RNA binding proteins in spermatogenesis: an in depth focus on the Musashi family

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Controlled gene regulation during gamete development is vital for maintaining reproductive potential. During the complex process of mammalian spermatogenesis, male germ cells experience extended periods of the inactive transcription despite heavy translational requirements for continued growth and differentiation. Hence, spermatogenesis is highly reliant on mechanisms of posttranscriptional regulation of gene expression, facilitated by RNA binding proteins (RBPs), which remain abundantly expressed throughout this process. One such group of proteins is the Musashi family, previously identified as critical regulators of testis germ cell development and meiosis in Drosophila, and also shown to be vital to sperm development and reproductive potential in the mouse. This review describes the role and function of RBPs within the scope of male germ cell development, focusing on our recent knowledge of the Musashi proteins in spermatogenesis. The functional mechanisms utilized by RBPs within the cell are outlined in depth, and the significance of sub-cellular localization and stage-specific expression in relation to the mode and impact of posttranscriptional regulation is also highlighted. We emphasize the historical role of the Musashi family of RBPs in stem cell function and cell fate determination, as originally characterized in Drosophila and Xenopus, and conclude with our current understanding of the differential roles and functions of the mammalian Musashi proteins, Musashi-1 and Musashi-2, with a primary focus on our findings in spermatogenesis. This review highlights both the essential contribution of RBPs to posttranscriptional regulation and the importance of the Musashi family as master regulators of male gamete development.

Keywords: gene regulation; Musashi; Musashi-1; Musashi-2; posttranscriptional control; RNA binding proteins; spermatogenesis; splicing; testis; translation

INTRODUCTION
Spermatogenesis
Spermatogenesis defines the maturation process of male gametes and is one of the most complex differentiation events that occur within developmental biology, necessitating the controlled regulation of gene expression (Figure 1). Within the male testis, spermatogenesis commences shortly after birth with the differentiation of prespermatogonial gonocytes to spermatogonia. These spermatogonia provide the pool of stem cells essential for the continual production of spermatooza throughout postpubertal life. Spermatogonial stem cells undergo a series of mitotic amplifications to produce primary spermatocytes. These spermatocytes then go through two rounds of meiosis to form haploid round spermatids. Postmeiotic spermatid differentiation (spermiogenesis) defines the profound morphological changes that mark transition into elongating spermatids and the progressive development of immature spermatooza.

During the process of spermatogenesis, two phases of the inactive transcription have been described. The first of these phases takes place during homologous recombination of spermatocytes entering early meiosis,¹ whereby the genome is damaged and repaired for crossing over and transcription is blocked.² The second phase marks the cessation of mRNA synthesis, and takes place in late elongating spermatids, at the time of chromatin condensation in spermiogenesis, denoted by the mass degradation of mRNAs and the gradual decline of translation.³,⁴ These extended periods of transcriptional cessation that occur during the continued differentiation program of spermatogenesis necessitate extensive regulation of translation.⁵ Consequently, the mechanisms of posttranscriptional regulation, controlled primarily by RNA binding proteins (RBPs), become of utmost importance. In instances where the mechanisms for posttranscriptional control function abnormally, spermatogenesis can fail resulting in the production of nonviable gametes.⁶,⁷

RNA binding proteins
RBPs are an extensive class of proteins defined by their ability to recognize and bind to specific sequences of RNA, and regulate the function utilizing an array of mechanisms. They contain at least one RNA recognition site responsible for identifying a particular motif within target sequences.

The sub-cellular level at which RBPs are expressed is important in determining the function. Consequently, RBPs can be divided into nuclear RBPs and cytoplasmic RBPs. Nuclear RBPs localize to the nucleus where they primarily regulate nascent mRNA (pre-mRNA) processing events, including capping, polyadenylation, and splicing.⁷ Cytoplasmic RBPs bind mature mRNA sequences in the cytoplasm as

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they are released from the nucleus. Cytoplasmic RBPs operate more directly in the translation via: directing mRNA transport, competitive or co-operative interactions with translation machinery, and regulating mRNA stability. A number of RBPs are both nuclear and cytoplasmic and can, therefore, be involved in a combination of the previously mentioned processes, as well as uniquely in mRNA nuclear export.

Importantly RBPs are highly expressed throughout spermatogenesis and have been well documented as being essential to posttranscriptional control during all stages of germ cell development. Characterization studies using transgenic models of testis-expressed RBP disruption commonly identify an irregular spermatogenesis phenotype and often exhibit various stages of spermatogenic arrest and resultant sterility. Piecing together the mechanisms that these RBPs utilize to make them fundamental during testis germ cell development is vital to our overall understanding of spermatogenesis and male factor infertility.

FUNCTIONAL MECHANISMS OF RNA BINDING PROTEINS

Within the cell, RBPs function at all levels of RNA metabolism. Assembling on nascent and processed mRNAs, RBPs have the ability to govern gene regulation at the posttranscriptional level in both health and disease. Here, we divide the mechanisms utilized by RBPs into six sub-categories in terms of posttranscriptional regulatory processes (Figure 2).

Capping

Message RNA production requires synthesis of a pre-mRNA and processing of the nascent precursor by 5’ capping, splicing of introns, and 3’ cleavage/polyadenylation to make mature mRNA. Although these processes are interconnected: the 5’ cap can enhance splicing of the first intron and 3’ processing, while 3’ processing depends on splicing of the last intron, it is important to assess each step individually. Capping describes the addition of a 7-methylguanosine linked via a triphosphate bridge, to the first nucleotide of the 5’ end of the transcript. This process is essential in the production of different mRNA isoforms. This process, known as alternative splicing, has the capacity to alter gene coding and affect RNA stability and has been recognized as a mechanism for increasing the functional diversity of the proteome, altering the expression of a protein 3- to 4-fold. Both constitutive and alternative splicing can occur co- and post-transcriptionally and is catalyzed by the spliceosome, a macromolecular complex comprised of the U1-U6 family of small nuclear ribonucleoparticle (snRNPs) in conjunction with >100 additional proteins. Unsurprisingly, mutations in spliceosome components and subsequent splicing errors underlie a large number of human diseases.

Spliceosome proteins are a well-studied class of RBPs. There are also a number of RBPs involved in alternative splicing that bind pre-mRNA either to encourage or block specific splicing events. DExd/H-type RNA-dependent ATPases/helicases have long been implicated in rearrangements within the spliceosome and act at discrete
stages of splicing including single-strand RNA translocation, strand annealing, and protein displacement.\textsuperscript{29}

Despite being composed of a large number of RBPs, very few mutations in core spliceosome components have been found, suggesting that such mutations are nonviable either at the cellular level or in early development.\textsuperscript{21} A diverse set of diseases is also associated with even moderate changes in expression of any number of RBPs involved in splicing and splicing regulation.\textsuperscript{22,23} Recently, an unbiased genetic screen for essential male fertility genes in the mouse identified the RBP, RBM5, as an essential regulator of haploid male germ cell pre-mRNA splicing and fertility. Mice carrying a missense mutation in the second RNA recognition motif of RBM5, affecting pre-mRNA splicing of putative targets, exhibited spermatid differentiation arrest, which led to azoospermia and male sterility.\textsuperscript{24}

Endonucleolytic cleavage marks the final stage of transcription and is followed by addition of a poly(A) tail at the 3’-end. Similar to the 5’ cap, the poly(A) tail is important for the stability and translational efficiency of the mRNA transcript.\textsuperscript{25} And like splicing, transcripts can be alternatively polyadenylated, altering stability, localization, and transport. More than half of the genes in the human genome are estimated to be subject to alternative 3’-end processing, generating isoforms that differ in 3’ UTR length or encoding different proteins.\textsuperscript{26}

With the exception of replication-dependent histone genes, all protein encoding mRNAs contain a uniform 3’-end consisting of around 200 adenosine residues. The formation of this poly(A) tail is directed by sequences present on the pre-mRNA and the polyadenylation machinery. In mammals this consists of six multimeric proteins which come together to firstly mediate cleavage of the nascent mRNA 3’-end, and secondly facilitate coupled polyadenylation, namely: cleavage and polyadenylation specificity factor, cleavage stimulation factor (CstF), cleavage factors I and II (CFI and CFII), poly(A) polymerase, and poly(A)-binding protein II.\textsuperscript{27}

A number of reports suggest that there are testis-specific mechanisms which support nuclear polyadenylation in male germ cells. One example of this is the testis-specific CstF paralog, τCstF-64 (gene name: Cstf2t), considered necessary for germ-cell polyadenylation and gene expression during spermatogenesis.\textsuperscript{28} Targeted deletion of Cstf2t resulted in male infertility due to aberrant meiotic and postmeiotic development.\textsuperscript{29}

**mRNA export**

Transport through the nuclear pore complex (NPC) represents the link between the nucleus and cytoplasm. With nuclear export of mature mRNAs likely to involve distinct docking, translocation and release steps from the NPC.\textsuperscript{30} Export is mediated by protein factors associated with the mRNA, and mRNAs without the necessary adaptor and export
RNA binding proteins in spermatogenesis

JM Sutherland et al

Factors remain held in the nucleus. The mRNA export machinery includes numerous RBPs, ATPase/RNA helicases, and NPC-associated proteins. Most of these are essential, with conditional mutations for many of these genes in yeast showing rapid and strong defects in mRNA export.1

Following mRNA export from the nucleus, interacting RBPs either remain in the nucleus or accompany the transcript into the cytoplasm, where the transcript either remains bound to the same RBPs or is recruited by others. This ultimately determines the cytoplasmic compartmentalization.7

mRNA stability

Maintenance of mRNA stability is essential for the translation of essential proteins and also the proteasomal-mediated degradation of unwanted transcripts. Stability of mRNAs can be rapidly modulated to alter the expression of specific genes, providing flexibility in affecting changes in patterns of protein synthesis. Degradation of transcripts is highly variable and thought to be controlled mainly by RBPs. An important step commonly observed in the regulation of stability is an alteration in the length of the poly(A) tail, which often precedes decapping, and Singh of the poly(A).32 It is often the case that RBPs that accompany the transcript from the nucleus to the cytoplasm, with the assistance of newly assembled translation initiation factors, aid in the recruitment of the translation machinery.33 In contrast, mRNA binding of the miRNA-induced silencing complex brings about decapping, deadenylation, and translational repression of the mRNA.34

Translation

Perhaps the best-described and also most important event that RBPs control is translation. Regulation of translation is controlled through three main mechanisms: poly(A) tail modification, association with RNPs, and competition with translation machinery. Lengthening the poly(A) tail of a transcript can significantly increase translation rates. This is achieved through the regulation of binding of the RBP poly(A)-binding protein (PABP), which recruits factors essential for translation initiation.35 The most significant mechanism involves directing the transcripts to either cytoplasmic compartments - correlated with translational inhibition, or polyribosomes - known sites of translation.36 Cytoplasmic compartmentalization of transcripts in RNPs represents a targeted location for processing, sorting, storage, or degradation. Generally associated with translational arrest (stress granules), some RBPs are associated with exonucleases (exosome complexes) and are thus sites for mRNA degradation, while others represent locations where transcripts can be protected and remain translationally silent until they are needed (processing bodies, intermitochondrial cement/nuage, and chromatoid bodies).37 Polyribosomes or polysomes are groups of ribosomes clustered around a single mRNA transcript, allowing for simultaneous translation of the transcript, resulting in rapid protein production.38 Polysome aggregates represent the main sites of translation, controlled to correspond with cell needs.

STAGE-SPECIFIC EXPRESSION OF RNA BINDING PROTEINS DURING SPERMATOGENESIS

The stage at which RBPs are expressed during spermatogenesis is also essential in determining their role. Given the sequential process of germ cell differentiation, defining the expression patterns of testis-expressed RBPs in terms of the differentiation status is relatively straightforward. We can sub-divide these RBPs into three major germ cell categories: mitotic, meiotic, and postmeiotic and in the following section explore all three groups in depth, providing examples of each (Table 1). It is important to note that some RBPs are expressed throughout all stages of spermatogenesis, quite often serving a variety of functions.

Mitotic RNA binding proteins

Mitotic RBPs consist of those expressed during early stage spermatogenesis, primarily in gonocytes and spermatogonia. Transcription remains active during this period and, as a result, the cell is not as heavily reliant upon posttranscriptional gene regulation. Fewer RBPs have been identified during this stage; however those that are expressed appear to be essential.

Two well-characterized RBPs expressed during mitosis are Nanos2 and Nanos3. Nanos2 is predominantly expressed in early male germ cells, and the elimination of this gene results in a complete loss of spermatogonia.37 Nanos3 is expressed slightly earlier, in migrating gonocytes,38 with deletion of this factor resulting in complete germ cell loss.39

Similarly, the RBP DAZ1, present only in higher primates, is known to be expressed in gonocytes, with increasingly high levels in observed spermatogonial stem cells plateauing at the first wave of meiosis.40 This unique pattern of expression indicates that DAZ1 participates in differentiation, proliferation, or maintenance of germ cells during early spermatogenesis. Deletion of DAZ1 is associated with azoospermia, strongly suggesting that DAZ1 plays a critical role in normal spermatogenesis,41 with a predicted function in translational activation.42

HnRNP proteins are a class of nuclear pre-mRNA binding proteins with important roles in the biogenesis of mRNA. In the testis, hnRNP proteins are abundant, yet their expression remains tightly regulated. Specifically, hnRNP A1 is highly expressed only in early spermatogonia and absent in later stages.43 Although no hnRNP A1 mutations have been described, it is predicted based on its role in splicing regulation in other cell systems,44,45 that this protein would serve a similar essential function during spermatogenesis.

Meiotic RNA binding proteins

These RBPs are present in cells undergoing either of the two meiotic divisions that occur during spermatogenesis. These cells, spermatocytes, initially undergo homologous recombination to differentiate their genome from the parental ones. During this period, the genome is damaged and repaired for crossing over and transcription is blocked.2 Following this, pachytene spermatocytes begin to synthesize large amounts of stored mRNAs into proteins that will allow them to sustain two consecutive rounds of cell divisions without a real interphase.4 Round spermatids are the resultant germ cells. Given the importance of posttranscriptional regulation during this period, it is not surprising that there is an abundance of RBPs expressed in meiotic spermatocytes.

TRBP2 (formerly PRBP) is a protamine-1 RBP present in the cytoplasmic compartment of late-stage meiotic cells and haploid round spermatids. Recombinant TRBP2 protein inhibits the translation of multiple mRNAs, suggesting that TRBP2 acts as a translational repressor during spermatogenesis.46 Mice that carry a targeted disruption of Tarbp2 are sterile and severely oligospermic due to failure of late-stage spermiogenesis.47

Khdrbs1 (formerly SAM68) belongs to the STAR family of RBPs, which regulate a range of processes, including RNA stability, export, splicing, and mRNA translation.48 The expression of Khdrbs1 protein during spermatogenesis peaks in spermatocytes as the meiotic cells prepare for division. Khdrbs1 is a nuclear protein during most of spermatogenesis but is found within the cytoplasm in meiosis.49 Analysis of the reproductive phenotype of Khdrbs1 knockout mice
revealed that males are completely infertile due to azoospermia, indicating the requirement of Khdbs1 expression in fertility.

**Postmeiotic RNA binding proteins**

Postmeiotic RBPs describe those expressed in both round and elongating spermatids during the differentiation process known as spermiogenesis. The cessation of mRNA synthesis during spermidogenesis necessitates extensive posttranscriptional regulation as a number of new proteins appear during the later stages of this process.5

Adad1 (formerly TENR) is a testis-specific nuclear RBP, detected in postmeiotic cells, primarily in round and early elongating spermatids, and in association with the nuclear scaffold.51 Adad1 is predicted to function in either in pre-mRNA editing or transport. Targeted mutation of the Adad1 gene causes male sterility, through a combination of reduced sperm number, decreased motility, and increased malformed heads in remaining spermatova, indicating an essential function in spermatid morphogenesis.52

The RBP Paip2 is a PABP binding partner expressed late elongated spermatids and acts in the translational repression of poly(A)-containing mRNAs.53 Paip2-KO mice exhibit male infertility via translational inhibition of essential development proteins, resulting in defective elongated spermatids.54

Ybx2 (also MSY2) is a germ cell-specific member of the Y-box family of proteins with broad DNA and mRNA binding capacity. Largely localized in the cytoplasm, Ybx2 exhibits maximal expression in postmeiotic round spermatids and is predicted to function in mRNA storage and translational delay.55 The inactivation of Ybx2 results in spermatogenic arrest and infertility, with incomplete nuclear condensation prominent in later-stage spermatids at the time of massive spermatid loss.56 This occurs due to increased mRNA instability, as indicated by polysomal redistribution resulting in decreases to the abundance of numerous mRNAs normally expressed in these germ cells.56

**MUSASHI FAMILY OF RNA BINDING PROTEINS**

Historically, the Musashi family of RBPs has well-established roles in stem cell function and cell fate determination. All Musashi family members contain two tandem RNA recognition motifs located at the N-terminal of the protein, each composed of two highly conserved motifs; RNP-1 and RNP-2, that bind to target mRNAs through a transcriptional activation regulator.56-60 Originally described in Drosophila, Musashi has also been well-characterized in Xenopus. Both of these model species have provided keen insights into elucidating the pivotal functions of Musashi in the mammalian system, specifically in stem cell maintenance, nervous system development, and tumorigenesis.

**Musashi in Drosophila**

The original Drosophila Musashi (dMsi), was discovered to function in the nucleus of adult external sensory organs. Loss-of-function dMsi mutants exhibit a phenotype of extra outer support cells.61 Consequently, dMsi was shown to play an essential role in the asymmetric division of the external sensory organs progenitor cells; the sensory organ precursor cells. This was later found to be achieved through dMsi-mediated mRNA binding and subsequent translational repression of the neural differentiation inhibitory factor Tramtrack69 (ttk69).62 Similarly, dMsi is required to negatively regulate ttk69 expression in the photoreceptor cells of the developing eye.63 dMsi is also expressed in the central nervous system (CNS) in proliferating neural stem/progenitor cells within the Drosophila larva brain with overexpression resulting in the proliferation of undifferentiated cells.64

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**Table 1: RBP expressed during mammalian spermatogenesis, function, and phenotype**

| RBP | Expression | Function | Phenotype | Reference |
|-----|------------|----------|-----------|-----------|
| RNA binding motif 5 (RBM5) | Nucleus and cytoplasm from spermatogonia to round spermatids | Pre-mRNA splicing | Sterile: spermatid arrest | 24 |
| Cleavage stimulation factor 64 testis-specific (CstF-64) | Nuclei of pachytene spermatocytes through to early spermatids | Nuclear polyadenylation (testis-specific) | Sterile: disrupted meiotic and post-meiotic development | 28 and 29 |
| Nanos homolog 2 (NANOS2) | Cytoplasmic p-bodies: gonocytes and spermatagonia | mRNA degradation | Sterile: complete loss of spermatagonia | 37 and 39 |
| Nanos homolog 3 (NANOS3) | Stress granules and p-bodies: primordial germ cells to spermatogonia | Translational repression | Sterile: total germ cell loss | 38 and 39 |
| Deleted in azoospermia 1(DAZ1)* | Predominately cytoplasmic: gonocytes, spermatagonia, and early spermatocytes | Translational activation | Deletion associated with idiopathic oligozoospermia | 40, 41, and 42 |
| Heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) | Spermatogonia nuclei | pre-mRNA splicing | Not described | 43, 44, and 45 |
| TAR (HIV) RNA binding protein 2 (TARBP2) | Cytoplasm of late-stage meiotic cells and round spermatids | Translational repression | Sterile: late stage failure of spermiogenesis | 46 and 47 |
| KH domain containing, RNA binding, signal transduction associated 1 (KHDRBS1) | Cytoplasm and nucleus of spermatocytes, decreased levels in round spermatid nuclei | Alternative splicing and translational activation | Sterile: reduced post-meiotic cells and defects in spermiogenesis | 49 and 50 |
| Adenosine deaminase domain containing 1 testis-binding (ADAD1) | Nucleus of round and early elongating spermatid cells | pre-mRNA processing | Sterile: defective spermatid morphogenesis | 51 and 52 |
| Polyadenylate-binding protein-interacting protein 2 (PAIP2) | Cytoplasm of late elongating spermatids | Major translational regulator | Sterile: multiple defects in late spermiogenesis | 53 and 54 |
| Y-box-binding protein 2 (YBX2) | Cytoplasm of post-meiotic round spermatids | mRNA stability | Sterile: late stage spermatid failure | 56 |
| Musashi RNA-binding protein 1 (Msi1) | Cytoplasm of spermatogonia, XY body of pachytene spermatocytes | Translational regulation | Not described | 100 |
| Musashi RNA-binding protein 2 (Msi2) | Nucleus of spermatocytes and spermatids | Pre-mRNA processing | Sterile: multiple defects in late spermiogenesis | 100 |

*Description of human RBP; **Testis-specific over-expression. Each RBP described is listed by full name and protein symbol, expression describes the sub-cellular localization of the RBP during mammalian spermatogenesis, function related to method of posttranscriptional regulation, and phenotype describes the spermatogenesis related effects from targeted mutation/knockout of RBP in mice, with related references. RBP: RNA binding proteins.
More recently, a second dMsi protein has been identified. Termed RNA-binding protein 6 (Rbp6), it is considered more closely related to the vertebrate Musashi proteins due to sequence homology.\(^5\) Rbp6 is expressed in multiple tissues throughout development, but interestingly, Rbp6 mutants are viable and fertile, exhibiting only a delay in the timing of larval development.\(^9\) Furthermore, this work showed no overlap in function between Rbp6 and dMsi.

**Musashi in Xenopus**

Studies in *Xenopus laevis* have uncovered evidence for the additional roles of vertebrate Musashi in posttranscriptional regulation, both in retinal development and oocyte maturation. The *Xenopus* Musashi-1 homolog (xMsi1) is a heterogeneous nuclear RBP specifically expressed in the nervous system.\(^9\) During *Xenopus* retina development xMsi1 is expressed in retinal stem cells, mitotically active neural precursors, postmitotic photoreceptors, and retinal pigment epithelium.\(^2\) In *Xenopus*, Musashi function is also considered essential to establishing the temporal order of maternal mRNA translation during the meiotic cell cycle in developing oocytes.\(^9\) Here, xMsi1 directs the activation of the mRNAs required for MAP kinase- and CDK-mediated promotion of cell cycle progression.\(^9\) In to oocyte, it has been demonstrated that *Xenopus* Musashi proteins auto-regulate xMsi1 translation,\(^7\) while Musashi-mediated translational activation of the proto-oncogene *Mos* is considered a necessary event for meiotic cell cycle progression.\(^6\)

**Musashi-1**

Mammalian Musashi-1 (Msi1) is strongly expressed in fetal and adult CNS and brain, with functional roles in the maintenance of stem-cell state, differentiation, and tumorigenesis.\(^6,7\) Msi1 null mice develop obstructive hydrocephalus and suffer early postnatal lethality.\(^7\) This indicates a vital role for Msi1 in the normal development of ependymal stem cells and highlights the importance of Msi1 expression in the proliferation and maintenance of CNS stem cell population.

In mouse neural stem cells, Msi1 inhibits translation by binding to consensus sequences of transcripts encoding Numb,\(^7\) the cell cycle regulator Cdkn1a,\(^7,24\) and Dcx.\(^7\) The mechanisms described for Msi1 translational repression competition with eIF4G for PABP binding demonstrates an essential mechanism for preventing the formation of the 80S ribosome.\(^26\) Conversely, in the hindbrain, Msi1 has been shown to facilitate the translational activation of *Robo3*, required for axonal midline crossing of precerebellar neurons.\(^7\)

Interestingly, Msi1 is often up-regulated in tumor cells, specifically in malignant gliomas and astrocytomas where Msi1 is highly enriched when compared with nonneoplastic brain tissue. Furthermore, the level of increased expression was positively correlated with the aggressiveness of the tumour.\(^28\) Considered a putative marker of intestinal stem cells,\(^29\) Msi1 expression is frequently detected at elevated levels in both premalignant gastric lesions and invasive gastric cancer.\(^30,31\) Up-regulation of Msi1 has also been linked with endometrial carcinoma\(^32\) and of lymph node metastases.\(^33,34\)

**Musashi-2**

Musashi-2 (Msi2) shares high sequence homology to mammalian Msi1, appearing to have arisen following gene duplication.\(^4,35\) Msi2-deficient mice demonstrate 50% embryonic lethality, and subfertility when crossed among themselves.\(^36\)

In neural precursor cells, Msi2 and Msi1 are strongly co-expressed and share similar RNA-binding target specificity, as well as being predicted to be co-operatively involved in the proliferation and maintenance of CNS stem cell population.\(^71\) Indeed, extensive studies of Musashi proteins in the CNS unequivocally favor a redundant and compensatory role for Msi2 that is indistinct from Msi1.\(^72,73,74\)

In instances involving primary regulation via Msi2, it is common for Msi1 expression to be effectively undetectable.\(^90,91\) However, a recent study in pancreatic islet (endocrine) cells did identify distinctive roles for Msi1 and Msi2.\(^92\)

Like Msi1, Msi2 has also been linked with tumorigenesis. Specifically, up-regulation of Msi2 has been implicated in brain tumor growth,\(^93\) leukemia progression,\(^94,95\) and the differentiation of hematopoietic stem cells (HSCs).\(^96\) Further studies in HSC utilizing a Msi2 conditional ablation model revealed failure of HSC maintenance and engraftment resulting from a loss of quiescence and increased commitment divisions.\(^97\)

**MUSASHI IN SPERMATOGENESIS**

Genetic screening of *Drosophila* genes involved in germ cell biology identified Musashi as a critical regulator of testis stem cell maintenance and meiosis.\(^98\) Using the fly testis as a model system, it has been demonstrated that loss of Musashi function disrupts the balance between germ-line stem cell renewal and later-stage differentiation, resulting in the premature differentiation of germ-line stem cells and meiotic defects.\(^98\) Studies in the mouse have identified differential expression of the two primary mammalian orthologs: Msi1 and Msi2, both at a developmental stage and sub-cellular level.\(^98,99\)

Recent work has by our group detailed the distinctive expression of mammalian Msi1 and Msi2 and explored the outcomes of aberrant expression of both RBPs during the process of male gamete development.\(^100\) The unique spatial and temporal expression patterns of the Musashi proteins throughout spermatogenesis indicate individual roles of both RBPs throughout during gamete development in the mammalian testes (Figure 3). Specifically, Msi1 predominately localizes to the cytoplasmic compartments of mitotic gonocytes and spermatagonia, while Msi2 shows nuclear expression in meiotic spermatocytes and differentiating spermatids. The two novel transgenic mouse models utilized, with germ cell-specific overexpression of full-length isoforms of Msi1 or Msi2, demonstrated that aberrant

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**Figure 3:** Musashi RBP expression during spermatogenesis. dMsi paralogs dMsi and Rbp6 are differentially expressed in the fly testis: dMsi is nuclear and expressed in early germ cells and spermatocytes while Rbp6 is cytoplasmic and localized to spermatogonial cyst cells. Mammalian Msi1 is expressed in the cytoplasm of early mitotic germ cells before translocating to the nucleus upon transition to meiosis. Mammalian Msi2 is entirely nuclear, expressed throughout meiosis and during spermatid differentiation. The dotted line refers to cytoplasmic localization while the solid line refers to nuclear-specific expression. RBP: RNA binding proteins; Msi1: Musashi-1; Msi2: Musashi-2; dMsi: *Drosophila* Musashi; Rbp6: RNA-binding protein 6.
expression of either gene was deleterious to normal spermatogenesis and detrimental to cell health. In addition to this, preliminary studies performed on human testicular seminoma tumors have provided further insights into the relevance of Msi1 and Msi2 over-expression as diagnostic markers to human stem cell cancers.\(^\text{100}\)

**SUMMARY**

Herein, we have highlighted various mechanisms utilized by RBPs within the cell and emphasized the importance of sub-cellular localization and stage-specific expression on the function of these master regulators of spermatogenesis, we have provided new evidence for the novel and unique roles of both Msi1 and Msi2 during male germ cell development.

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