

Sam68: A New STAR in the Male Fertility Firmament

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Abstract

Male infertility accounts for approximately 50% of the cases of sterile human couples and in many instances the genetic or molecular defects involved remain unknown. Studies conducted in animal models have elucidated the key role played by RNA binding proteins and by the post-transcriptional regulation of gene expression during spermatogenesis. Ablation of proteins involved in each of the steps required for the processing and the utilization of mRNAs impairs the production of fertile spermatozoa. Recent evidence indicates that the RNA binding protein Sam68 is absolutely required for the correct progression of spermatogenesis and for male fertility in the mouse. Sam68 belongs to the evolutionary conserved Signal Transduction and Activation of RNA (STAR) family of RNA binding proteins. The members of this family have been demonstrated to play crucial roles in cell differentiation and development, including male and female gametogenesis. In this review we will summarize the observations gathered on the functions of STAR proteins in different organisms, with particular emphasis on the role of Sam68 in male fertility.

1. Introduction

Spermatogenesis is a complex differentiation program that involves both genetic and morphological processes. In addition to a specific transcriptional regulation of gene expression (Eddy, 2002; Tanaka and Baba, 2005), germ cells have adopted a highly regulated post-transcriptional control of the messengers produced during spermatogenesis (Paronetto and Sette, 2009). These post-transcriptional events are orchestrated by a large number of RNA binding proteins (RBPs) that act at different steps of mRNA maturation and utilization. Since the onset of transcription, nascent mRNAs are coated with RBPs that protect them from degradation, avoid their base pair annealing with the template DNA and assist the processing events that will yield mature transcripts. Interactions of the pre-mRNA with specific RBPs are required for the modification of the 5' and 3' ends of the transcript, for the splicing of the introns, for RNA editing and for the export of the mature mRNA to the cytoplasm (Moore and Proudfoot, 2009). In addition, many processed mRNAs are stored in the nucleus of male germ cells (Kleene, 2001), likely in association with RBPs that protect them until they are needed. Their accumulation in ribonucleoprotein particles (mRNPs) occurs in late pachytene spermatocytes and round spermatids (Geremia et al., 1977). This storage is probably necessary to provide the mRNA templates for protein synthesis when transcription is repressed. For instance, RNA synthesis is low or absent in early meiosis, when the genome is engaged in homologous recombination, or during the nuclear compaction in late spermiogenesis (Geremia et al., 1977; Monesi, 1964). Once transported in the cytoplasm, the decision to repress or to activate translation of specific mRNAs is still operated by RBPs that associate with them,

and many mRNAs are shifted from the translationally inactive mRNPs to the polysomes during the progression of spermatogenesis (Iguchi et al., 2006).

Although these post-transcriptional events exist in every cell of the organism, they acquire particular relevance in highly differentiated cells such as neurons and gametes. As a consequence of this highly regulated post-transcriptional control, a large number of RBPs are either uniquely or highly expressed in germ cells (Venables and Eperon, 1999; Paronetto and Sette, 2009). The essential role played by these RBPs and by post-transcriptional regulation of gene expression during spermatogenesis has been highlighted by the infertile phenotype of mouse knockout models with genetic ablation of specific RBPs and by the occurrence of loss-of-function mutation in genes encoding several RBPs in infertile men (Paronetto and Sette, 2009). Remarkably, RBPs involved in all the steps of RNA processing, from alternative splicing (i.e. hnRNP G/T; Ehrmann et al., 2008), to 3' end modification (i.e. *tau*CSTF-64; Dass et al., 2007), to mRNA export and translation (i.e. MSY2; Yang et al., 2005; 2007) are essential for male fertility, suggesting that the fine-tuning of all the processes involved in post-transcriptional modification of mRNAs by specific RBPs is required to insure the production of a functional male gamete. Herein we will describe the functions of an emerging family of RBPs involved in cell differentiation events and in spermatogenesis: the STAR family.

2. The evolutionary conserved STAR family of RNA binding proteins

The STAR (Signal Transduction and Activation of RNA) family is a class of evolutionary conserved RNA-binding proteins (RBPs) that integrate signal transduction pathways to changes in RNA processing (Fig. 1A). The family includes the *A. salina* GRP33, the *C. elegans* GLD-1 and ASD-2, the *D. melanogaster* HOW and KEP1 and the mammalian QUAKING (QKI) proteins, Sam68, SLM-1, SLM-2 and SF1 (Vernet and Artzt, 1997; Lukong and Richard, 2003). STAR proteins are characterized by a GSG (GRP33/SAM68/GLD1) RNA-binding domain of about 200 amino acids, which comprises a single maxi KH (hnRNP K Homology) domain and two conserved flanking sequences referred to as NK (N-terminus of KH) and CK (C-terminus of KH) (Lukong and Richard, 2003). The NK and CK sequences, which consist of approximately of 80 and 30 amino acids, are conserved from yeast to humans and confer RNA-binding affinity and specificity to the GSG and the ability to homodimerize to the STAR proteins. In addition to their RNA-binding features, STAR proteins contain regions that suggest a functional role in signal transduction. These include proline-rich motifs, which represent binding sites for SH3 and WW domain-containing proteins, stretches of tyrosines in the C-terminal tail, which can be phosphorylated and form docking sites for proteins containing SH2 domains, and RG-rich regions, which can be methylated (Lukong and Richard, 2003). The ability of STAR proteins to undergo post-transcriptional modifications, together with their tissue-specific expression, suggested that they could participate to the spatial and temporal control of gene expression and thereby affect specific developmental processes

(Vernet and Artzt, 1997). Indeed, support to this original hypothesis has been gathered by observations in different model organisms, such as *C. elegans*, *D. melanogaster*, *X. laevis* and mammals (Fig. 1).

3. GLD-1: a prototype STAR protein required for oogenesis.

Perhaps the most studied role for a STAR protein in developmental processes is represented by GLD-1 in *C. elegans*. GLD-1 is a germline-specific cytoplasmic protein (Jones and Schedl, 1995) and is thought to repress gene expression at the translational level (Hansen *et al.*, 2006; Jan *et al.*, 1999). GLD-1 is essential for the progression of the meiotic prophase during oogenesis and it acts redundantly with the RNA polyA polymerase GLD-2 in the promotion of meiotic entry (Hansen and Schedl, 2006). In *Gld-1* null worms, germ cells enter meiosis but fail to progress through the first prophase and re-enter in mitosis. This event results in over-proliferation of germ cells and the consequent formation of gonadal tumors (Francis *et al.*, 1995a). GLD-1 also plays a non-essential function in male sexual fate in the hermaphrodite germ line, as shown by the fewer sperm produced in *gld-1(lf) XX* (Francis *et al.*, 1995b).

GLD-1 is localized in the cytoplasm of germ cells and has been shown to repress translation of target mRNAs. In particular, translational repression of proteins required for germ cell proliferation, such as the NOTCH-like protein GLP-1, allows germ cells to exit mitosis and enter the meiotic differentiation program (Marin and Evans, 2003; Hansen *et al.*, 2004). GLD-1 also protects germ cells from radiation-induced apoptosis through translational repression of CEP-1, an ancestor p53 gene that regulates many pro-apoptotic genes at the transcriptional level (Schumacher *et al.*, 2005). In addition, GLD-1 represses translation of a number of mRNAs produced at the pachytene stage that are required for late oocyte differentiation or early embryonic development (Lee and Schedl, 2001), suggesting that this STAR protein is required for the accumulation of specific transcripts and for their expression at stages when transcription might be repressed (Fig. 1B). Finally, GLD-1, together with another translational regulator, MEX-3, is required for the maintenance of totipotency in the *C. elegans* germ line (Ciosk *et al.*, 2006). These observations demonstrate that translational regulation of gene expression by GLD-1 plays a crucial role for important developmental decisions in this organism.

4. Functional roles of STAR protein in developmental processes

Other STAR proteins have been shown to regulate cell differentiation events in organisms as diverse as worms and humans. We will briefly describe the most elucidated examples.

The *C. elegans* *Asd-2* (Alternative Splicing Defective-2) gene encodes, through alternative splicing, two protein isoforms named ASD-2a and ASD-2b, which differ in their N-terminus sequence (Ohno *et al.*, 2008). ASD-2a is expressed in hypodermis and pharyngeal muscles, whereas ASD-2b is present in pharyngeal and body wall muscles. ASD-2b modulates the alternative splicing of *let-2* mRNA, which encodes for $\alpha 2$ (IV) collagen, by promoting the inclusion of exon 10 instead of exon 9 in the late

larval stages and in adult animals. Remarkably, this post-transcriptional event is required for the development of *C. elegans* body wall muscles (Ohno et al., 2008).

In *D. melanogaster*, the STAR protein HOW coordinates morphogenetic events during embryonic development (Volk et al., 2008). HOW is maternally inherited by the embryo but it is also expressed later by the embryonic genome in multiple tissues, where its activity is essential for promoting key developmental steps (Fig. 1B). *How* mutant phenotypes include abnormal function of the heart and somatic muscles (Baehrecke, 1997; Zaffran et al., 1997), aberrant maturation of tendon cells (Nabel-Rosen et al., 2002; Volohonsky et al., 2007), and defects in the maturation of glial cells (Edenfeld et al., 2006). The single *how* gene produces at least two protein isoform through alternative splicing: a longer HOW(L) isoform and a shorter HOW(S) isoform, which exert opposite effects on the mRNA targets. Although these proteins are identical throughout most of their sequence, they differ in the C-terminus. Moreover, HOW(L) is expressed in the nucleus of cells during early developmental stages, whereas HOW(S) is localized in the cytoplasm during late embryogenesis. The binding of HOW(L) to the 3'UTR of target mRNAs leads to destabilization and rapid degradation of the mRNA. By contrast, association of HOW(S) with the same sequences stabilizes the target mRNA. This suggests that the ratio between the two isoforms in a given tissue determines the direction of the specific developmental program through differential regulation of target mRNAs (Nabel-Rosen et al., 2002; Volk et al., 2008).

The *Xqua* gene in *X. laevis* generates two different mRNAs of 5 kb and 5.5 kb, which are homologous to the 5-kb and 6-kb transcripts of mouse *Qki* or *Quaking*. The 5 kb transcript is expressed only in the early embryo, whereas expression of the 5.5 mRNA is maintained throughout development (Zorn et al, 1997). The Xqua protein (pXqua) is localized both in the cytoplasm and the nucleus of cells starting from early gastrulation (Zorn et al., 1997). Over-expression of pXqua in the embryo enhances notochord development, whereas over-expression of a dominant negative allele blocks notochord development, demonstrating an essential function for this STAR protein during notochord differentiation (Zorn and Krieg, 1997) (Fig. 1B). Although the mechanism has not been elucidated, pXqua appears to favour the accumulation of mRNAs involved in notochord specification, such as *Xnot-2*, *Xbra* and *gooseoid* (Zorn and Krieg, 1997), suggesting that it regulates gene expression at the post-transcriptional level like other STAR proteins.

Mammalian STAR proteins can be divided in two different groups: the QUAKING proteins and the Sam68 subfamily. QUAKING (QKI) proteins have been implicated in alternative splicing, RNA export and translation (Chenard and Richard, 2008). Alternative splicing of the single *qki* gene yields at least five proteins, including QKI-5, QKI-6 and QKI-7, which differ only in their C-terminal domains. QKI-5 shows a nuclear localization and is predominantly expressed during early developmental stages, whereas QKI-6 and QKI-7 share a prominent cytoplasmic distribution, as well as a characteristic

expression pattern during late embryogenesis. Although the *Qki* gene is essential, *Qki* viable mutations exist and cause dysmyelination in the central nervous system (CNS) and mice develop a tremor defect few days after birth (Chenard and Richard, 2008) (Fig. 1B). QKI proteins bind the 3' UTR of the mRNA for the myelin basic protein, a major component of the myelin sheet, and direct its subcellular localization (Li et al., 2000). It was also shown that QKI expression is required for the nuclear export of the MBP mRNA (Larocque et al., 2002). In addition, QKI binds to and stabilizes the mRNA for the cyclin-dependent kinase inhibitor p27(Kip1) thereby enhancing its expression and inducing cell cycle arrest and oligodendrocyte differentiation in the CNS (Larocque et al., 2005). A similar QKI-dependent association and stabilization was observed for the *Map1b* mRNA (Zhao et al., 2006), which encodes a microtubule-associated protein that is essential for neural development. On the other hand, the nuclear QKI-5 isoform was shown to regulate the alternative splicing of the *Mag* pre-mRNA, encoding a myelin associated glycoprotein (Wu et al., 2002). Finally, high-throughput identification of QKI-target mRNAs demonstrated that a large proportion of them are implicated in cell differentiation and development (Galarneau and Richard, 2005). These observations reinforce the hypothesis that QKI proteins play a crucial role in the development of the central nervous system. Interestingly, some evidence has been provided that altered QKI function is linked to human genetic disease such as ataxia, glioblastoma multiforme and schizophrenia (reviewed in Chenard and Richard, 2008)

5. The Sam68 subfamily of STAR proteins

The Sam68 subfamily is composed of Sam68 (SRC-associated in mitosis, 68 kDa) (Fumagalli et al., 1994; Taylor and Shalloway, 1994) and its homologues Sam68-like mammalian protein 1 and 2 (SLM-1 and SLM-2, also named T-STAR in humans) (Di Fruscio et al., 1999; Venables et al., 1999). Sam68 is ubiquitously expressed, whereas SLM-1 and SLM-2 expression is restricted to few cell types or tissues (Paronetto and Sette, 2009). Although all three proteins have been implicated in alternative splicing of target pre-mRNAs (Stoss et al., 2001; 2004; Matter et al., 2002; Paronetto et al., 2007; Chawla et al., 2009), insights into their biological function have been so far obtained only for Sam68. It was demonstrated that Sam68 regulates cell cycle progression and apoptosis (Taylor et al., 2004; Paronetto et al., 2007) through both RNA-dependent and RNA-independent mechanisms. Moreover, Sam68 contributes to the growth, proliferation and invasion of prostate and breast cancer cells (Busà et al., 2007; Richard et al., 2008), as well as to the oncogenic potential of the Mixed Lineage Leukemia (MLL) transcriptional complex (Cheung et al., 2007). Sam68 was also shown to regulate neuronal differentiation of progenitors cells in culture (Chawla et al., 2009). Genetic ablation of the *Sam68* locus in mice reduced viability by unknown reasons. Although the majority of knockout animals survived to adulthood, they displayed defects in mesenchymal cell differentiation and bone metabolism and defects in locomotion (Richard et al., 2005; Lukong and Richard, 2008) (Fig. 1B). These observations indicate that Sam68, like

the other STAR proteins, is required for key differentiation events during development. Moreover, since Sam68 is post-translationally modified by acetylation (Babic et al., 2004), methylation (Coté et al., 2003) and serine/threonine (Matter et al., 2002; Paronetto et al., 2006) or tyrosine phosphorylation (Fumagalli et al., 1994; Taylor and Shalloway, 1994) in response to activation of signaling pathways or in cancer cells (Paronetto et al., 2004; Lukong et al., 2005), it represents an excellent example of the role of STAR proteins at the crossroad between intracellular signals and the modulation of RNA metabolism.

6. A novel role for Sam68 in spermatogenesis

Although Sam68 is a ubiquitous protein, its expression levels vary from tissue to tissue, with testis and ovary expressing higher levels of the protein (Fig. 2A). A developmental analysis of Sam68 expression in mouse testis revealed that its levels peak at 18-20 days post partum (dpp) (Paronetto et al., 2006), when the bulk of germ cells approach their first wave of meiotic divisions. The analysis of Sam68 expression in purified germ cell populations confirmed that the highest levels are present in late pachytene spermatocytes. Moreover, immunohistochemistry revealed that this STAR protein displays an oscillatory pattern of expression in the seminiferous tubules. Sam68 is expressed in mitotic spermatogonia at 4 and 7 dpp, it disappears in early meiotic germ cells (leptotene/zygotene stages) at 12 dpp and it strongly accumulates in mid/late pachytene spermatocytes (Paronetto et al., 2006). After meiosis, Sam68 is still expressed in round spermatids but it disappears in elongating spermatids after stage IX of the seminiferous tubule. Moreover, these analyses revealed that, although prevalently nuclear, Sam68 is localized in the cytoplasm of secondary spermatocytes and early round spermatids (Fig. 2B) (Paronetto et al., 2006; 2009). The oscillatory pattern of expression and localization of Sam68 is maintained in the adult testis, suggesting that this STAR protein plays a functional role during spermatogenesis.

6.1 Sam68 is required for male fertility

The physiological function of proteins is often established by the analysis of phenotypes produced by their ablation in mice. The analysis of the reproductive phenotype of *Sam68*^{-/-} mice revealed that males were completely infertile (Paronetto et al., 2009), indicating the requirement of Sam68 expression for fertility. In contrast with what observed in several infertile male knockout mice, Sam68 depletion did not cause gross testicular atrophy and the testis/body weight ratio was only slightly decreased. Nevertheless, histological examination revealed several abnormalities, with a large decrease in post-meiotic haploid cells. The defects in spermatogenesis of *Sam68*^{-/-} mice started to appear after the first meiotic divisions, when the decrease in haploid cells correlated with a strongly increased rate of apoptosis in germ cells. The surviving spermatids displayed several morphological abnormalities at the ultrastructural level, such as aberrant nuclear divisions or multiple flagella. Although *Sam68*^{-/-} mice still produced few mature spermatozoa, these were either immotile or moved aberrantly. Moreover, the

knockout spermatozoa were unable to fertilize ovulated oocytes even under in vitro conditions (Paronetto et al., 2009), indicating that Sam68 is absolutely required for the production of a functional male gamete.

6.2 Sam68 regulates the expression of specific mRNAs in mouse testis

Ablation of Sam68 affects the expression of a limited number (approximately 2%) of the mRNAs in the testis. Among the transcripts affected, there was a significant enrichment in mRNAs encoding for proteins involved in cell cycle, cell death and cancer (Paronetto et al., 2009). Since Sam68 is known to regulate cell proliferation and apoptosis also in somatic cells (Taylor et al., 2004; Paronetto et al., 2007), it is likely that its function relies on the regulation of such transcripts. Moreover, recent evidence have strongly suggested a role for Sam68 in prostate carcinoma, in which it contributes to proliferation and survival of cancer cells to genotoxic stress (Busà et al., 2007) and it regulates the transcriptional activity of the androgen receptor (Rajan et al., 2008). Sam68 is also required for the oncogenic properties of the Mixed Lineage Leukaemia (MLL)-EEN fusion protein, through its ability to bridge this transcription factor to the arginine methyltransferase PRMT1, thereby affecting gene expression (Cheung et al., 2007). Finally, it was demonstrated that Sam68 enhanced the onset and progression of breast tumorigenesis (Richard et al., 2008). Since the function of Sam68 and the RNA targets affected by this protein in cancer cells remain to be established, it will be interesting to determine whether the transcripts affected by Sam68 in mouse testis are also altered in these tumours in which Sam68 plays a role.

6.3 Sam68 is required for translation of its target mRNAs.

Interestingly, in wild type germ cells Sam68 associated with several of the transcripts that were altered in the knockout testis, suggesting that the regulated mRNAs are direct targets of this RBP. Since Sam68 ablation mainly affected post-meiotic haploid cells, after this RBP translocates to the cytoplasm (Paronetto et al., 2006), it was suggested that it could play a role in translation. In support to this hypothesis, Sam68 associates with the translation initiation complex eIF4F (reviewed in Richter and Sonenberg, 2005) in secondary spermatocytes and early round spermatids. Moreover, a pool of the protein was stably associated with mRNAs on the polysomes in these cells (Paronetto et al., 2009), suggesting that Sam68 accompanies its target mRNAs in their journey toward the translational machinery, in a manner similar to what was shown for the polyA-binding protein PABP1 (Collier et al., 2005). Notably, although ablation of Sam68 did not affect general translation, the polysomal recruitment of several targets, such as the *Spag16*, *Nedd1* and *Spdya* mRNAs, were strongly decreased and this correlated with lower levels of the corresponding proteins in knockout germ cells (Paronetto et al., 2009). In cultured spermatocytes, the cytoplasmic translocation and polysomal association of Sam68 during the meiotic divisions favoured the translation of target mRNAs. Remarkably, this effect of Sam68 on

translation strongly correlated with phosphorylation by the ERK1/2 kinases in meiotic germ cells and in somatic cells, as determined by using a luciferase reporter construct containing the *Spag16* 3' UTR (Paronetto et al., 2009). Thus, phosphorylation of Sam68 by ERKs stimulates translation of target mRNAs in male meiotic and post-meiotic germ cells.

Sam68 might play a role in mRNA translation also in somatic cells under specific circumstances. For instance, its association with polysomes and its effect on translation of bound mRNAs has been reported in depolarizing neurons (Grange et al., 2004; 2009). Moreover, Sam68 is exported from the nucleus during viral infections (McBride et al., 1996) and it enhances cytoplasmic utilization of viral RNA (Coyle et al., 2003), whereas a dominant-negative mutant of the protein sequesters a target viral mRNA in stress granules and represses its translation (Henao-Mejia et al., 2009). Finally, Sam68 might also play a role in translation at mitosis, when it is released in the cytoplasm upon breakdown of the nuclear envelope, as it has been shown for other RBPs (Schepens et al., 2007; van den Bogaart et al., 2009). Although in some of these examples the role of Sam68 in mRNA translation has been less firmly connected with a specific function, in germ cells defects in Sam68-mediated translational regulation likely contribute to the infertility phenotype of male mice. Indeed, *Sam68* knockout germ cells express low levels of SPAG16 protein, a component of the sperm axoneme whose deficiency causes reduced spermatozoa motility and male infertility (Zhang et al., 2006). Hence, the defects in meiotic divisions and haploid cell differentiation likely stem from the combined effect of reduced translation of several of the identified Sam68 targets (Paronetto et al., 2009). However, since Sam68 resides in the nucleus in premeiotic male germ cells and it has been implicated in nuclear processing events, such as alternative splicing (Lukong and Richard, 2003; Paronetto and Sette, 2009), it is also possible that additional functions of this RBP contribute to the phenotype observed.

7. Concluding remarks

STAR proteins are evolutionary conserved in eukaryotes and play relevant roles during developmental switches in both embryos and adult animals. Their ability to integrate signal transduction pathways with changes in mRNA processing, export, stabilization or translation allows STAR proteins to be finely modulated in cell exposed to various environmental cues or stresses, or morphogenetic determinants. A new example of such flexibility is illustrated by the role played by Sam68 during mouse spermatogenesis. Although Sam68 is prevalently nuclear in the cell, its phosphorylation by the ERK1/2 serine/threonine kinases during the meiotic divisions of male germ cells allows its interaction with the polysomes in secondary spermatocytes and early round spermatids. This translocation of the protein is required for the translation of target mRNAs bound by Sam68 in male germ cells. Remarkably, since *Sam68* knockout germ cells are defective in their differentiation into mature spermatozoa, it is likely that this role of the STAR protein is essential for male fertility. Sam68 deficiency elicits an oligospermic

phenotype in the mouse, without dramatically affecting the size of the testis. Because this phenotype resembles some cases of human infertility (Matzuk and Lamb, 2008), it is possible that mutations in the human *Sam68* gene underlie to the sterility of some idiopathic patients.

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Figure Legends

Figure 1. Schematic representation of the structural and functional features of the STAR proteins.

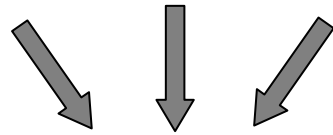
A) STAR proteins are thought to integrate signals deriving from environmental cues and with modifications of the metabolism of their target mRNAs. Changes in subcellular localization, RNA affinity and activity of STAR proteins can be modulated by post-translational modifications and/or protein-protein interactions. The effect on RNA processing described for some STAR proteins in different organisms are listed. The structural domains that characterize STAR proteins are schematically illustrated.

B) Description of the known functional roles described for STAR proteins in different organisms, from the nematode *C. elegans* to the mouse animal models.

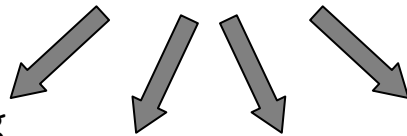
Figure 2. Expression, localization and function of the STAR protein Sam68. A) Western blot analysis of Sam68 expression in different mouse tissue (30 µg of proteins in each lane). B) Immunohistochemistry with a anti-Sam68 antibody reveals that this STAR protein is localized in the cytoplasm of mouse secondary spermatocytes and round spermatids in stage XII seminiferous tubule sections. C) Schematic model of the functions of Sam68 in meiotic germ cells. Before meiosis, Sam68 is strictly nuclear and might participate to pre-mRNA processing; during meiosis, Sam68 is released in the cytoplasm and favours translation of target mRNAs through its interaction with the translation initiation complex eIF4F and the polysomes.

A

signalling pathways,
post-translational modifications,
protein-protein interactions



STAR proteins



splicing

Sam68
QKI
ASD-2
SF-1

export

QKI
Sam68

stability

QKI
HOW

translation

GLD-1
QKI
Sam68

B

GLD-1
(*C. elegans*)

switch from proliferation to meiotic differentiation of germ cells;
translational repression and storage of oocyte- and embryo-specific mRNAs during oogenesis;

HOW
(*D. melanogaster*)

development of heart, somatic muscles and tendons;
regulation of mRNA stability

pXqua
(*X. laevis*)

regulation of notochord formation

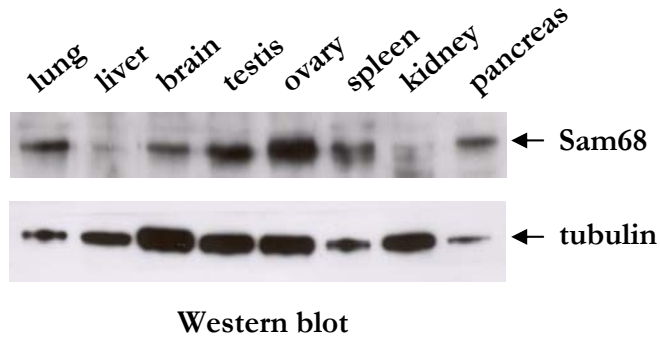
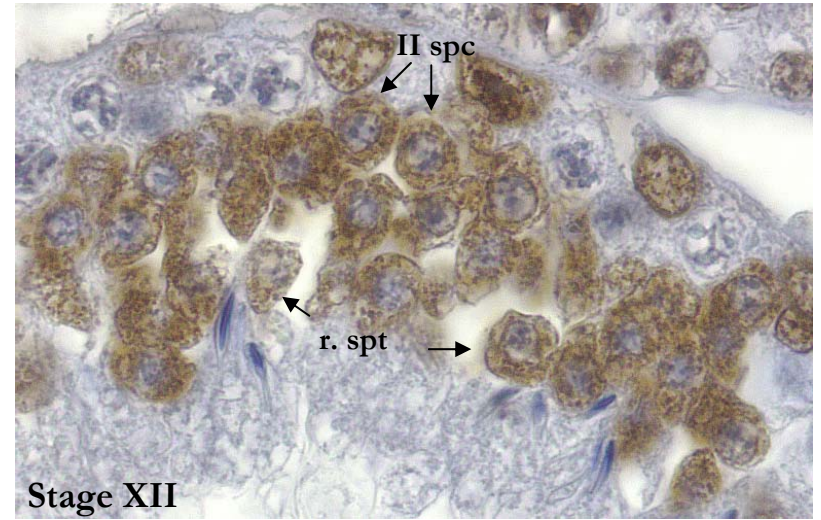
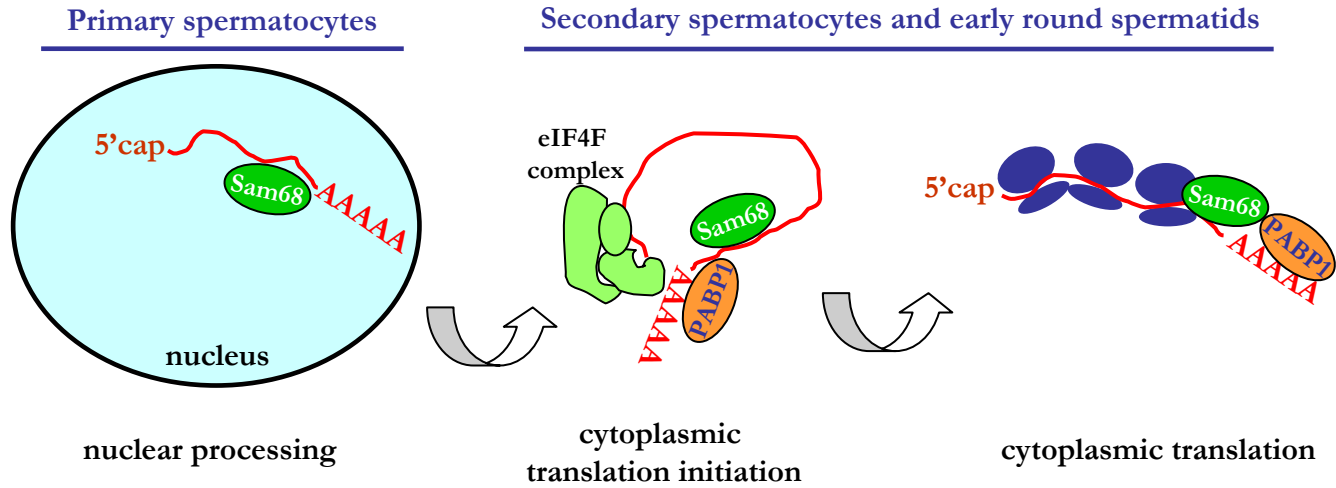
QKI
(*mammals*)

myelination during CNS development;
regulation of mRNA splicing, export stability and translation

Sam68
(*mammals*)

bone metabolism, male fertility, locomotion;
regulation of mRNA splicing, export and translation

Figure 1

A**B****C****Figure 2**