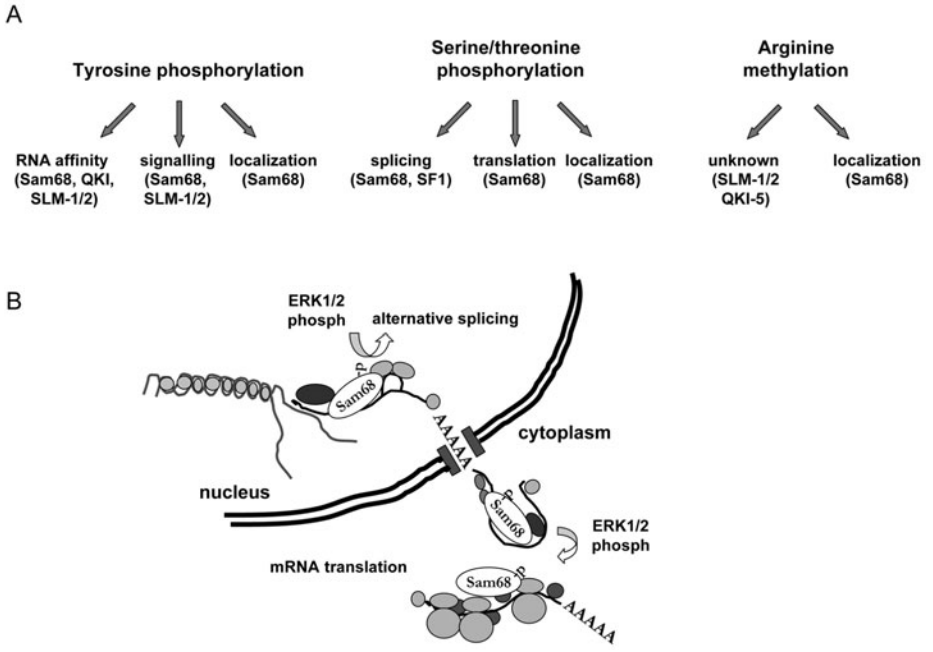

Figure 1. Post-translational modifications of STAR proteins affect their biological functions. A) Schematic representation of the best known post-translational modifications that affect the subcellular localization or activity of specific STAR proteins. B) Schematic representation of the effects of ERK1/2 phosphorylation on the activities of Sam68 in the nucleus and in the cytoplasm. In the nucleus, it was reported that ERK1/2 phosphorylation stimulated the splicing activity of Sam68;[10] in the cytoplasm, it was demonstrated that this phosphorylation event enhanced translation of specific mRNAs in germ cells.[12]

response to specific stimuli.[3,4] More recently, Sam68 has been demonstrated to regulate RNA metabolism at different steps. First, it was shown that it enhanced export and cytoplasmic utilization of viral RNAs,[14,15] complementing the function of the HIV Rev protein. This activity is likely important, because the virus replicates poorly in cells depleted of Sam68 or expressing a dominant-negative Sam68 protein.[16-18] On the other hand, this STAR protein is also implicated in normal nuclear events, such as transcriptional and post-transcriptional regulation of selected cellular transcripts. Through its association with transcription factors or regulators, Sam68 modulates the transcription of target genes.[19-21] This activity could be linked to the effect of Sam68 on alternative splicing,[21,22] since the two process are tightly linked.[23] Indeed, Sam68 was shown to regulate the choice of alternatively spliced exons in *CD44*, *Bcl-x* and a subset of transcripts required for neurogenesis.[5,10,24] Finally, Sam68 was detected in the cytoplasm of primary neurons[25,26] and germ cells,[11,12,27] where it associated with the translation initiation complex eIF4F and the polyribosomes, thereby enhancing translation of a subset of mRNAs (Fig. 1B). This function of Sam68 is likely essential, at least in germ cells, because its genetic ablation leads to defects in germ cell differentiation and to male infertility,[12] see Chapter 5. Thus, the many tasks carried out by Sam68 in different cell types suggest that the activity and subcellular localization of this multifunctional RBP needs to be finely tuned according to the specific requirements of the cell. In line with this hypothesis, Sam68 has been shown

to undergo many post-translational modifications under various conditions and in most cases these modifications have an impact on Sam68 function and/or localization. Hence, it is appropriate to begin with a review of the literature illustrating the high versatility of the most studied of the STAR proteins.

REGULATION OF Sam68 FUNCTIONS BY TYROSINE PHOSPHORYLATION

Sam68 was originally identified by virtue of its tyrosine phosphorylation in cells transformed with the Src oncogene[28] and erroneously named p62GAP associated protein.[29] A few years later, two groups independently identified this RBP as a phosphoprotein that associated with Src in mitotic cells (Src Associated in Mitosis protein, of 68 kDa) and renamed it Sam68.[30,31] Sam68 was highly phosphorylated in tyrosine residues in mitotic cells transformed with an oncogenic form of Src and its association with the SH3 and SH2 domains of the kinase was required for phosphorylation. Moreover, these studies showed that Sam68 could bind polyribonucleotides in vitro and suggested that Src might regulate the processing of cellular RNAs through its interaction with this RBP.[30] It was subsequently demonstrated that Sam68 could also associate with the Src-related kinase Fyn in a similar manner.[13] Tyrosine phosphorylation of Sam68 likely promotes the formation of multimolecular complexes that enhance propagation of intracellular signals, as indicated by the active role it plays in antigen-stimulated T-cells.[32-34] Moreover, tyrosine phosphorylation of Sam68 is part of the signaling events triggered by engagement of the insulin and prolactin receptors.[35,36] This suggests that this post-translational modification insures correct hormonal response of target cells. Beside Src family kinases (SFKs), a number of signaling proteins, like PLCγ1, PI3K and the adaptor molecules NCK and GRB2, bind to tyrosine-phosphorylated Sam68.[3,4] In the cell, Sam68 forms two multimolecular complexes of different size and tyrosine phosphorylation stimulates its association with the smaller complex, which also depends on RNA binding and might correlate with the splicing activity of this STAR protein (Huot ME, Vogel G and Richard S; personal communication).

Regulation of signal transduction pathways by Sam68 might play a role in tumorigenesis. Indeed, it was observed that decreased expression of Sam68 delayed the onset of mammary tumors in vivo and that this effect was correlated with increased Src activity in tissue.[37] Remarkably, phosphorylation of Sam68 was increased in specimens from patients affected by breast[38] and prostate cancer.[39] In this latter tumor type, phosphorylation of Sam68 correlated with expression of a truncated form of the c-kit receptor and activation of Src.[39] Notably, Sam68 was previously shown to promote a complex between these kinases.[40] Furthermore, tyrosine phosphorylation of Sam68 was strongly induced by RET/PTC2, an oncogene implicated in thyroid cancers,[41] which acts upstream of SFKs. Finally, Sam68 could play an additional role as modulator of SFK activity in cancer cells. For instance, it was proposed that Sam68 regulates the dynamic assembly of the actin cytoskeleton at the plasma membrane through modulation of the Src signaling pathway and that depletion of Sam68 causes aberrant activation of the Rho small GTPase.[42]

In addition to SFKs, other tyrosine kinases have been shown to interact with and phosphorylate Sam68. The Tec family kinases ITK[43] and BTK[44], which are specifically expressed in T-cells and B-cells, respectively, ZAP-70[45] and BRK[46] all associate with and phosphorylate Sam68 in tyrosine residues. BRK is particularly interesting for its

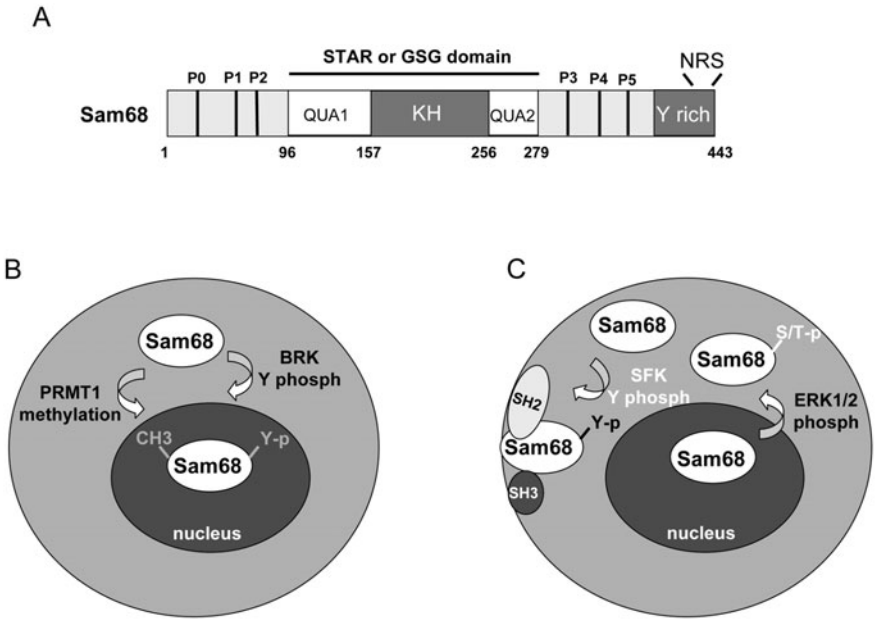

Figure 2. Regulation of Sam68 subcellular localization by post-translational modifications. A) Scheme of the structure of Sam68. P0-P5 identify the position of the proline-rich sequences that are known to interact with SH3 and WW domains; the position of the KH domain for RNA binding and the QUA1 and QUA2 regions of homology with other GSG domains are indicated; Y-rich indicates the region enriched in tyrosine residues that are sites of phosphorylation by BRK, Tec kinases and SFKs; NRS indicates the position of the nuclear retention signal. B) Schematic representation of the stimulation of Sam68 nuclear localization exerted by PRMT1-driven arginine methylation or by BRK-driven tyrosine phosphorylation. C) Schematic representation of the stimulation of Sam68 nuclear export exerted by ERK1/2-driven phosphorylation and of the interaction with signaling proteins containing SH2 or SH3 domains near the plasma membrane exerted by SFK-driven tyrosine phosphorylation.

subcellular localization. Indeed, this tyrosine kinase is mainly localized in the nucleus and accumulates in the same nuclear bodies as Sam68. This suggests that it could be responsible for regulation of the bulk of Sam68, which also resides in the nucleus of interphase cells. Tyrosine phosphorylation by either SFKs or BRK leads to decreased affinity of Sam68 for RNA.[7,46] However, although overexpression studies have shown that the cytoplasmic Fyn can phosphorylate Sam68 and modulate its nuclear activities, such as alternative splicing of target pre-mRNAs[5] or association with the splicing factor YT521-B,[47] it is likely that the endogenous proteins remain separated in the cell by the nuclear envelope. On the other hand, endogenous BRK colocalizes with Sam68 and might represent a better regulator of its nuclear activities. In line with this hypothesis, BRK can repress the ability of Sam68 to export viral RNAs and to promote their cytoplasmic utilization.[15] Moreover, it has been shown that BRK-mediated tyrosine phosphorylation of Sam68 promotes its nuclear translocation in breast cancer cells under stimulation with epidermal growth factor (EGF) (Fig. 2B).[38] Interestingly, BRK is aberrantly regulated in prostate cancer,[48] a tumor type in which Sam68 is up-regulated[49] and hyperphosphorylated[39] and its expression supports growth and survival of the neoplastic cells.[49] These results suggest that aberrant regulation of the BRK/Sam68 pathway might play a role in oncogenesis.

Thus, the evidence above strongly indicates that tyrosine phosphorylation of Sam68 by SFKs or Tec kinases in the cytoplasm (Fig. 2C) and by BRK in the nucleus (Fig. 2B) affects the ability of this RBP to function as a signaling protein and as an RNA modulator.

REGULATION OF Sam68 FUNCTIONS BY SERINE/THREONINE PHOSPHORYLATION

Although tyrosine phosphorylation of Sam68 has been intensively studied in various experimental settings, in several instances it was observed as a consequence of overexpression of SFKs or other tyrosine kinases, or even expression of their constitutively active forms. An attempt to investigate the changes in phosphorylation of the endogenous Sam68 during the cell cycle was originally done by David Shalloway and collaborators.[50] In this study, it was shown that Sam68 is phosphorylated on serine during interphase and in mitotic cells and on threonine only in mitotic cells. The kinase responsible for threonine phosphorylation in mitosis was identified as Cdc2, whereas the kinase(s) responsible for serine phosphorylation in interphase and mitotic cells was not identified. Under these conditions, no tyrosine phosphorylation of Sam68 was detected, possibly due to technical problems.[50] It is conceivable that serine phosphorylation in interphase is exerted by the extracellular regulated kinases 1 and 2 (ERK1/2), members of the mitogen activated protein kinase (MAPK) family. Indeed, it was shown that Sam68 is phosphorylated by ERK1/2 during stimulation of T-lymphoma cells with phorbol ester.[10] Notably, phosphorylation of Sam68 by ERK1/2 affected the splicing activity of this STAR protein (Fig. 1A,B), enhancing the inclusion of the variable exon 5 (v5) in the CD44 mRNA.[10] Since inclusion of this variable exon positively correlates with neoplastic transformation,[51] modulation of the splicing activity of Sam68 by the MAPK-dependent phosphorylation might represent another cancer-related event involving this multifunctional STAR protein. Mechanistically, it was proposed that Sam68 formed a complex with the splicing factor U2AF65, which recognizes the 3′ splice site, thereby enhancing its recruitment on the v5 exon.[52] Phosphorylation of Sam68 by ERK1/2 would favour the dynamic recruitment of other spliceosomal components through changes in affinity of the Sam68/U2AF65 complex for the v5 RNA.[52]

Serine/threonine phosphorylation of Sam68 also occurs in male germ cells undergoing the meiotic divisions.[11] Similarly to somatic cells, phosphorylation was due to the activity of Cdc2 and ERK1/2 and correlated with the localization of Sam68 in the cytoplasm and its association with the polyribosomes (Figs. 1B and 2C). Using specific inhibitors, it was shown that the ERK1/2-mediated phosphorylation was the main regulator of the association of Sam68 with the translational machinery.[11] A subsequent study indicated that translocation of Sam68 to the cytoplasm and its association with polyribosomes was required for translational activation of a subset of mRNAs that are target of this STAR protein in germ cells (Fig. 1B).[12] This effect could be recapitulated in somatic cells by transfecting a constitutively active form of RAS, which led to activation of ERK1/2, enhanced phosphorylation of Sam68 and its association with polyribosomes as in germ cells.[12] Remarkably, since germ cells ablated of Sam68 express lower levels of the proteins encoded by these target mRNAs and Sam68 knockout male mice were sterile and produced few spermatozoa,[12] it is likely that this post-translational regulation of Sam68 is essential for male fertility in vivo.

These observations suggest that serine/threonine phosphorylation of Sam68 mainly affects its RNA-binding activity and the functions related to it.

REGULATION OF Sam68 FUNCTIONS BY METHYLATION

The proline-rich sequences in Sam68 are flanked by RG (arginine-glycine) repeats that are consensus for methylation by the Type I of protein arginine methyltransferases (Type I PRMTs).[53] It was shown that methylation of these RG repeats occurs in vitro by incubation of Sam68 with PRMT1. Proline-rich sequences bind SH3 and WW domains. Interestingly it was demonstrated that methylation of Sam68 in the RG repeats that flanked the proline-rich sequences decreased binding to SH3 domains but not WW domains,[53] suggesting that methylation could affect the choice of partners by Sam68 in the cell. In support of a role for methylation in vivo, Sam68 associated with PRMT1 and was constitutively methylated in live cells.[54] Moreover, methylation was required for efficient nuclear localization of the protein (Figs. 1A and 2B) and for its ability to favour the export of viral RNAs,[54] suggesting that it is an essential modification required for Sam68 function. Decreased methylation of Sam68 might occur under pathological conditions. It was shown that peroxynitrite, a pro-atherogenic substance known to induce endothelial dysfunction, inhibited arginine methylation of this STAR protein. The authors reported that reduced methylation of Sam68 did not affect its RNA binding activity, or its levels of tyrosine phosphorylation. However, they showed that it correlated with increased rate of apoptosis and premature senescence of endothelial cells.[55] By contrast, reduction of RNA-binding by methylation of the Sam68 RG repeats was shown by another group.[56] They suggested that the RG repeats provide an additional RNA-binding motif to STAR proteins, outside of the GSG domain and that methylation can modify the affinity of the RG repeats for RNA.[56] However, since methylation of Sam68 appears to be a constitutive event,[54] it seems unlikely that it impedes RNA binding. Although both studies employed poly-uridine synthetic oligonucleotides to test Sam68 affinity for RNA, they reached opposite conclusions. Thus, more physiological RNA substrates need to be tested to fully understand the influence of methylation on Sam68 RNA-binding affinity.

Remarkably, the ability of Sam68 to interact with PRMT1 is exploited by an oncogene to elicit neoplastic transformation. The MLL-EEN translocation causes Mixed Lineage Leukemia (MLL) in humans. A recent study showed that the SH3 domain of EEN is the only part of this protein required for MLL oncogenesis.[57] A screen for proteins interacting with this SH3 domain identified Sam68 and subsequent experiments demonstrated that recruitment of Sam68 to MLL-EEN by the SH3 domain was crucial to elicit neoplastic transformation.[57] Interestingly, the activity of Sam68 important for cell transformation was its ability to associate with PRMT1. Indeed, direct fusion of PRMT1 with MLL bypassed the requirement of both EEN and Sam68. Thus, MLL-EEN induced transformation through the recruitment of a complex formed by Sam68 and PRMT1 with the SH3 domain of EEN. This complex allows PRMT1 to be recruited to MLL-sensitive promoters and to alter the epigenetic status of the responsive genes, hence modifying gene expression.[57] This study links the scaffold function of Sam68 and its connection with methyltransferases with oncogenesis and highlights how a versatile STAR protein can be used by the cell for unexpected functions, such as those set in motion by a mutated oncogene.

REGULATION OF Sam68 FUNCTIONS BY ACETYLATION AND SUMOYLATION

Another post-translational modification that can affect Sam68 function in the cell is acetylation. It was initially observed that Sam68 associated with the histone acetyltransferase CBP on specific promoters.[19] Few years later, it was observed that Sam68 is preferentially acetylated in breast cancer cell lines as compared to normal breast epithelial cells.[58] Moreover, acetylation of Sam68 positively correlated with its ability to bind poly-uridine synthetic RNA in extracts obtained from these cell lines. The authors also showed that CBP could acetylate Sam68 in vitro, mainly on lysines present in the QUA1 region and the first half of the KH domain, hence increasing RNA binding and that similar results are obtained by overexpressing CBP in transfected cells.[58] However, the role played by acetylation in the biological functions of Sam68 still needs to be addressed.

Sam68 was shown to form complexes with transcriptional regulators and modulate transcription of reporter genes.[19,22] One of the transcriptional targets of Sam68 in normal and cancer cells is cyclin D1.[20,49] This activity of Sam68 might also be subject to regulation by a post-translational modification. It was reported that Sam68 is modified by covalent link with the small ubiquitin-like protein SUMO.[59] This reaction was catalyzed by the SUMO E3 ligase known as PIAS1 and occurred on lysine 96 of Sam68. Since mutation of this acceptor site enhanced the pro-apoptotic activity of Sam68, which is dependent on its RNA-binding activity,[5,20] it is possible that sumoylation affects the binding of Sam68 to its cellular mRNA targets. On the other hand, sumoylation increased the repression of the cyclin D1 promoter by Sam68. A SUMO1-Sam68 fusion protein recapitulated these events, causing stronger repression of cyclin D1 expression and lower levels of apoptosis.[59] Thus, it is possible that sumoylation of endogenous Sam68 finely tunes the ability of this protein to regulate cell cycle progression and apoptosis in response to specific signals. However, the mechanism by which sumoylation affects Sam68 activity is still unknown and more work is needed to ascertain the biological importance of this modification in live cells.

POST-TRANSLATIONAL MODIFICATIONS OF SLM-1 AND SLM-2

The STAR proteins that are more related to Sam68 are SLM-1 and SLM-2 (Sam68 Like Mammalian protein 1 and 2).[60] SLM-2 was independently cloned by another group and named T-STAR and étoile in human and mouse, respectively.[61] These proteins share approximately 65-70% sequence identity with Sam68 in their STAR domain and have similar SH2 and SH3 domain binding sites.[60] Moreover, many of the post-translational modifications described for Sam68 apply also to SLM-1 and SLM-2 (Fig. 1A). However, some differences exist. For instance, SLM-2 was not phosphorylated by SFKs and did not interact with the SH3 domains of several signaling proteins tested,[60] suggesting that it lacked the scaffold function of Sam68 and SLM-1. On the other hand, both SLM-1 and SLM-2 were phosphorylated by the nuclear BRK and this modification decreased their affinity for synthetic RNA in vitro.[8] Another feature in common between Sam68 ans SLM-2 was their methylation by PRMT1,[54] which was suggested to decrease their affinity for RNA in the same in vitro study in which Sam68 was analysed.[56] In terms of biological roles, they also share many features with Sam68. SLM proteins synergize with the HIV protein Rev in stimulating gene expression of responsive genes, but, differently

from Sam68, they could not substitute for the Rev function.[62] Both SLM-1 and SLM-2 function in alternative splicing like Sam68,[9,63] but only SLM-1 activity is inhibited by SFK-mediated tyrosine phosphorylation.[9] Thus, SLM-1 and 2 appear to be very close homologues of Sam68 that share many of its features and activities. Since Sam68 knockout mice are viable,[64] it is possible that they compensate for lack of their cousin STAR protein and support viability of these mice.

POST-TRANSLATIONAL MODIFICATIONS OF THE QKI PROTEINS

The mouse quaking (*Qk*) gene is essential for central nervous system (CNS) myelination and for survival of the early embryo.[65] The proteins encoded by this gene (QKI5, QKI6 and QKI7) show the typical structure of STAR proteins, combining RNA-binding with signal transduction properties and are expressed in myelin-forming cells and in astrocytes.[66] QKI6 and QKI7 are localized to cytoplasm, whereas a nuclear localization signal in the C-terminus of QKI5 allows its import in the nucleus.[67] Mutations in the *Qk* gene that are compatible with life, named *qk(v)*, cause severe dysmyelination in mice, which correlates with aberrant expression of the QKI proteins in different subsets of glial cells.[66] On the other hand, a mutation in the STAR domain that impairs homodimerization is lethal in vivo,[68] highlighting the importance of this self-interaction in the function of STAR proteins. QKI proteins mediate post-transcriptional regulation of mRNAs encoding several proteins involved in the formation of the myelin sheet. For instance, the nuclear QKI5 isoform regulates alternative splicing of exon 12 in the myelin-associated glycoprotein (MAG) pre-mRNA and this splicing event is altered in the quaking mice.[69] Moreover, the QKI proteins are required for the stabilization and export of the mRNAs encoding the myelin basic protein (MBP) isoforms, thereby causing accumulation of this protein in cells undergoing myelinogenesis.[70,71] Notably, the interaction between QKI and MBP mRNA is regulated by SFKs in the developing CNS. It was shown that, similarly to Sam68, phosphorylation of the C-terminal tyrosine residues in QKI proteins by Src or Fyn decreased their affinity for target RNA in vivo and in vitro (Fig. 1A).[72] When examined in the developing brain, tyrosine phosphorylation of QKI proteins was maximal at day 7 postnatal and rapidly declined from day 7 to day 20, concomitantly with the strong induction in MBP mRNA and protein levels and with myelinogenesis.[72] In addition, expression of Fyn was elevated in oligodendrocyte precursor cells, whereas the activity of this SFK declined later on during myelin accumulation.[73] Notably, Fyn and QKI activity seemed also to antagonistically regulate alternative splicing of MBP mRNA isoforms.[73] These observations strongly indicated that post-translational regulation of QKI proteins affected the accumulation of one component of the myelin sheet in developing neurons.

In addition to phosphorylation, QKI5 was weakly methylated in arginines in vivo (Fig. 1A). However, in contrast to Sam68, this STAR protein did not associate with protein methyltransferase activity and with PRMT1.[54] These observations suggested that QKI-5 is a target for a different methyltransferase in vivo and that this post-translational modification might not require a stable interaction.[54] The consequences of methylation on QKI-5 activity and whether or not other QKI isoforms are methylated in vivo, remain to be established. Moreover, no information on additional post-translational modifications of these STAR proteins has been reported, suggesting that more studies are required to fully understand QKI regulation in vivo.

POST-TRANSLATIONAL MODIFICATIONS OF SF1

The more distantly related STAR protein SF1 (Splicing Factor 1) is a branchpoint binding protein involved in early steps of the splicing reaction.[1,74,75] Differently from Sam68, SLMs and QKI proteins, the proline-rich sequences of SF1 were shown to preferentially interact with the tyrosine kinase Abl rather than with SFKs.[1] Moreover, these regions allow SF1 to interact with WW domains of proteins that may link transcription to pre-mRNA processing.[76] SF1 binds the intron branch site and associates with the splicing regulator U2AF65.[77] Interestingly, the cGMP-dependent protein kinase-I (PKG-I) phosphorylates SF1 at serine 20, thereby impairing its interaction with U2AF65 and spliceosomal assembly in neuronal cells.[78] This observation suggested that signaling events that alter the levels of cGMP in neurons can exert an effect on splicing through regulation of SF1. On the other hand, the protein kinase KIS (kinase interacting stathmin) interacts with SF1 and phosphorylates two serine residues (aa 80 and 82) flanked by a proline (SPSP motif) and these phosphorylation events enhanced the interaction of SF1 with U2AF65.[79] Thus, the splicing activity of SF1 can be tightly regulated through phosphorylation by different kinases that exert opposite effects on the recruitment of U2AF65 by this STAR protein (Fig. 1A).

CONCLUSION

Since their first identification, the structural features of members of the STAR family suggested a role for these proteins at the crossroad between signal transduction pathways and RNA metabolism.[1] Much work has been done in the past decade to confirm this initial hypothesis. Several STAR proteins have been demonstrated to be post-translationally modified in response to external and internal cues (Fig. 1A). Moreover, examples of their ability to bind RNA and to affect transcriptional and post-transcriptional processing of target mRNAs have been illustrated. Nevertheless, much remains to be done, especially for those proteins lacking genetic support for their in vivo function(s). In addition, the biological influence of some modifications, such as sumoylation or acetylation for Sam68, needs to be further investigated. Another aspect to be clarified is the potential redundancy of Sam68, SLM-1 and SLM-2. These STAR proteins are highly homologous and share many features in terms of signal transduction and RNA activities. However, some differences at the level of post-translational modifications have been demonstrated (i.e., association with PRMT activity for SLM-1; tyrosine phosphorylation by SFKs for SLM-2). Notably, although Sam68 has been implicated in many crucial biological processes, cells can proliferate and survive without it and, in addition, knockout mice can develop and live throughout adulthood, albeit at lower rates than their littermates.[64] It is possible that SLM-1 and SLM-2 constitute a backup activity that supports cell viability in the absence of Sam68 and that part of the defects observed in the knockout animals are due to the slightly different post-translational modifications occurring in the SLM proteins. Finally, post-translational modifications of other members have not been reported yet, but hints indicate that they might occur. For instance, the *D. melanogaster* HOW(L) nuclear protein contains the HPYR motif that is conserved in the nuclear localization signal of other nuclear STAR proteins like QKI-5 and Sam68 and its mutation caused mislocalization of HOW(L) in the cytoplasm.[80] This observation suggests that, similarly to Sam68 (Fig. 2B), the nuclear localization of HOW(L) could be regulated by post-translational modifications.

Thus, future studies are warranted to fully determine the impact of post-translational control on the many activities played by the STAR proteins and to elucidate the many functions of this highly regulated family of RBPs in cells and live organisms.

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