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Roles of RNA-binding Proteins and Post-transcriptional Regulation in Driving Male Germ Cell Development in the Mouse

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Abstract

Tissue development and homeostasis are dependent on highly regulated gene expression programs in which cell-specific combinations of regulatory factors determine which genes are expressed and the post-transcriptional fate of the resulting RNA transcripts. Post-transcriptional regulation of gene expression by RNA-binding proteins has critical roles in tissue development—allowing individual genes to generate multiple RNA and protein products, and the timing, location, and abundance of protein synthesis to be finely controlled. Extensive post-transcriptional regulation occurs during mammalian gametogenesis, including high levels of alternative mRNA expression, stage-specific expression of mRNA variants, broad translational repression, and stage-specific activation of mRNA translation. In this chapter, an overview of the roles of RNA-binding proteins and the importance of post-transcriptional regulation in male germ cell development in the mouse is presented.

Keywords

Post-transcriptional regulation; Alternative mRNA processing; Splicing; Polyadenylation; Translational control; RNA-binding proteins; Gametogenesis

1 Introduction

Post-transcriptional regulation of gene expression is a central and widespread mechanism that alters genetic output during cell differentiation and tissue development. Transcriptional controls define which combinations of genes are transcribed in a cell and the magnitude of each gene's transcriptional output, while post-transcriptional controls include regulatory events that act on the RNA products of transcription. These include regulation of nuclear pre-mRNA splicing and polyadenylation, and cytoplasmic mRNA localization, stability, translation, and degradation. In different cell types and stages of development, specific combinations of RNA-binding proteins (RBPs) establish and modulate post-transcriptional gene regulatory networks. The importance of RBPs in tissue homeostasis and function is highlighted by a growing list of human diseases associated with aberrant expression of RBPs and disruption of post-transcriptional regulatory events [1].

Alternative mRNA processing (the production of alternatively spliced and polyadenylated mRNAs from a single gene) has major roles in transcriptome diversification in different tissues [2]. Tissue-specific differences also exist in the extent to which mRNAs are translationally controlled. The mammalian testis stands out from other tissues with respect to

transcriptome complexity and widespread post-transcriptional regulation [3–5]. Within the testis are seminiferous tubules where germ cells proceed through a well-characterized series of developmental steps to generate haploid gametes. Mouse models have identified essential roles for RBPs and post-transcriptional regulation in nearly all steps of germ cell development, from the earliest embryonic stages to the formation and release of mature spermatozoa [6].

In this chapter, the importance of RBPs and post-transcriptional regulation of protein coding genes in gametogenesis is reviewed, with a focus on male germ cell development in the mouse. As mouse and human male germ cell development are broadly comparable, studies of germ cell development in the mouse have provided insights on many different aspects of human cellular and reproductive biology [7, 8]. This includes new insights into regulatory mechanisms that control mammalian cell differentiation, apoptosis, chromosome biology, infertility, and testicular cancer. In the first section, an overview of the different stages of male germ cell development is provided. In the second section, different types of post-transcriptional regulation and examples of their impact on germ cell gene expression are presented. In the final section, evidence of the importance of specific RBPs and their roles in germ cell development is reviewed.

2 Male Germ Cell Development

2.1 Overview of Pathway

Germ cell development can be broadly divided into distinct stages and involves a number of well-characterized cellular division and differentiation events driven by intrinsic factors and extrinsic cues [9] (Fig. 6.1). The embryonic stage of germ cell development includes the specification (or ‘birth’) of germ-line cells, their migration to the genital ridge (the site of the future gonads), and sex determination. The remaining stages of germ cell development occur postnatally and include: (1) a proliferative stage where spermatogonial cells either self-renew or undergo a number of proliferative divisions to yield spermatocytes that enter meiosis; (2) a meiotic stage where four genetically distinct haploid cells (spermatids) are generated from each spermatocyte; and (3) a differentiation stage (called spermiogenesis) where haploid spermatids transform into spermatozoa. Here, an overview of the major stages of male germ cell development in the mouse, with an emphasis on steps in which RBPs and post-transcriptional control of gene expression have critical roles is described.

2.2 Embryonic Stages of Germ Cell Development

Germ-line cells are first detectable at approximately day 7 of embryogenesis (E7) as a cluster of cells in the epiblast [10]. These primordial germ cells (PGCs) proliferate and migrate to the genital ridge, during which time they remain sexually bi-potent, able to commit to either the male or female program of germ cell development. Germ cell sex-determination depends on whether gonadal support cells express the SRY gene encoded on chromosome Y. In the absence of SRY expression (XX embryos), the supporting gonadal cells differentiate into female granulosa cells. In XY embryos, SRY expression induces differentiation of gonadal support cells into male Sertoli cells. Sex-specific gonadal support cells (granulosa cells or Sertoli cells) provide extracellular signals that determine whether

PGCs progress towards a female program of development and differentiate to meiotic oocytes, or commit to a male program of germ cell development in which PGCs become gonocytes (also called prospermatogonia) and proliferate briefly before undergoing cell cycle arrest at G1/G0 and quiescence for the remainder of embryogenesis (Fig. 6.1a) [11, 12]. In the postnatal testis, Sertoli cells remain in close contact with germ cells, providing structural and nutritional support throughout spermatogenesis via testis-specific junctions [13].

2.3 Postnatal Germ Cell Development

2.3.1 Spermatogonia Proliferation, Renewal, and Differentiation—A few days after birth, the quiescent gonocytes resume mitotic proliferation and differentiate into ‘type A’ spermatogonial cells (Fig. 6.1b) [14–16]. Single type A spermatogonia (A_{single} or A_s) are thought to have stem cell potential and undergo self-renewing divisions to produce two new A_s cells. Alternatively, A_s cells can divide to produce a pair of spermatogonial cells (A_{paired} or A_{pr}) that remain connected to one another via intercellular bridges resulting from incomplete cytokinesis. Subsequent divisions yield chains of 4–16, and even 32 spermatogonial cells called A_{aligned} (A_{al}). The differentiation of A_{al} cells into A1 spermatogonia begins the strictly time-regulated stages of spermatogenesis involving mitotic divisions to generate interconnected chains of A2, A3, A4, Intermediate, and finally type B spermatogonia. B spermatogonia undergo a final mitotic division to yield pre-leptotene spermatocytes that enter meiosis.

2.3.2 Meiosis—The second major phase of postnatal germ cell development is meiosis, in which spermatocytes undergo a single genome duplication event followed by two successive divisions (meiosis I and meiosis II) to generate haploid cells called spermatids (Fig. 6.1c). During the prolonged prophase of meiosis I, chromosomes condense and homologous pairs of chromosomes recognize one another and align. The recognition, juxtaposition, and synapsis of homologous chromosomes allows them to physically exchange genetic information through the repair of double strand breaks [17]. Recombination between homologous sequences on maternally- and parentally- inherited chromosomes creates new combinations of alleles and therefore generates genetic diversity. Additional diversity arises from the independent assortment (segregation) of homologous pairs into daughter cells during meiosis I. Meiosis II is significantly shorter than meiosis I and is similar to mitosis in that sister chromatids are separated from one another and segregate into daughter cells.

2.3.3 Spermiogenesis (Spermatid Differentiation)—Round spermatids (the haploid products of meiosis) undergo an ordered series of cytological and morphological changes to produce spermatozoa—slender, elongated cells that consist of three main regions: (1) a flagellum for motility, (2) a midpiece region lined with mitochondria to provide ATP for motility, and (3) a head region consisting of a compact nucleus whose anterior is encased with a granular vesicle (the acrosome) that contains hydrolytic enzymes necessary for oocyte penetration (Fig. 6.1d) [18].

The process of spermatid differentiation (called spermiogenesis) takes ~13.5 days to complete in the mouse, and consists of 16 steps that can be roughly divided into the round

spermatid steps (steps 1–8) and elongating spermatid steps (steps 9–16). During spermatid elongation, extensive chromatin remodeling and compaction results from the successive replacement of histones with transition proteins, followed by the replacement of transition proteins with protamines. A consequence of chromatin compaction is transcriptional inactivation in elongating spermatids [19, 20]. As a result, synthesis of new proteins during spermatid elongation is dependent on a reservoir of translationally-repressed mRNAs synthesized days earlier in transcriptionally active round spermatids (discussed in greater detail below) [3]. In the final stages of spermiogenesis, mature spermatids shed excess cytoplasm, detach from Sertoli cells as cell junctions are severed, and are released into the lumen of the seminiferous tubule [21]. Mature spermatids (called spermatozoa) then transit out of the testis and into the epididymis where further maturation occurs.

3 Complexity and Post-transcriptional Regulation of the Developing Germ Cell Transcriptome

3.1 Modulating Gene Output Via Alternative Splicing and Polyadenylation

Nearly all protein-coding genes in higher eukaryotes have a ‘split-gene’ organization in which the sequences present in the resulting mRNA (expressed sequences or exons) are interrupted in the gene by longer intervening sequences (introns) [2, 22]. Consequently, production of a mature mRNA template for translation requires the removal of intronic sequences from the mRNA precursor (pre-mRNA) and the precise joining (or splicing) of exons. Alternative splicing is the process in which specific exons are differentially spliced into the mature transcript. Nearly all multi-exon genes in mammals yield alternatively spliced mRNA isoforms, most of which are expressed in a specific tissue or stage of development [23–26].

In some cases, alternative splicing results in modest changes in the primary sequence and functional properties of the encoded protein, while in others alternative splicing can have a profound effect on biology and act as a switch that controls the production of protein isoforms with antagonist activities (Fig. 6.2). For example, several genes encoding regulators of apoptosis can yield both anti- and pro- apoptotic isoforms as a result of alternative pre-mRNA splicing [27]. Tissue-restricted alternative splicing events frequently alter regions of proteins that are phosphorylated thus altering the range of targets for specific kinases in each tissue [26, 28], as well as regions that specify tissue-specific protein-protein interaction networks [29, 30]. Regulated alternative splicing can also be coupled to changes in mRNA abundance as the inclusion or exclusion of some alternative exons results in a coding frame-shift and the introduction of a premature termination codon which then targets the mRNA for degradation by the nonsense-mediated mRNA decay pathway [31].

With few exceptions, all mRNAs receive a polyadenosine tract (polyA tail) at the 3' end [32]. The addition of the polyA tail is functionally linked to transcription termination and involves two tightly coupled steps [33]. In the first step of 3' end formation, pre-mRNA is endonucleolytically cleaved to expose a free 3' hydroxyl that will be the substrate for the second step, the non-templated addition of a polyA tail. The majority of mammalian genes yield alternative mRNA variants that can be cleaved and polyadenylated at one of multiple

positions [34]. The most common alternative polyA site variants arise from selection of one of multiple polyA sites that are present in tandem in the same exon (Fig. 6.2). In these alternative mRNAs, selection of a proximal or distal site for polyA tail addition does not alter protein coding sequences, but does alter 3'UTR length. Thus, alternative polyadenylation has the potential to switch mRNAs from one cytoplasmic fate to another, by altering the repertoire of 3'UTR *cis* regulatory sequences associated with post-transcriptional mRNA control including regulation by small RNAs (miRNAs and possibly piRNAs [35]) or RBPs that control mRNA localization, translation, and decay (Fig. 6.2) [36–39]. Alternatively polyadenylated mRNAs can also arise from differential use of polyA sites located in different 3' terminal exons. This form of alternative polyadenylation is coupled to changes in exon splicing of the pre-mRNA and results in mRNAs that have distinct 3'UTRs and code for proteins with different C-termini. Thus alternative polyadenylation coupled to alternative splicing can yield alternative mRNAs from the same gene yet code for different protein isoforms and subject to different post-transcriptional controls.

Similar to splice variants, most alternatively polyadenylated mRNAs are expressed in a developmentally regulated or tissue-specific manner [4, 24, 40, 41]. In addition, tissue-specific biases in alternative polyadenylation have been identified. For example, mRNAs with long 3'UTRs (selection of distal polyA sites) are most abundant in neural tissues, while mRNAs with short 3'UTRs (selection of proximal polyA sites) are prevalent in testis. Important roles for alternative polyadenylation have been identified during T-cell stimulation [37], neuronal signaling [42], and in proliferation of tumor cell lines in culture [36]. In general, proliferating and undifferentiated cells tend to express mRNAs with short 3'UTRs while non-proliferating and/or differentiating cells (neurons and 'resting' T-cells, for example) generate mRNAs with long 3'UTRs [37, 43, 44]. Interestingly, 3'UTRs of a large number of germ cell mRNAs switch from long to short as cells progress through spermatogenesis [45–47], with the selection of proximal polyA sites being a common feature of mRNAs expressed in round spermatids [48, 49]. It is not known whether accelerated decay of long 3'UTR mRNAs contributes to differences in the relative levels of long and short 3'UTR variants in different stages of spermatogenesis.

3.2 Functional Consequences of Alternative Processing of Germ Cell mRNAs

Compared to other tissues, the testis expresses higher numbers of alternatively spliced mRNAs including testis-specific mRNA variants, and mRNAs that exhibit stage-specific patterns of alternative splicing [23, 24, 50–52]. In addition to extensive stage-specific alternative splicing, changes in 3'UTRs caused by alternative polyadenylation are prevalent during spermatogenesis [45, 46].

The number of alternatively spliced mRNAs expressed in a given tissue generally correlate with the number of genes expressed (including those encoding splicing factors), suggesting that higher numbers of alternatively spliced mRNA variants result from increased combinations of splicing regulatory proteins [53]. In mice and humans, more genes are expressed in the testis (~84 % of RefSeq genes) than any other tissue [4]. Strikingly, the majority of RNA present in whole testis preparations is contributed by two germ cell types:

pachytene spermatocytes (germ cells in meiotic prophase I where chromatin condensation and homologous recombination occurs) and round spermatids (the haploid products of meiosis). In these cells, a more open chromatin state facilitates promiscuous transcription of the genome including protein-coding and non-coding genes and intergenic elements (SINEs, LINEs, and LTRs) [54]. Collectively, these observations raise questions regarding the biologic importance of the expression of high numbers of alternatively spliced germ cell mRNAs and stage-specific changes in alternative splicing and polyadenylation during spermatogenesis. Nevertheless, specific examples of functional differences in alternatively processed germ cell mRNAs have been described.

3.2.1 LIG3, SOX17, and CREM—Representative examples of genes that yield alternative mRNAs that encode functionally distinct protein isoforms in mouse germ cells include LIG3, SOX17, and CREM. LIG3 encodes two isoforms (α and β) of DNA ligase III through utilization of distinct 3' terminal exons. Both isoforms are highly expressed in testis, with DNA ligase III β mRNA being the predominant species. In somatic cells, both isoforms are expressed at low levels and DNA ligase III α mRNA is the predominant species. The α and β mRNAs yield polypeptides with different C-termini, and while both proteins are active as DNA joining enzymes, the β form (unlike the α form) is unable to interact with the DNA repair protein XRCC1, suggesting distinct cellular functions for the α and β isoforms of DNA ligase III [55, 56].

The SOX17 gene encodes a transcription factor bearing a high mobility group (HMG) box region in its N-terminus. In mouse testis, Sox17 is present in spermatogonia and has decreased expression in pachytene spermatocytes. Reduced Sox17 levels in pachytene cells is accompanied by increased expression of t-Sox17, an alternatively spliced variant that lacks the exon that codes for the majority of the HMG box region. As a result, t-Sox17 mRNA encodes a truncated protein which lacks an intact HMG box region and is unable to bind DNA or stimulate transcription of a luciferase reporter gene in co-transfection experiments [57].

Although the analysis of LIG3 and SOX17 mRNAs expressed in germ cells highlight the ability of alternative pre-mRNA processing to generate biochemically distinct polypeptides, the functional importance of these alternative isoforms (as with most germ cell alternative mRNAs that have been identified) has not been investigated *in vivo*. Transgenic mouse models that disrupt the balance of specific alternative mRNA variants or delay the expression of stage-specific isoforms are needed to determine whether specific changes in alternative mRNA isoforms are functionally important for mammalian germ cell development.

Studies of the transcription factor CREM (cAMP-responsive element modulator) highlight one of the best characterized examples of the importance of alternative pre-mRNA processing in spermatogenesis. Through selection of alternative promoters, alternative exon splicing, and alternative polyadenylation, the CREM gene can give rise to multiple mRNA and protein variants. In pre-meiotic germ cells and early prophase spermatocytes, the predominant CREM isoforms expressed (β and γ) are capable of binding CRE sequences of target genes but lack the glutamine-rich domains important for transactivation and thus

function as suppressors of cAMP-induced transcription [58]. In pachytene spermatocytes, a switch in the pattern of CREM pre-mRNA splicing results in the production of CREM τ , which differs from β by the presence of two inserted glutamine-rich amino acid regions that confer transactivation function to CREM τ [59]. In addition to the conversion of CREM from suppressor to activator by alternative splicing, an alternative polyadenylation switch from distal to proximal polyA site use in the CREM τ 3'UTR eliminates multiple mRNA-destabilizing elements and is associated with a robust increase in CREM τ mRNA levels in spermatids [60]. Thus, multiple changes in CREM pre-mRNA processing modulate protein function and mRNA abundance. Importantly, in mice homozygous for a null allele of CREM, spermatids fail to differentiate and there is an increase in germ cell apoptosis, suggesting that CREM τ is functionally important in transcriptional activation of genes in postmeiotic germ cells [61, 62].

3.2.2 Different Fates for Alternatively Polyadenylated Germ Cell mRNAs—In

addition to CREM τ , several genes have been identified that yield alternative polyA variants in mouse testis with a bias towards selection of proximal sites (mRNAs with shorter 3'UTRs) in later stages of germ cell development [45, 62, 63]. The functional significance of 3'UTR shortening of large numbers of germ cell mRNAs is not understood.

Representative examples of genes that yield alternative 3'UTR variants with different cytoplasmic fates include RNF4 and DAZAP1. RNF4 encodes the small nuclear ring finger protein 4, a ubiquitin E3 ligase [64]. In spermatocytes and spermatids, two alternative RNF4 mRNA variants (of ~1.6 and 3.0 kb) are generated due to alternative use of different polyA sites present in the same 3' terminal exon. Both the long and short 3'UTR mRNA isoforms of RNF4 are present at comparable levels in spermatocytes, while there is a significant increase in the abundance of the shorter ~1.6 kb RNF4 mRNA variant in spermatids [65]. Northern blot analysis of RNF4 mRNAs following sucrose density gradient centrifugation and fractionation of adult testis (a widely used method to assess the translational status of specific mRNAs) demonstrated that the long 3'UTR RNF4 isoform is polysome-associated while the short 3'UTR isoform is predominantly in the non-translating mRNP fraction. Thus, alternatively polyadenylated variants of RNF4 mRNA exhibit differences in ribosome association.

Differences in polysome-association for long and short 3'UTR variants have also been described for DAZAP1 mRNAs. DAZAP1 (DAZ associated protein 1) encodes a ubiquitously expressed RBP that is implicated in transcription, RNA splicing, and translation [66]. Two DAZAP1 mRNA isoforms generated by alternative polyadenylation at sites within the same 3' terminal exon are present at comparable levels throughout testis development [67]. Both DAZAP1 mRNAs exhibit similar levels of polysome-association in early stages of postnatal testis development. However, as testis development proceeds in prepubertal mice and postmeiotic germ cells appear and gradually comprise a larger proportion of the total germ cell population, the short 3'UTR isoform is progressively reduced in the polysome fractions and localizes predominantly in the non-translating mRNP fractions. In contrast, the long 3'UTR DAZAP1 mRNA exhibits a modest decrease in polysome association with testis development, however the majority of the long 3'UTR isoform remains polysome-associated in adult testis. Thus, alternatively polyadenylated variants of

DAZAP1 mRNA exhibit differences in ribosome-association in different stages of spermatogenesis.

Studies of alternative polyadenylation in T-cells and cancer cell lines in culture have posited that 3'UTR shortening due to selection of proximal alternative polyA sites functions as a mechanism to allow mRNAs to escape negative regulation imposed by elements present in long 3'UTRs [36]. In the examples presented here, the short 3'UTR variant of mRNAs derived from the RNF4 and DAZAP1 genes exhibited low levels of polysome-association in later stages of spermatogenesis. These observations indicate that 3'UTR shortening may not be obligatorily coupled to increased translation in all mammalian cell types. Multiple scenarios could account for the translational differences observed in these alternatively polyadenylated variants. One possibility is that reduced translation of the short 3'UTR isoforms in late stages of spermatogenesis results from the absence of specific 'translation-promoting' sequences that are present only in the long 3'UTR variants and may counteract 'translation-repressing' sequences present upstream of the proximal polyA site. The observation that the DAZAP1 short 3'UTR mRNA is competent for efficient translation in earlier stages of spermatogenesis, suggests the involvement of stage-specific cofactors that determine if an mRNA is translationally active or repressed. Transgenic mouse models have shown that mRNAs expressed in spermatids can undergo sequence-independent assembly into translationally-repressed mRNP particles [68]. In addition, selection of proximal polyA sites is a common feature of mRNAs expressed in spermatids [45]. Thus, differences in the translation or repression of alternative polyA variants could be due, in part, to differences in the timing of their synthesis whereby widespread repression of the majority of mRNA is coincident with the synthesis of mRNAs with short 3'UTRs due to selection of proximal sites for 3' end cleavage and polyadenylation. Insights into the molecular mechanisms and functional consequences of alternative polyadenylation during germ cell development awaits comprehensive measurements of the timing and dynamics of the synthesis of alternative mRNAs, their movement into and out of the translating and non-translating fractions, and their decay.

3.3 Translational Control: Global and Message-Specific

A third widespread form of post-transcriptional gene control in developing germ cells is the regulation of mRNA translation. This includes repression of the majority of germ cell mRNAs and translational activation of select mRNAs at specific stages of development. While the extent of repression varies between individual mRNA species, germ cell mRNAs generally show lower levels of polysome association compared to mRNAs expressed in somatic cells [69]. Nearly two-thirds of the total polyadenylated RNA present in isolated spermatocytes [70], spermatids [71], and in whole testis [72] is present in non-polysomal mRNP fractions, indicating that a significant portion of germ cell mRNAs are not involved in protein synthesis. Accordingly, a survey of eight tissues showed that the testis exhibits the lowest correlation between proteome and transcriptome, consistent with widespread translational repression of the majority of germ cell transcripts [73]. Global repression of mRNA translation is hypothesized to protect against over-production of proteins due to 'leaky or promiscuous' expression of large numbers of genes and higher mRNA levels compared to somatic cells [5, 54].

Regulation of mRNA translation has important roles throughout mammalian gametogenesis, from early steps of germ cell development during embryogenesis to release of spermatozoa into the lumen of seminiferous tubules in adults. For example, as germ cells transition from gonocytes to spermatogonia in the neonatal testis, ~50 genes show expression level changes while ~3000 mRNAs exhibit at least a twofold increase in translation efficiency [74, 75]. Thus, increased translation of a large cohort of mRNAs, rather than expansive changes in transcribed genes, remodels the germ cell proteome in the neonatal testis.

Stage-specific changes in mRNA translation have been described in multiple stages of spermatogenesis, but have been most intensively studied during spermatid differentiation [76, 77], where the assembly of mRNAs into translationally-repressed mRNPs and stage-specific release of specific mRNAs from this repression is essential. During spermatid elongation, chromatin compaction results in the cessation of transcription, thus translation of new polypeptides necessary for the completion of spermatogenesis is dependent on mRNAs synthesized days earlier and stored in mRNPs [3, 78–80]. Examples of well-studied transcripts whose synthesis and translation are temporally disconnected include mRNAs encoding transition proteins (Tnp1 and Tnp2), protamine proteins (Prm1 and Prm2), and the sperm mitochondrial associated cysteine-rich protein (Smcp), all of which are encoded by genes that are essential for proper germ cell development in mice [81–85].

Prm1 and Tnp2 mRNAs are first detected in round spermatids but are not translated until several days later in elongating spermatids [69, 86–88]. Interestingly, these mRNAs only exhibit a partial release from translational repression (for example, less than half of the total Prm1 and Prm2 mRNAs become polysome-associated in elongating spermatids). The importance of UTR elements in temporal and stage-specific control of mRNA repression and translation was demonstrated in mice containing Prm1 or Tnp2 transgenes in which their respective UTRs were replaced, resulting in premature translation of Prm1 and Tnp2 mRNAs and germ cell developmental abnormalities [90, 91].

Transgenic mouse models have also provided key insights into potential regulatory mechanisms of mRNP assembly and stage-specific release of mRNAs from translational repression. To better understand mRNA assembly into and release from mRNPs in spermatids, a series of transgenic mice have been generated that contain a reporter gene (GFP or hGh) with various 5' and/or 3' UTRs, including those from translationally controlled mRNAs. Analyses of these transgenes revealed that mRNAs expressed in spermatids can undergo sequence-independent assembly into translationally-repressed mRNPs [68], and that specific sequences in the UTRs of Scmp and Prm1 participate in controlling the timing of mRNA release from mRNP particles and association with ribosomes [92–95]. Studies by Braun and colleagues identified two sequences in the Prm1 3'UTR that can delay translation of a reporter mRNA, including a translational control element (TCE) and a Y-box recognition sequence (YRS, UCCAUCA) that is recognized by Y-box proteins (discussed below) [94–97]. Interestingly, both the TCE and the YRS must be in close proximity to the polyA tail to function, however the molecular basis remains unknown.

Studies by Kleene and colleagues revealed a role for uORFs in translational control during spermiogenesis while demonstrating that both the 5' and 3' UTRs of Smcp are required to recapitulate the strength and duration of translational control observed with endogenous Smcp mRNAs [92, 93]. Additional evidence that 5'UTRs can influence translation of germ cell transcripts in spermatids comes from the analysis of alternative mRNAs derived from the AKAP4, TBP, and SOD1 genes. Selection of different sites of transcription initiation generates multiple alternative mRNA variants in germ cells from each of these genes. Interestingly, alternative transcription site use does not alter AKAP4, TBP, or SOD1 protein coding sequences, but does generate mRNAs that differ in their 5'UTRs and in translation efficiency [98–100]. These examples highlight an important role for alternative transcription start site selection in regulation of protein abundance.

3.4 Post-transcriptional Control Through PolyA Tail Length Regulation

The polyA tail present at the 3' end of nearly all mRNAs has important roles in several steps in the mRNA lifecycle including nuclear export, translational control, and mRNA stability [101, 102]. Many factors that impact post-transcriptional regulation of gene expression do so by directly or indirectly modifying polyA tail length. Deadenylation (polyA tail shortening) is the first step in the degradation of the majority of mRNAs [103]. Multiple post-transcriptional regulatory factors control their mRNA targets by affecting the recruitment and activity of deadenylases to specific mRNAs.

The importance of cytoplasmic polyA tail lengthening in mouse gametogenesis is illustrated by spermatogenic defects in knockout mice lacking a cytoplasmic polyA polymerase [104]. In addition, the cytoplasmic polyadenylation element binding protein (CPEB) important for cytoplasmic polyadenylation of specific mRNAs is essential in mouse germ cells. (discussed below, [105]). For some mRNAs, polyA tail lengthening in the cytoplasm occurs in response to specific cellular cues and is associated with increased mRNA translation. Translational activation resulting from polyA tail lengthening is believed to be due to stabilization of a circular 'closed loop' mRNA structure via interactions between polyA-binding protein (PABP) bound to the polyA tail and the eIF4E translation initiation complex bound to mRNA 5' end 'cap' [101]. A requirement for 5'UTR and 3'UTR sequences in temporal control of translation in differentiating spermatids (discussed above) is consistent with a closed loop model of translational control of some germ cell mRNAs. Furthermore, mouse models have indicated that stage-specific modulation of PABP levels has an important role in the temporal control of translation and is critical for proper germ cell development [106].

A striking observation from northern blot analysis of translationally regulated mRNAs in spermatids is a difference in the electrophoretic mobility of specific mRNA species in ribosome-free mRNP and polysome-associated sucrose gradient fractions from mouse testis [86, 88, 107]. In the mRNP fractions, mRNAs encoding Prm1 or Tnp1 for example, appear as homogenous transcripts. However, the corresponding transcripts in the polysome fractions migrate as heterogeneous species or 'RNA smear' that results from polyA tail shortening. It remains unclear as to why translation of some mRNAs is accompanied by polyA tail shortening. Shortening does not appear to be absolutely required however, as full length mRNAs (comparable in size to those in the mRNP fractions) do appear in the polysome

fractions in addition to the isoforms with shorter polyA tails. It is not known whether partial deadenylation promotes release from translational repression, or if deadenylation occurs while mRNAs are being translated to act as a translational 'timer' whereby the polyA tail is progressively shortened until a critical length is reached that can no longer support interactions between the 5' and 3' ends and the mRNA.

4 Roles of RNA-binding Proteins in Germ Cell mRNA Regulation

The molecular mechanisms that control alternative mRNA expression and temporal control of translation during germ cell development remain poorly understood. Each step in the lifecycle of an mRNA is dependent on the combination and positions of bound RBPs. In the nucleus, co-transcriptional loading of specific RBPs onto nascent pre-mRNA can alter alternative splicing and alternative polyadenylation. In some cases, RBPs can function as either positive or negative-acting factors depending on their position relative to an alternative exon or polyA site [108]. RBPs also control downstream post-transcriptional events such as regulation of mRNA stability, localization, or translation. Thus, RBPs can regulate and integrate multiple layers of gene regulation to control which protein variants are made and modulate the timing, location, and dosage of mRNA translation. Accordingly, variations in the RBPs expressed in each cell type underlie tissue-specific differences in post-transcriptional gene regulation.

Additional modulation of post-transcriptional fate is achieved through changes in the levels and/or activity of core RNA regulatory factors as well as auxiliary factors that do not directly bind RNA [108]. Core factors include those that are broadly expressed and have central roles in mRNA regulation. For example, members of the SR and hnRNP families of RBPs are widely expressed and generally function in activation or repression respectively, of exon splicing. Auxiliary factors modulate the activity of core factors through either direct binding to specific mRNAs (for example, miRNAs and tissue-specific RBPs) or indirectly through post-translational modifications of regulatory factors. An example of the latter includes members of the CLK/STY family of protein kinases that can phosphorylate SR proteins resulting in alterations in their localization and splicing activity [109–111]. Coincidentally, all four genes that encode CLK/STY kinases yield alternative mRNAs that are expressed in different tissue-specific combinations, with high levels of expression in the testis [112, 113]. It is not known whether differential expression of CLK/STY kinases contributes to stage specific changes in alternative splicing during spermatogenesis.

The conversion of an mRNA from a translationally-silent to active state is poorly understood, but likely to involve the selective removal and/or addition of specific RBPs [114, 115]. In male germ cells, remodeling of mRNPs is thought to have important roles in regulating the timing of mRNA stabilization, translation, and decay. RNA helicases are thought to reshape protein-RNA complexes—removing some RBPs and allowing others to bind [116]. Many RNA helicases localize to chromatoid bodies, dynamic spermatid-specific structures suggested to function as RNA processing centers involved in post-transcriptional RNA regulation [115, 117, 118].

Below, representative examples of RBPs believed to function as major contributors to post-transcriptional gene regulatory programs at specific stages of male gametogenesis are described. This includes factors that have either been shown to be essential for specific stages of germ cell development as well as RBPs that are suspected to have multiple key roles in post-transcriptional control of germ cell mRNAs.

4.1 Elavl1/HuR

The embryonic lethal abnormal vision 1 protein (Elavl1, also known as HuR) is one of four related proteins of the Elavl/Hu family that have multiple roles in post-transcriptional regulation, including mRNA processing, export, translation, and decay [119]. Elavl1/HuR is broadly expressed, while the other members of the family (Elavl2/HuB, Elavl3/HuC, and Elavl3/HuD) are predominantly expressed in the nervous system. Elavl proteins bind their mRNA targets via interaction with U-rich sequences. In mouse brain and in mammalian cell culture, the Elavl RBPs binds thousands of transcripts via interactions in introns and 3'UTRs and regulate alternative mRNA splicing and mRNA stability [120–122]. In mouse spermatogenic cells, Elavl1/HuR is expressed in pachytene spermatocytes and round spermatids. Conditional inactivation of Elavl1 expression in germ cells results in male sterility due to defects in the completion of meiosis and failure of round spermatids to differentiate into elongated spermatids [123]. As a result, spermatozoa are absent in the epididymides. The direct role(s) of Elavl1 in mouse spermatogenic cells remain to be determined, however it is probable that Elavl1 is involved in multiple post-transcriptional regulatory events in germ cells since many regulatory roles have been attributed to Elavl proteins in different cellular contexts. To date, the functional consequence of Elavl1 deletion has been investigated on a single mRNA, HSP2A, that encodes a heat shock protein whose deletion results in a phenotype similar to Elavl1-null germ cells. Elavl1 binds HSP2A mRNA and loss of Elavl1 results in reduced levels of HSP2A mRNA on polysomes [123]. The molecular mechanism by which Elavl1 promotes HSP2A translation is not known.

4.2 CELF (CUGBP, ELAV-Like Family) Proteins

The CELF family of RBPs (CELF1-6; related to the ELAV family of RBPs) are multifunctional with roles in alternative splicing, mRNA translation, and mRNA deadenylation [124]. CELF proteins bind GU-rich elements (GREs). The roles of CELF proteins are dependent on the position of GRE-CELF interactions within mRNA targets, as has been demonstrated with other RBPs. Binding in the 5'UTR and 3'UTR has been linked to roles in mRNA translation and stability respectively, while binding within intronic sequences flanking alternative exons is associated with regulation of exon splicing [124, 125]. In mammals, CELF proteins have been shown to have important roles in regulation of developmentally regulated tissue- specific alternative mRNA splicing. In addition, CELF proteins have an evolutionarily conserved role in promoting mRNA decay through interactions with GREs in mRNA 3'UTRs [126]. In the mouse testis, CELF1 is expressed in both somatic cells and germ cells. In mixed background CELF1-null mice, a range of spermatogenic cell defects are observed including increased germ cell apoptosis and the absence of elongated spermatids [127]. While the direct roles of CELF proteins in post-transcriptional control in germ cells are not known, the ability of CELF proteins to regulate multiple steps in mRNA metabolism suggest that CELF may also be multifunctional in

mouse germ cells and coordinate pre-mRNA processing and cytoplasmic control of specific mRNAs.

4.3 Sam68

Sam68 is a member of the STAR (signal transduction and activation of RNA) family of RBPs that link signal transduction pathways to post-transcriptional regulation of mRNA [128]. In response to activation of signaling pathways, Sam68 can be phosphorylated resulting in a change in Sam68 subcellular localization and/or its activity on its mRNA targets. For example, depolarization of neurons results in the activation of the calcium/calmodulin dependent kinase IV (which has an important role in activity-dependent alternative mRNA splicing of many neuronal pre-mRNAs) as a result of the phosphorylation of Sam68 [129]. Among the mRNAs whose splicing is altered in an activity-dependent manner include those that encode the synaptic receptors Neurexin-1, -2, and -3 (encoded by three separate genes yet capable of yielding ~1000 mRNA isoforms due to extensive alternative splicing) [130]. Sam68 regulates Neurexin pre-mRNA splicing *in vitro*, while the absence of Sam68 *in vivo* results in the failure of neurexin pre-mRNAs to be spliced in response to neuronal activation [129]. Collectively, these observations indicate that Sam68 is an important mediator of activity-dependent changes in mRNA processing in the brain.

In the testis, Sam68 is expressed in spermatogenic cells and Sertoli cells [131]. Interestingly, Sam68 localization within germ cells differs in different cell types with nuclear expression in spermatogonia, pachytene spermatocytes and round spermatids, and cytoplasmic localization during meiotic divisions where it associates with polysomes [132]. Translocation of Sam68 to the cytoplasm coincides with its phosphorylation. Sam68 knockout mice are infertile and exhibit a range of spermatogenic defects including high numbers of apoptotic cells, aberrant nuclear divisions, and misshapen and immotile spermatozoa [133]. Multiple defects in mRNA regulation have been identified in Sam68 null testis. This includes up-regulation and down-regulation of ~100 and ~300 genes respectively, decreased polysome-association of specific mRNAs [133], and aberrant alternative mRNA splicing [134]. Thus, Sam68 is a multifunctional RBP in male germ cell development. Interestingly, a second member of the STAR family of RBPs, T-STAR (also called SLM2), is highly expressed in testis yet is dispensable for spermatogenesis. The overlapping expression of Sam68 and T-STAR in mouse germ cells suggests that Sam68 may functionally compensate for the loss of T-STAR, however T-STAR is not able to compensate for the loss of Sam68 in spermatogenic cells [131].

4.4 PTB (Polypyrimidine Tract Binding) Family of RBPs

The PTB family of RBPs includes Ptbp1 (more commonly known as PTB), Ptbp2 (also called brain or neuronal PTB, brPTB and nPTB respectively), and Ptbp3 (also called ROD1). Studies in several model systems (from *in vitro* assays to mammalian tissue) have identified multiple roles for Ptbp1 in mRNA regulation, including control of mRNA splicing, mRNA stability, and localization (for review, see [135]). While the role of PTB proteins in mammalian germ cell development have not been described, multiple lines of evidence suggest that they are likely to have important roles in post-transcriptional control of germ cell mRNAs.

In the mouse testis, Ptbp1 expression is restricted to spermatogonia while Ptbp2 is expressed in spermatocytes and spermatids [136]. The reciprocal expression of Ptbp1 and Ptbp2 in different phases of spermatogenesis suggests that Ptbp1 and Ptbp2 have distinct roles in different stages of germ cell development. Recent analyses of sequences associated with alternative exons that are differentially spliced in 6- and 21-day old testis (where the most advanced germ cells are spermatogonia and spermatids, respectively) revealed an enrichment of motifs that match binding sites for PTB proteins, suggesting that one or both of the PTB proteins expressed in different stages of spermatogenesis may have important roles in temporal control of germ cell mRNA splicing [137]. In the embryonic brain Ptbp2 functions predominantly as a silencer of alternative exon splicing through interactions upstream of and/or within alternative exons [138]. Furthermore, exons that are repressed by Ptbp2 in the embryonic brain correspond to exons that are activated (spliced) in adult brain. Whether Ptbp2 has a similar role in temporal control of alternative exons during germ cell development remains to be determined.

A splicing-independent role for PTB proteins in germ cell post-transcriptional control has also been proposed. In one report, incubation of *in vitro* synthesized RNA probes in mouse testis lysate identified Ptbp2 binding to a specific region of the 3'UTR of Pgc2 mRNA, an mRNA that is first detected in meiotic cells whereas PGK2 protein is first detected in post-meiotic cells [136]. In HeLa cells and *in vitro*, Ptbp2 was able to increase PGK2 mRNA half-life suggesting a role for Ptbp2 in stabilization of this mRNA during spermatogenesis. mRNA stabilization is an important component of temporal mRNA control, ensuring that mRNAs are available as templates for translation in mid- to late-spermiogenesis [78–80]. Whether Ptbp2 regulates the stability of Pgc2 and other mRNAs in germ cells is not known and will require comparative analysis of mRNA steady state levels in wild type and Ptbp2-null testes.

4.5 τ -Cstf64

τ -Cstf64 (encoded by CSTF2T) is a retrotransposed paralog of the CSTF2 gene that encodes the 64 kilodalton subunit of the CSTF (cleavage stimulatory factor) complex required for 3' end cleavage and polyadenylation. Due to the location of CSTF2 on the X chromosome, meiotic sex chromosome inactivation results in a loss of Cstf64 expression in pachytene spermatocytes. τ -Cstf64 is expressed in pachytene spermatocytes (where it is proposed to functionally compensate for the loss of Cstf64) and continues through the early spermatid stages [139]. Interestingly, expression of τ -Cstf64 coincides with increased selection of proximal alternative polyadenylation sites, which generally contain a non-canonical polyA signal [43, 45]. Together, these observations led to the hypothesis that selection of proximal polyA sites in germ cells results from differences in the relative levels and activity of Cst64 and τ -Cstf64. However, recent analyses indicate that Cst64 and τ -Cstf64 have highly similar RNA-binding specificities and overlapping functionality [140–142], indicating that other factors and regulatory mechanisms may contribute to changes in the alternative polyA site selection during spermatogenesis. For example, inactivation of the gene encoding BRDT, a member of the BET (bromodomain and extra terminal motif) family of chromatin-interacting regulators of transcription, results in reduced accumulation of mRNAs processed at proximal polyA sites, indicating that alternative polyA site selection in developing germ

cells may be coupled to transcriptional activity and/or chromatin state [143]. Nonetheless, τ -Cstf64 expression is necessary for proper germ cell development as several spermatogenic defects are observed in CSTF2T-null mice [144, 145].

4.6 Y-Box Proteins

The Y-box proteins are functionally conserved DNA and RNA-binding proteins with important roles in binding and regulating mRNAs in germ cells [146, 147, 148]. Three separate Y-box genes are present in mice: YBX1 (also called MSY1), YBX2 (MSY2), and YBX3 (MSY4) which yields two protein isoforms (long and short, YBX3L and YBX3S respectively) that are expressed at comparable levels in mouse testis and are derived from alternative exon splicing. In mouse testis, Y-box proteins are found in association with translationally repressed mRNP fractions [146, 149]. Approximately 75 % of testis polyadenylated RNA is complexed with YBX2 and YBX3. YBX2 and YBX3 are expressed in meiotic and post-meiotic cells and are essential for spermatogenesis [146–148]. Interestingly, transgenic mice engineered to prolong YBX3 expression beyond the round spermatid stage interfered with translational activation of temporal controlled mRNAs bearing a 3'UTR Y-box recognition sequence [146]. Collectively, these studies indicate that Y-box proteins mediate the storage and masking of mRNAs during mouse spermatogenesis.

4.7 CPEB (Cytoplasmic Polyadenylation Element Binding Protein)

The cytoplasmic polyadenylation element (CPE) is a U-rich 3'UTR sequence (usually $U_{4-5}A_{1-2}U$) that has important roles in translational regulation. The CPE is recognized by CPE-binding protein (CPEB), which has dual roles in translation by acting as a central component of regulatory pathways that promote or repress mRNA translation. In response to phosphorylation, CPEB bound to a CPE that is close to a polyA signal element (usually AAUAAA located 10–30 bases upstream of the site of cleavage) can promote the formation of an active cytoplasmic polyadenylation complex, resulting in polyA tail lengthening and activation of translation [150]. CPEB bound to a CPE can repress translation through interactions with factors that prevent translation initiation or recruit components of the deadenylation and mRNA decay machinery [101, 151]. The specific action of CPEB on a given mRNA (as activator or repressor) as well as the magnitude of translational regulation is dictated by the combination and positions of CPEs as well as binding elements for other RBPs in the 3'UTR. For example, the presence of a PBE (UGUANAUA, the binding site for the Pumilio family of RBPs, discussed below) can enhance CPEB-mediated translational activation with additional PBEs exerting a positive effect [152]. Combinatorial regulation of CPE-containing mRNAs allows for coordinated control of networks of co-regulated mRNAs and ensures that not all CPE-containing mRNAs are repressed and/or translated at the same time. Additional regulatory complexity can be achieved by recruitment of CPEB to mRNAs that lack detectable CPEs via interactions with other RBPs bound to their cognate binding sites in a 3'UTR [153].

The importance of CPEB in mouse germ cells is demonstrated by spermatogenic arrest in CPEB1-null mice. In the absence of CPEB1 (one of four mouse CPEB genes), male germ cells contain fragmented and dispersed chromatin, consistent with defects in chromosome synapsis and recombination [105]. Importantly, the SYCP1 and SYCP3 mRNAs encoding

components of the synaptonemal complex required for pairing of sister chromatids, contain CPEs in their 3'UTRs. In mouse oocytes, SYCP1 and SYCP3 mRNA levels are unaffected by the loss of CPEB1, however their polyA tail lengths are reduced and the corresponding proteins absent [105]. These observations suggest a direct role for CPEB1 in the regulation of polyA tail length and production of synaptonemal complex proteins in mouse spermatogenesis. Interestingly, mouse CPEB paralogs display overlapping yet distinct patterns of expression during spermatogenesis [105, 154] raising the possibility that additional complexity of CPEB-mediated control via specific CPEB isoforms.

4.8 Pumilio and Nanos

The Pumilio (PUM) proteins are members of the PUF (Pumilio and FBF) family of RBPs that are structurally and functionally conserved from yeast to mammals and plants [155, 156]. PUM proteins function as critical post-transcriptional regulators of cell fate and developmental programs of gene expression. PUM proteins regulate their mRNA targets through interactions with one or more PUM binding elements (PBEs, with the consensus sequence UGUANAUA) present in the 3'UTR. While positive roles for PUM proteins have been described in CPEB-mediated translation regulation (see above), most analyses of PUM proteins have focused on their roles in translational repression and de-stabilization of specific mRNAs.

The mouse and human genomes each contain genes for two PUM proteins (Pum1 and Pum2) with widespread and overlapping expression in different tissues [155]. Pum2 appears to be dispensable for male germ cell development in mice, as fertility is unaffected in animals that are homozygous for a genetrap mutation that abrogates Pum2 expression [157]. In contrast, Pum1 knockout mice exhibit spermatogenic defects including reduced numbers of spermatozoa and increased numbers of apoptotic spermatocytes [158]. Analysis of RNAs that co-precipitate with Pum1 from adult testis provided insights into the molecular basis of the Pum1-null testis phenotype. Among the 1527 genes whose mRNA products are bound by Pum1, is an enrichment of genes encoding regulators of the cell cycle and p53. In the absence of Pum1, mRNAs encoding eight activators of p53 are upregulated resulting in activation of p53. Thus, Pum1 appears to have a role in safeguarding spermatogenic cells from apoptotic programs.

mRNA regulation by PUM proteins involves direct and indirect interactions with components of mRNA degradation complexes. For example, physical interactions between PUF proteins and components of the deadenylation machinery have been described from yeast to human [158]. Deadenylation factors can also be indirectly recruited to mRNAs with PUM bound to a 3'UTR PBE through other PUM-interacting factors. For example, the Nanos proteins are evolutionarily conserved post-transcriptional regulatory factors that interact with and are dependent on PUM in order to be recruited to specific mRNAs. Biochemical purification of Nanos proteins from mouse testis identified several components of the CCR4-NOT deadenylation complex as co-purified proteins, indicating that Nanos proteins down-regulate their mRNA targets through recruitment of the mRNA deadenylation complex [159].

Gene knockouts and transgenic strains have demonstrated essential roles for two mouse *Nanos* genes (*Nanos2* and *Nanos3*) in mouse germ cell development [160]. In the absence of *Nanos3*, PGCs that have migrated to the genital ridge exhibit progressive reductions in number and eventually are all lost [161]. A similar failure of PGCs to proliferate and/or survive has also been described in mouse knockouts of the RBPs *TIAL1* and *DND1* [162, 163]. Germ cell loss also occurs in *Nano2*-deficient mice [161]. In spermatogonia, *Nanos2* has a critical role in regulating the balance between self-renewal and differentiation, as postnatal deletion of *Nanos2* results in depletion of undifferentiated spermatogonia, whereas *Nanos2* overexpression results in the accumulation of undifferentiated spermatogonia [164].

4.9 Dazl

Dazl (Deleted in Azoospermia [*Daz*]-Like) is the autosomal homolog of the primate-specific *Daz* gene (deleted in azoospermia) present on chromosome Y and deleted in a subset of men with spermatogenic failure (~5–10 %; ranging from complete) absence of germ cells to oligozoospermia) [165, 166]. *Daz*, *Dazl*, and *Boule* comprise the *Daz* family of proteins, all of which have a single RRM-type RNA-binding protein, have overlapping yet distinct expression patterns in male germ cells, and are required for germ cell development in a variety of model organisms [166]. Importantly, the human *Daz* gene can partially rescue defects in *Dazl*-null mice, indicating that these highly conserved proteins have considerable functional conservation [167]. In mice with a mixed genetic background (CD1 and C57BL/6J), *Dazl*-null testes exhibit reduced numbers of germ cells, most of which appear to arrest at early stages of spermatogonial proliferation and differentiation [168–170]. The few *Dazl*-null germ cells that do enter meiosis, arrest in an early (pre-leptotene) stage of meiosis I. In a pure C57BL/6J genetic background, *Dazl*-null germ cells exhibit earlier defects including impaired primordial germ cell development and migration [171].

Multiple lines of evidence (reviewed in [166]) support a role for *Dazl* as a positive regulator of mRNA translation via interactions with *Dazl* RNA-binding sites (short polyU-rich tracts interspersed by G nucleotides, or C to a lesser extent) in the 3'UTR of target mRNAs. Of the ~20 genes whose mRNA products either co-purify with or interact with *Dazl* in mouse testis lysate, few have been directly assayed for protein levels in *Dazl*-null germ cells. For two target genes (*Sycp3* and *Mvh*) immunofluorescence assays demonstrate reduced protein levels, although the magnitude of the reduction is variable between cells [172, 173]. Positive roles for *Dazl* in translation have also been observed using reporter mRNAs in oocytes and transfected cells [166]. Interestingly, in some cases identical reporter mRNA (containing a 3'UTR from a *Dazl* mRNA target) exhibits opposite responses to co-transfection of *Dazl*-expressing constructs in different cell lines. For example, *lacZ* reporter constructs bearing the *Mvh* 3'UTR show increased translation when co-transfected with *Dazl* expression vector in GC-1 cells, and reduced levels compared to no *Dazl* control cells in embryonic stem cells [174]. These findings indicate that *Dazl* may also have a negative role in translation and that cell context may be a determinant of *Dazl* function. Additional evidence of multiple roles for *Dazl* in post-transcriptional regulation is suggested by the presence of *Dazl* in both non-translating and polysomal fractions prepared from adult testis [175], the accumulation of *Dazl* in stress granules following heat stress of mouse testis, and a requirement of *Dazl* for such granules to form [176].

5 Conclusion

The importance of post-transcriptional control in mammalian germ cell development is evident from the large number of mouse models that have identified essential roles for specific RBPs at every stage of germ cell development, from the survival and proliferation of embryonic germ cells to the release of mature spermatozoa into the lumen of seminiferous tubules. In addition, transgenic mice have demonstrated the necessity of stage-specific post-transcriptional control, and revealed specific sequences in mRNAs that are essential for such regulation. Despite significant progress in identifying RBPs and post-transcriptional regulatory events that are essential for germ cell development, significant questions and challenges remain. What mRNAs are directly bound by specific RBPs and what are the functional consequences of such interactions? How are networks of co-regulated mRNAs controlled at specific stages of development? How are changes in alternative mRNA isoform expression regulated, and what are the functional consequences of such regulation? While cell culture and *in vitro* assays can provide some information, the inability of such approaches to recapitulate regulatory events or accurately predict roles of specific RBPs *in vivo* (as described by Kleene [6]) limits their utility. Continued use of transgenic and knockout models in combination with cellular and biochemical enrichment tools and high throughput sequencing methodologies (including CLIP, RNA-Seq, and ribosome profiling, for example) promises to provide new insights into mechanisms of stage-specific post-transcriptional regulation in a transcriptome-wide manner. The demonstration of essential roles for germ cell RBPs in an array of cellular processes including control of self renewal, proliferation, entry into meiosis and differentiation highlights mouse spermatogenesis as a powerful model system to investigate how post-transcriptional controls drive mammalian cell development *in vivo*.

References

1. Cooper TA, Wan L, Dreyfuss G. RNA and disease. *Cell*. 2009; 136:777–793. [PubMed: 19239895]
2. Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing. *Nature*. 2010; 463:457–463. [PubMed: 20110989]
3. Kleene KC. Patterns, mechanisms, and functions of translation regulation in mammalian spermatogenic cells. *Cytogenet Genome Res*. 2003; 103:217–224. [PubMed: 15051942]
4. Ramskold D, Wang ET, Burge CB, Sandberg R. An abundance of ubiquitously expressed genes revealed by tissue transcriptome sequence data. *PLoS Comput Biol*. 2009; 5:e1000598. [PubMed: 20011106]
5. Kleene KC. A possible meiotic function of the peculiar patterns of gene expression in mammalian spermatogenic cells. *Mech Dev*. 2001; 106:3–23. [PubMed: 11472831]
6. Kleene KC. Connecting cis-elements and trans-factors with mechanisms of developmental regulation of mRNA translation in meiotic and haploid mammalian spermatogenic cells. *Reproduction*. 2013; 146:R1–R19. [PubMed: 23579190]
7. Cooke HJ, Saunders PT. Mouse models of male infertility. *Nat Rev Genet*. 2002; 3:790–801. [PubMed: 12360237]
8. Jamsai D, O'Bryan MK. Mouse models in male fertility research. *Asian J Androl*. 2011; 13:139–151. [PubMed: 21057516]
9. Rossi P, Dolci S. Paracrine Mechanisms Involved in the Control of Early Stages of Mammalian Spermatogenesis. *Front Endocrinol (Lausanne)*. 2013; 4:181. [PubMed: 24324457]
10. Ginsburg M, Snow MH, McLaren A. Primordial germ cells in the mouse embryo during gastrulation. *Development*. 1990; 110:521–528. [PubMed: 2133553]

11. McLaren A. Mammalian germ cells: birth, sex, and immortality. *Cell Struct Funct.* 2001; 26:119–122. [PubMed: 11565803]
12. Nagano R, Tabata S, Nakanishi Y, Ohsako S, Kurohmaru M, Hayashi Y. Reproliferation and relocation of mouse male germ cells (gonocytes) during prespermatogenesis. *Anat Rec.* 2000; 258:210–220. [PubMed: 10645968]
13. Kopera IA, Bilinska B, Cheng CY, Mruk DD. Sertoli-germ cell junctions in the testis: a review of recent data. *Philos Trans R Soc Lond B Biol Sci.* 2010; 365:1593–1605. [PubMed: 20403872]
14. de Rooij DG, Griswold MD. Questions about spermatogonia posed and answered since 2000. *J Androl.* 2012; 33:1085–1095. [PubMed: 22879526]
15. Song HW, Wilkinson MF. Transcriptional control of spermatogonial maintenance and differentiation. *Semin Cell Dev Biol.* 2014; 30:14–26. [PubMed: 24560784]
16. Yoshida S. Spermatogenic stem cell system in the mouse testis. *Cold Spring Harb Symp Quant Biol.* 2008; 73:25–32. [PubMed: 19150967]
17. Youds JL, Boulton SJ. The choice in meiosis—defining the factors that influence crossover or non-crossover formation. *J Cell Sci.* 2011; 124:501–513. [PubMed: 21282472]
18. Gupta SK, Bhandari B. Acrosome reaction: relevance of zona pellucida glycoproteins. *Asian J Androl.* 2011; 13:97–105. [PubMed: 21042299]
19. Monesi V, Geremia R, D’Agostino A, Boitani C. Biochemistry of male germ cell differentiation in mammals: RNA synthesis in meiotic and postmeiotic cells. *Curr Top Dev Biol.* 1978; 12:11–36. [PubMed: 352625]
20. Kierszenbaum AL, Tres LL. Structural and transcriptional features of the mouse spermatid genome. *J Cell Biol.* 1975; 65:258–270. [PubMed: 1127016]
21. Upadhyay RD, Kumar AV, Ganeshan M, Balasinor NH. Tubulobulbar complex: cytoskeletal remodeling to release spermatozoa. *Reprod Biol Endocrinol.* 2012; 10:27. [PubMed: 22510523]
22. Gilbert W. Why genes in pieces? *Nature.* 1978; 271:501. [PubMed: 622185]
23. Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. *Nat Genet.* 2008; 40:1413–1415. [PubMed: 18978789]
24. Wang ET, Sandberg R, Luo S, et al. Alternative isoform regulation in human tissue transcriptomes. *Nature.* 2008; 456:470–476. [PubMed: 18978772]
25. Barbosa-Morais NL, Irimia M, Pan Q, et al. The evolutionary landscape of alternative splicing in vertebrate species. *Science.* 2012; 338:1587–1593. [PubMed: 23258890]
26. Merkin J, Russell C, Chen P, Burge CB. Evolutionary dynamics of gene and isoform regulation in Mammalian tissues. *Science.* 2012; 338:1593–1599. [PubMed: 23258891]
27. Miura K, Fujibuchi W, Unno M. Splice variants in apoptotic pathway. *Exp Oncol.* 2012; 34:212–217. [PubMed: 23070006]
28. Zhang C, Frias MA, Mele A, et al. Integrative modeling defines the Nova splicing- regulatory network and its combinatorial controls. *Science.* 2010; 329:439–443. [PubMed: 20558669]
29. Ellis JD, Barrios-Rodiles M, Colak R, et al. Tissue-specific alternative splicing remodels protein-protein interaction networks. *Mol Cell.* 2012; 46:884–892. [PubMed: 22749401]
30. Buljan M, Chalancon G, Eustermann S, et al. Tissue-specific splicing of disordered segments that embed binding motifs rewires protein interaction networks. *Mol Cell.* 2012; 46:871–883. [PubMed: 22749400]
31. McGlincy NJ, Smith CW. Alternative splicing resulting in nonsense-mediated mRNA decay: what is the meaning of nonsense? *Trends Biochem Sci.* 2008; 33:385–393. [PubMed: 18621535]
32. Proudfoot NJ. Ending the message: poly(A) signals then and now. *Genes Dev.* 2011; 25:1770–1782. [PubMed: 21896654]
33. Bentley DL. Coupling mRNA processing with transcription in time and space. *Nat Rev Genet.* 2014; 15:163–175. [PubMed: 24514444]
34. Shi Y. Alternative polyadenylation: new insights from global analyses. *RNA.* 2012; 18:2105–2117. [PubMed: 23097429]
35. Rouget C, Papin C, Boureux A, et al. Maternal mRNA deadenylation and decay by the piRNA pathway in the early *Drosophila* embryo. *Nature.* 2010; 467:1128–1132. [PubMed: 20953170]

36. Mayr C, Bartel DP. Widespread shortening of 3'UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. *Cell*. 2009; 138:673–684. [PubMed: 19703394]
37. Sandberg R, Neilson JR, Sarma A, Sharp PA, Burge CB. Proliferating cells express mRNAs with shortened 3' untranslated regions and fewer microRNA target sites. *Science*. 2008; 320:1643–1647. [PubMed: 18566288]
38. Ghosh T, Soni K, Scaria V, Halimani M, Bhattacharjee C, Pillai B. MicroRNA-mediated up-regulation of an alternatively polyadenylated variant of the mouse cytoplasmic {beta}-actin gene. *Nucleic Acids Res*. 2008; 36:6318–6332. [PubMed: 18835850]
39. Mignone F, Gissi C, Liuni S, Pesole G. Untranslated regions of mRNAs. *Genome Biol*. 2002; 3:REVIEWS0004. [PubMed: 11897027]
40. Tian B, Hu J, Zhang H, Lutz CS. A large-scale analysis of mRNA polyadenylation of human and mouse genes. *Nucleic Acids Res*. 2005; 33:201–212. [PubMed: 15647503]
41. Tian B, Manley JL. Alternative cleavage and polyadenylation: the long and short of it. *Trends Biochem Sci*. 2013; 38:312–320. [PubMed: 23632313]
42. Flavell SW, Kim TK, Gray JM, et al. Genome-wide analysis of MEF2 transcriptional program reveals synaptic target genes and neuronal activity-dependent polyadenylation site selection. *Neuron*. 2008; 60:1022–1038. [PubMed: 19109909]
43. Zhang H, Lee JY, Tian B. Biased alternative polyadenylation in human tissues. *Genome Biol*. 2005; 6:R100. [PubMed: 16356263]
44. Ji Z, Lee JY, Pan Z, Jiang B, Tian B. Progressive lengthening of 3' untranslated regions of mRNAs by alternative polyadenylation during mouse embryonic development. *Proc Natl Acad Sci U S A*. 2009; 106:7028–7033. [PubMed: 19372383]
45. Liu D, Brockman JM, Dass B, et al. Systematic variation in mRNA 3'-processing signals during mouse spermatogenesis. *Nucleic Acids Res*. 2007; 35:234–246. [PubMed: 17158511]
46. McMahon KW, Hirsch BA, MacDonald CC. Differences in polyadenylation site choice between somatic and male germ cells. *BMC Mol Biol*. 2006; 7:35. [PubMed: 17038175]
47. Wang H, Sartini BL, Millette CF, Kilpatrick DL. A developmental switch in transcription factor isoforms during spermatogenesis controlled by alternative messenger RNA 3'-end formation. *Biol Reprod*. 2006; 75:318–323. [PubMed: 16723505]
48. O'Brien DA, Welch JE, Fulcher KD, Eddy EM. Expression of mannose 6-phosphate receptor messenger ribonucleic acids in mouse spermatogenic and Sertoli cells. *Biol Reprod*. 1994; 50:429–435. [PubMed: 8142560]
49. Shaper NL, Wright WW, Shaper JH. Murine beta 1,4-galactosyltransferase: both the amounts and structure of the mRNA are regulated during spermatogenesis. *Proc Natl Acad Sci U S A*. 1990; 87:791–795. [PubMed: 1689054]
50. Yeo G, Holste D, Kreiman G, Burge CB. Variation in alternative splicing across human tissues. *Genome Biol*. 2004; 5:R74. [PubMed: 15461793]
51. Clark TA, Schweitzer AC, Chen TX, et al. Discovery of tissue-specific exons using comprehensive human exon microarrays. *Genome Biol*. 2007; 8:R64. [PubMed: 17456239]
52. Grosso AR, Gomes AQ, Barbosa-Morais NL, et al. Tissue-specific splicing factor gene expression signatures. *Nucleic Acids Res*. 2008; 36:4823–4832. [PubMed: 18653532]
53. de la Grange P, Gratadou L, Delord M, Dutertre M, Auboeuf D. Splicing factor and exon profiling across human tissues. *Nucleic Acids Res*. 2010; 38:2825–2838. [PubMed: 20110256]
54. Soumillon M, Necsulea A, Weier M, et al. Cellular source and mechanisms of high transcriptome complexity in the mammalian testis. *Cell Rep*. 2013; 3:2179–2190. [PubMed: 23791531]
55. Mackey ZB, Ramos W, Levin DS, Walter CA, McCarrey JR, Tomkinson AE. An alternative splicing event which occurs in mouse pachytene spermatocytes generates a form of DNA ligase III with distinct biochemical properties that may function in meiotic recombination. *Mol Cell Biol*. 1997; 17:989–998. [PubMed: 9001252]
56. Nash RA, Caldecott KW, Barnes DE, Lindahl T. XRCC1 protein interacts with one of two distinct forms of DNA ligase III. *Biochemistry*. 1997; 36:5207–5211. [PubMed: 9136882]
57. Kanai Y, Kanai-Azuma M, Noce T, et al. Identification of two Sox17 messenger RNA isoforms, with and without the high mobility group box region, and their differential expression in mouse spermatogenesis. *J Cell Biol*. 1996; 133:667–681. [PubMed: 8636240]

58. Foulkes NS, Borrelli E, Sassone-Corsi P. CREM gene: use of alternative DNA-binding domains generates multiple antagonists of cAMP-induced transcription. *Cell*. 1991; 64:739–749. [PubMed: 1847666]
59. Foulkes NS, Mellstrom B, Benusiglio E, Sassone-Corsi P. Developmental switch of CREM function during spermatogenesis: from antagonist to activator. *Nature*. 1992; 355:80–84. [PubMed: 1370576]
60. Foulkes NS, Schlotter F, Pevet P, Sassone-Corsi P. Pituitary hormone FSH directs the CREM functional switch during spermatogenesis. *Nature*. 1993; 362:264–267. [PubMed: 7681549]
61. Nantel F, Monaco L, Foulkes NS, et al. Spermiogenesis deficiency and germ-cell apoptosis in CREM-mutant mice. *Nature*. 1996; 380:159–162. [PubMed: 8600390]
62. Blendy JA, Kaestner KH, Weinbauer GF, Nieschlag E, Schutz G. Severe impairment of spermatogenesis in mice lacking the CREM gene. *Nature*. 1996; 380:162–165. [PubMed: 8600391]
63. Yang G, Zhang YL, Buchold GM, Jetten AM, O'Brien DA. Analysis of germ cell nuclear factor transcripts and protein expression during spermatogenesis. *Biol Reprod*. 2003; 68:1620–1630. [PubMed: 12606326]
64. Grocock LM, Nie M, Prudden J, et al. RNF4 interacts with both SUMO and nucleosomes to promote the DNA damage response. *EMBO Rep*. 2014; 15:601–608. [PubMed: 24714598]
65. Pero R, Lembo F, Chieffi P, et al. Translational regulation of a novel testis-specific RNF4 transcript. *Mol Reprod Dev*. 2003; 66:1–7. [PubMed: 12874792]
66. Hsu LC, Chen HY, Lin YW, et al. DAZAP1, an hnRNP protein, is required for normal growth and spermatogenesis in mice. *RNA*. 2008; 14:1814–1822. [PubMed: 18669443]
67. Yang CK, Yen P. Differential translation of Dazap1 transcripts during spermatogenesis. *PLoS One*. 2013; 8:e60873. [PubMed: 23658607]
68. Schmidt EE, Hanson ES, Capecchi MR. Sequence-independent assembly of spermatid mRNAs into messenger ribonucleoprotein particles. *Mol Cell Biol*. 1999; 19:3904–3915. [PubMed: 10207114]
69. Cataldo L, Mastrangelo MA, Kleene KC. A quantitative sucrose gradient analysis of the translational activity of 18 mRNA species in testes from adult mice. *Mol Hum Reprod*. 1999; 5:206–213. [PubMed: 10333353]
70. Gold B, Stern L, Bradley FM, Hecht NB. Gene expression during mammalian spermatogenesis. II. Evidence for stage-specific differences in mRNA populations. *J Exp Zool*. 1983; 225:123–134. [PubMed: 6187891]
71. Stern L, Kleene KC, Gold B, Hecht NB. Gene expression during mammalian spermatogenesis. III. Changes in populations of mRNA during spermiogenesis. *Exp Cell Res*. 1983; 143:247–255. [PubMed: 6186508]
72. Gold B, Hecht NB. Differential compartmentalization of messenger ribonucleic acid in murine testis. *Biochemistry*. 1981; 20:4871–4877. [PubMed: 6170318]
73. Cagney G, Park S, Chung C, et al. Human tissue profiling with multidimensional protein identification technology. *J Proteome Res*. 2005; 4:1757–1767. [PubMed: 16212430]
74. Shima JE, McLean DJ, McCarrey JR, Griswold MD. The murine testicular transcriptome: characterizing gene expression in the testis during the progression of spermatogenesis. *Biol Reprod*. 2004; 71:319–330. [PubMed: 15028632]
75. Chappell VA, Busada JT, Keiper BD, Geyer CB. Translational activation of developmental messenger RNAs during neonatal mouse testis development. *Biol Reprod*. 2013; 89:61. [PubMed: 23926285]
76. Iguchi N, Tobias JW, Hecht NB. Expression profiling reveals meiotic male germ cell mRNAs that are translationally up- and down-regulated. *Proc Natl Acad Sci U S A*. 2006; 103:7712–7717. [PubMed: 16682651]
77. Braun RE. Post-transcriptional control of gene expression during spermatogenesis. *Semin Cell Dev Biol*. 1998; 9:483–489. [PubMed: 9813196]
78. Steger K. Haploid spermatids exhibit translationally repressed mRNAs. *Anat Embryol (Berl)*. 2001; 203:323–334. [PubMed: 11411307]

79. Braun RE. Temporal control of protein synthesis during spermatogenesis. *Int J Androl.* 2000; 23(Suppl 2):92–94. [PubMed: 10849508]
80. Hecht NB. Molecular mechanisms of male germ cell differentiation. *Bioessays.* 1998; 20:555–561. [PubMed: 9723004]
81. Yu YE, Zhang Y, Unni E, et al. Abnormal spermatogenesis and reduced fertility in transition nuclear protein 1-deficient mice. *Proc Natl Acad Sci U S A.* 2000; 97:4683–4688. [PubMed: 10781074]
82. Zhao M, Shirley CR, Yu YE, et al. Targeted disruption of the transition protein 2 gene affects sperm chromatin structure and reduces fertility in mice. *Mol Cell Biol.* 2001; 21:7243–7255. [PubMed: 11585907]
83. Adham IM, Nayernia K, Burkhardt-Gottges E, et al. Teratozoospermia in mice lacking the transition protein 2 (Tnp2). *Mol Hum Reprod.* 2001; 7:513–520. [PubMed: 11385107]
84. Cho C, Willis WD, Goulding EH, et al. Haploinsufficiency of protamine-1 or -2 causes infertility in mice. *Nat Genet.* 2001; 28:82–86. [PubMed: 11326282]
85. Nayernia K, Adham IM, Burkhardt-Gottges E, et al. Asthenozoospermia in mice with targeted deletion of the sperm mitochondrion-associated cysteine-rich protein (Smcp) gene. *Mol Cell Biol.* 2002; 22:3046–3052. [PubMed: 11940662]
86. Kleene KC. Poly(A) shortening accompanies the activation of translation of five mRNAs during spermiogenesis in the mouse. *Development.* 1989; 106:367–373. [PubMed: 2512111]
87. Mali P, Kaipia A, Kangasniemi M, et al. Stage-specific expression of nucleoprotein mRNAs during rat and mouse spermiogenesis. *Reprod Fertil Dev.* 1989; 1:369–382. [PubMed: 2636425]
88. Kleene KC, Distel RJ, Hecht NB. Translational regulation and deadenylation of a protamine mRNA during spermiogenesis in the mouse. *Dev Biol.* 1984; 105:71–79. [PubMed: 6468765]
89. Shih DM, Kleene KC. A study by in situ hybridization of the stage of appearance and disappearance of the transition protein 2 and the mitochondrial capsule seleno-protein mRNAs during spermatogenesis in the mouse. *Mol Reprod Dev.* 1992; 33:222–227. [PubMed: 1418993]
90. Lee K, Haugen HS, Clegg CH, Braun RE. Premature translation of protamine 1 mRNA causes precocious nuclear condensation and arrests spermatid differentiation in mice. *Proc Natl Acad Sci U S A.* 1995; 92:12451–12455. [PubMed: 8618919]
91. Tsenden K, Topaloglu O, Meinhardt A, et al. Premature translation of transition protein 2 mRNA causes sperm abnormalities and male infertility. *Mol Reprod Dev.* 2007; 74:273–279. [PubMed: 16967499]
92. Bagarova J, Chowdhury TA, Kimura M, Kleene KC. Identification of elements in the Smcp 5' and 3' UTR that repress translation and promote the formation of heavy inactive mRNPs in spermatids by analysis of mutations in transgenic mice. *Reproduction.* 2010; 140:853–864. [PubMed: 20876225]
93. Hawthorne SK, Busanelli RR, Kleene KC. The 5' UTR and 3' UTR of the sperm mitochondria-associated cysteine-rich protein mRNA regulate translation in spermatids by multiple mechanisms in transgenic mice. *Dev Biol.* 2006; 297:118–126. [PubMed: 16759650]
94. Fajardo MA, Haugen HS, Clegg CH, Braun RE. Separate elements in the 3' untranslated region of the mouse protamine 1 mRNA regulate translational repression and activation during murine spermatogenesis. *Dev Biol.* 1997; 191:42–52. [PubMed: 9356170]
95. Zhong J, Peters AH, Kafer K, Braun RE. A highly conserved sequence essential for translational repression of the protamine 1 messenger rna in murine spermatids. *Biol Reprod.* 2001; 64:1784–1789. [PubMed: 11369609]
96. Giorgini F, Davies HG, Braun RE. MSY2 and MSY4 bind a conserved sequence in the 3' untranslated region of protamine 1 mRNA in vitro and in vivo. *Mol Cell Biol.* 2001; 21:7010–7019. [PubMed: 11564883]
97. Braun RE, Peschon JJ, Behringer RR, Brinster RL, Palmiter RD. Protamine 3'-untranslated sequences regulate temporal translational control and subcellular localization of growth hormone in spermatids of transgenic mice. *Genes Dev.* 1989; 3:793–802. [PubMed: 2744463]
98. Nipper RW, Chennothukuzhi V, Tutuncu L, Williams CJ, Gerton GL, Moss SB. Differential RNA expression and polyribosome loading of alternative transcripts of the Akap4 gene in murine spermatids. *Mol Reprod Dev.* 2005; 70:397–405. [PubMed: 15685631]

99. Schmidt EE, Schibler U. Developmental testis-specific regulation of mRNA levels and mRNA translational efficiencies for TATA-binding protein mRNA isoforms. *Dev Biol.* 1997; 184:138–149. [PubMed: 9142990]
100. Gu W, Morales C, Hecht NB. In male mouse germ cells, copper-zinc superoxide dismutase utilizes alternative promoters that produce multiple transcripts with different translation potential. *J Biol Chem.* 1995; 270:236–243. [PubMed: 7814380]
101. Weill L, Belloc E, Bava FA, Mendez R. Translational control by changes in poly(A) tail length: recycling mRNAs. *Nat Struct Mol Biol.* 2012; 19:577–585. [PubMed: 22664985]
102. Eckmann CR, Rammelt C, Wahle E. Control of poly(A) tail length. *Wiley Interdiscip Rev RNA.* 2011; 2:348–361. [PubMed: 21957022]
103. Doidge R, Mittal S, Aslam A, Winkler GS. Deadenylation of cytoplasmic mRNA by the mammalian Ccr4-Not complex. *Biochem Soc Trans.* 2012; 40:896–901. [PubMed: 22817755]
104. Kashiwabara S, Noguchi J, Zhuang T, et al. Regulation of spermatogenesis by testis- specific, cytoplasmic poly(A) polymerase TPAP. *Science.* 2002; 298:1999–2002. [PubMed: 12471261]
105. Tay J, Richter JD. Germ cell differentiation and synaptonemal complex formation are disrupted in CPEB knockout mice. *Dev Cell.* 2001; 1:201–213. [PubMed: 11702780]
106. Yanagiya A, Delbes G, Svitkin YV, Robaire B, Sonenberg N. The poly(A)-binding protein partner Paip2a controls translation during late spermiogenesis in mice. *J Clin Invest.* 2010; 120:3389–3400. [PubMed: 20739757]
107. Gu W, Kwon YK, Hecht NB. In postmeiotic male germ cells poly (A) shortening accompanies translation of mRNA encoding gamma enteric actin but not cytoplasmic beta and gamma actin mRNAs. *Mol Reprod Dev.* 1996; 44:141–145. [PubMed: 9115710]
108. Licatalosi DD, Darnell RB. RNA processing and its regulation: global insights into biological networks. *Nat Rev Genet.* 2010; 11:75–87. [PubMed: 20019688]
109. Colwill K, Pawson T, Andrews B, et al. The Clk/Sty protein kinase phosphorylates SR splicing factors and regulates their intranuclear distribution. *EMBO J.* 1996; 15:265–275. [PubMed: 8617202]
110. Duncan PI, Stojdl DF, Marius RM, Bell JC. In Vivo regulation of alternative pre-mRNA splicing by the Clk1 protein kinase. *Mol Cell Biol.* 1997; 17:5996–6001. [PubMed: 9315658]
111. Prasad J, Colwill K, Pawson T, Manley JL. The protein kinase Clk/Sty directly modulates SR protein activity: both hyper- and hypophosphorylation inhibit splicing. *Mol Cell Biol.* 1999; 19:6991–7000. [PubMed: 10490636]
112. Howell BW, Afar DE, Lew J, et al. STY, a tyrosine-phosphorylating enzyme with sequence homology to serine/threonine kinases. *Mol Cell Biol.* 1991; 11:568–572. [PubMed: 1986248]
113. Nayler O, Stamm S, Ullrich A. Characterization and comparison of four serine- and arginine-rich (SR) protein kinases. *Biochem J.* 1997; 326:693–700. [PubMed: 9307018]
114. Arkov AL, Ramos A. Building RNA-protein granules: insight from the germline. *Trends Cell Biol.* 2010; 20:482–490. [PubMed: 20541937]
115. Kotaja N, Sassone-Corsi P. The chromatoid body: a germ-cell-specific RNA- processing centre. *Nat Rev Mol Cell Biol.* 2007; 8:85–90. [PubMed: 17183363]
116. Putnam AA, Jankowsky E. DEAD-box helicases as integrators of RNA, nucleotide and protein binding. *Biochim Biophys Acta.* 1829; 2013:884–893.
117. Kleene KC, Cullinane DL. Maybe repressed mRNAs are not stored in the chromatoid body in mammalian spermatids. *Reproduction.* 2011; 142:383–388. [PubMed: 21673073]
118. Meikar O, Da Ros M, Korhonen H, Kotaja N. Chromatoid body and small RNAs in male germ cells. *Reproduction.* 2011; 142:195–209. [PubMed: 21652638]
119. Pascale A, Govoni S. The complex world of post-transcriptional mechanisms: is their deregulation a common link for diseases? Focus on ELAV-like RNA-binding proteins. *Cell Mol Life Sci.* 2012; 69:501–517. [PubMed: 21909784]
120. Ince-Dunn G, Okano HJ, Jensen KB, et al. Neuronal Elav-like (Hu) proteins regulate RNA splicing and abundance to control glutamate levels and neuronal excitability. *Neuron.* 2012; 75:1067–1080. [PubMed: 22998874]

121. Mukherjee N, Corcoran DL, Nusbaum JD, et al. Integrative regulatory mapping indicates that the RNA-binding protein HuR couples pre-mRNA processing and mRNA stability. *Mol Cell*. 2011; 43:327–339. [PubMed: 21723170]
122. Lebedeva S, Jens M, Theil K, et al. Transcriptome-wide analysis of regulatory interactions of the RNA-binding protein HuR. *Mol Cell*. 2011; 43:340–352. [PubMed: 21723171]
123. Chi MN, Auriol J, Jegou B, et al. The RNA-binding protein ELAVL1/HuR is essential for mouse spermatogenesis, acting both at meiotic and postmeiotic stages. *Mol Biol Cell*. 2011; 22:2875–2885. [PubMed: 21737689]
124. Vlasova-St Louis I, Dickson AM, Bohjanen PR, Wilusz CJ. CELFish ways to modulate mRNA decay. *Biochim Biophys Acta*. 1829; 2013:695–707.
125. Dasgupta T, Ladd AN. The importance of CELF control: molecular and biological roles of the CUG-BP, Elav-like family of RNA-binding proteins. *Wiley Interdiscip Rev RNA*. 2012; 3:104–121. [PubMed: 22180311]
126. Vlasova-St Louis I, Bohjanen PR. Coordinate regulation of mRNA decay networks by GU-rich elements and CELF1. *Curr Opin Genet Dev*. 2011; 21:444–451. [PubMed: 21497082]
127. Kress C, Gautier-Courteille C, Osborne HB, Babinet C, Paillard L. Inactivation of CUG-BP1/CELF1 causes growth, viability, and spermatogenesis defects in mice. *Mol Cell Biol*. 2007; 27:1146–1157. [PubMed: 17130239]
128. Sanchez-Jimenez F, Sanchez-Margalet V. Role of Sam68 in post-transcriptional gene regulation. *Int J Mol Sci*. 2013; 14:23402–23419. [PubMed: 24287914]
129. Iijima T, Wu K, Witte H, et al. SAM68 regulates neuronal activity-dependent alternative splicing of neuexin-1. *Cell*. 2011; 147:1601–1614. [PubMed: 22196734]
130. Missler M, Sudhof TC. Neurexins: three genes and 1001 products. *Trends Genet*. 1998; 14:20–26. [PubMed: 9448462]
131. Ehrmann I, Dalglish C, Liu Y, et al. The tissue-specific RNA-binding protein T-STAR controls regional splicing patterns of neuexin pre-mRNAs in the brain. *PLoS Genet*. 2013; 9:e1003474. [PubMed: 23637638]
132. Paronetto MP, Zalfa F, Botti F, Geremia R, Bagni C, Sette C. The nuclear RNA-binding protein Sam68 translocates to the cytoplasm and associates with the polysomes in mouse spermatocytes. *Mol Biol Cell*. 2006; 17:14–24. [PubMed: 16221888]
133. Paronetto MP, Messina V, Bianchi E, et al. Sam68 regulates translation of target mRNAs in male germ cells, necessary for mouse spermatogenesis. *J Cell Biol*. 2009; 185:235–249. [PubMed: 19380878]
134. Paronetto MP, Messina V, Barchi M, Geremia R, Richard S, Sette C. Sam68 marks the transcriptionally active stages of spermatogenesis and modulates alternative splicing in male germ cells. *Nucleic Acids Res*. 2011; 39:4961–4974. [PubMed: 21355037]
135. Sawicka K, Bushell M, Spriggs KA, Willis AE. Polypyrimidine-tract-binding protein: a multifunctional RNA-binding protein. *Biochem Soc Trans*. 2008; 36:641–647. [PubMed: 18631133]
136. Xu M, Hecht NB. Polypyrimidine tract binding protein 2 stabilizes phosphoglycerate kinase 2 mRNA in murine male germ cells by binding to its 3'UTR. *Biol Reprod*. 2007; 76:1025–1033. [PubMed: 17329592]
137. Schmid R, Grellscheid SN, Ehrmann I, et al. The splicing landscape is globally reprogrammed during male meiosis. *Nucleic Acids Res*. 2013; 41:10170–10184. [PubMed: 24038356]
138. Licatalosi DD, Yano M, Fak JJ, et al. Ptbp2 represses adult-specific splicing to regulate the generation of neuronal precursors in the embryonic brain. *Genes Dev*. 2012; 26:1626–1642. [PubMed: 22802532]
139. Wallace AM, Denison TL, Attaya EN, MacDonald CC. Developmental distribution of the polyadenylation protein CstF-64 and the variant tauCstF-64 in mouse and rat testis. *Biol Reprod*. 2004; 70:1080–1087. [PubMed: 14681198]
140. Yao C, Choi EA, Weng L, et al. Overlapping and distinct functions of CstF64 and CstF64tau in mammalian mRNA 3' processing. *RNA*. 2013; 19:1781–1790. [PubMed: 24149845]

141. Yao C, Biesinger J, Wan J, et al. Transcriptome-wide analyses of CstF64-RNA interactions in global regulation of mRNA alternative polyadenylation. *Proc Natl Acad Sci U S A*. 2012; 109:18773–18778. [PubMed: 23112178]
142. Martin G, Gruber AR, Keller W, Zavolan M. Genome-wide analysis of pre-mRNA 3' end processing reveals a decisive role of human cleavage factor I in the regulation of 3' UTR length. *Cell Rep*. 2012; 1:753–763. [PubMed: 22813749]
143. Berkovits BD, Wang L, Guarnieri P, Wolgemuth DJ. The testis-specific double bromodomain-containing protein BRDT forms a complex with multiple spliceosome components and is required for mRNA splicing and 3'-UTR truncation in round spermatids. *Nucleic Acids Res*. 2012; 40:7162–7175. [PubMed: 22570411]
144. Tardif S, Akrofi AS, Dass B, Hardy DM, MacDonald CC. Infertility with impaired zona pellucida adhesion of spermatozoa from mice lacking TauCstF-64. *Biol Reprod*. 2010; 83:464–472. [PubMed: 20463354]
145. Dass B, Tardif S, Park JY, et al. Loss of polyadenylation protein tauCstF-64 causes spermatogenic defects and male infertility. *Proc Natl Acad Sci U S A*. 2007; 104:20374–20379. [PubMed: 18077340]
146. Giorgini F, Davies HG, Braun RE. Translational repression by MSY4 inhibits spermatid differentiation in mice. *Development*. 2002; 129:3669–3679. [PubMed: 12117816]
147. Yang J, Medvedev S, Yu J, Schultz RM, Hecht NB. Deletion of the DNA/RNA-binding protein MSY2 leads to post-meiotic arrest. *Mol Cell Endocrinol*. 2006; 250:20–24. [PubMed: 16413673]
148. Yang J, Morales CR, Medvedev S, Schultz RM, Hecht NB. In the absence of the mouse DNA/RNA-binding protein MSY2, messenger RNA instability leads to spermatogenic arrest. *Biol Reprod*. 2007; 76:48–54. [PubMed: 17035640]
149. Tafuri SR, Familiarì M, Wolffe AP. A mouse Y box protein, MSY1, is associated with paternal mRNA in spermatocytes. *J Biol Chem*. 1993; 268:12213–12220. [PubMed: 8505341]
150. Mendez R, Murthy KG, Ryan K, Manley JL, Richter JD. Phosphorylation of CPEB by Eg2 mediates the recruitment of CPSF into an active cytoplasmic polyadenylation complex. *Mol Cell*. 2000; 6:1253–1259. [PubMed: 11106762]
151. Hosoda N, Funakoshi Y, Hirasawa M, et al. Anti-proliferative protein Tob negatively regulates CPEB3 target by recruiting Caf1 deadenylase. *EMBO J*. 2011; 30:1311–1323. [PubMed: 21336257]
152. Pique M, Lopez JM, Foissac S, Guigo R, Mendez R. A combinatorial code for CPE-mediated translational control. *Cell*. 2008; 132:434–448. [PubMed: 18267074]
153. Campbell ZT, Menichelli E, Friend K, et al. Identification of a conserved interface between PUF and CPEB proteins. *J Biol Chem*. 2012; 287:18854–18862. [PubMed: 22496444]
154. Kurihara Y, Tokuriki M, Myojin R, et al. CPEB2, a novel putative translational regulator in mouse haploid germ cells. *Biol Reprod*. 2003; 69:261–268. [PubMed: 12672660]
155. Spassov DS, Jurecic R. Cloning and comparative sequence analysis of PUM1 and PUM2 genes, human members of the Pumilio family of RNA-binding proteins. *Gene*. 2002; 299:195–204. [PubMed: 12459267]
156. Zamore PD, Williamson JR, Lehmann R. The Pumilio protein binds RNA through a conserved domain that defines a new class of RNA-binding proteins. *RNA*. 1997; 3:1421–1433. [PubMed: 9404893]
157. Xu EY, Chang R, Salmon NA, Reijo Pera RA. A gene trap mutation of a murine homolog of the *Drosophila* stem cell factor Pumilio results in smaller testes but does not affect litter size or fertility. *Mol Reprod Dev*. 2007; 74:912–921. [PubMed: 17219433]
158. Chen D, Zheng W, Lin A, Uyhazi K, Zhao H, Lin H. Pumilio 1 suppresses multiple activators of p53 to safeguard spermatogenesis. *Curr Biol*. 2012; 22:420–425. [PubMed: 22342750]
159. Suzuki A, Igarashi K, Aisaki K, Kanno J, Saga Y. NANOS2 interacts with the CCR4-NOT deadenylation complex and leads to suppression of specific RNAs. *Proc Natl Acad Sci U S A*. 2010; 107:3594–3599. [PubMed: 20133598]
160. Saga Y. Function of Nanos2 in the male germ cell lineage in mice. *Cell Mol Life Sci*. 2010; 67:3815–3822. [PubMed: 20652721]

161. Tsuda M, Sasaoka Y, Kiso M, et al. Conserved role of nanos proteins in germ cell development. *Science*. 2003; 301:1239–1241. [PubMed: 12947200]
162. Beck AR, Miller IJ, Anderson P, Streuli M. RNA-binding protein TIAR is essential for primordial germ cell development. *Proc Natl Acad Sci U S A*. 1998; 95:2331–2336. [PubMed: 9482885]
163. Youngren KK, Coveney D, Peng X, et al. The Ter mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. *Nature*. 2005; 435:360–364. [PubMed: 15902260]
164. Sada A, Suzuki A, Suzuki H, Saga Y. The RNA-binding protein NANOS2 is required to maintain murine spermatogonial stem cells. *Science*. 2009; 325:1394–1398. [PubMed: 19745153]
165. Reynolds N, Cooke HJ. Role of the DAZ genes in male fertility. *Reprod Biomed Online*. 2005; 10:72–80. [PubMed: 15705297]
166. VanGompel MJW, Xu EY. The roles of the DAZ family in spermatogenesis: more than just translation? *Spermatogenesis*. 2011; 1:36–46. [PubMed: 22523742]
167. Vogel T, Speed RM, Ross A, Cooke HJ. Partial rescue of the Dazl knockout mouse by the human DAZL gene. *Mol Hum Reprod*. 2002; 8:797–804. [PubMed: 12200456]
168. Saunders PT, Turner JM, Ruggiu M, et al. Absence of mDazl produces a final block on germ cell development at meiosis. *Reproduction*. 2003; 126:589–597. [PubMed: 14611631]
169. Schrans-Stassen BH, Saunders PT, Cooke HJ, de Rooij DG. Nature of the spermatogenic arrest in Dazl $-/-$ mice. *Biol Reprod*. 2001; 65:771–776. [PubMed: 11514340]
170. Ruggiu M, Speed R, Taggart M, et al. The mouse Dazla gene encodes a cytoplasmic protein essential for gametogenesis. *Nature*. 1997; 389:73–77. [PubMed: 9288969]
171. Lin Y, Page DC. Dazl deficiency leads to embryonic arrest of germ cell development in XY C57BL/6 mice. *Dev Biol*. 2005; 288:309–316. [PubMed: 16310179]
172. Reynolds N, Collier B, Bingham V, Gray NK, Cooke HJ. Translation of the synaptonemal complex component Sycp3 is enhanced in vivo by the germ cell specific regulator Dazl. *RNA*. 2007; 13:974–981. [PubMed: 17526644]
173. Reynolds N, Collier B, Maratou K, et al. Dazl binds in vivo to specific transcripts and can regulate the pre-meiotic translation of Mvh in germ cells. *Hum Mol Genet*. 2005; 14:3899–3909. [PubMed: 16278232]
174. Xu X, Tan X, Lin Q, Schmidt B, Engel W, Pantakani DV. Mouse Dazl and its novel splice variant functions in translational repression of target mRNAs in embryonic stem cells. *Biochim Biophys Acta*. 1829; 2013:425–435.
175. Tsui S, Dai T, Warren ST, Salido EC, Yen PH. Association of the mouse infertility factor DAZL1 with actively translating polyribosomes. *Biol Reprod*. 2000; 62:1655–1660. [PubMed: 10819768]
176. Kim B, Cooke HJ, Rhee K. DAZL is essential for stress granule formation implicated in germ cell survival upon heat stress. *Development*. 2012; 139:568–578. [PubMed: 22223682]

Fig. 6.1.

Overview of male germ cell development in the mouse. **(a)** During embryogenesis, primordial germ cells (PGCs) migrate to the genital ridge where they receive signals (red dashed arrow) from gonadal support cells (Sertoli cells or granulosa cells in XY and XX embryos, respectively) and commit to a male or female program of development. **(b)** During the first postnatal stage of germ cell development, spermatogonia type A cells self renew or undergo a series of proliferative and differentiating divisions to generate chains of cells that will enter meiosis. **(c)** Meiosis involves a single genome duplication event followed by two

successive divisions to generate haploid spermatids. **(d)** Spermiogenesis, the process by which round spermatids progress through 16 steps to transform into spermatozoa that are released into the lumen of the seminiferous tubule

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Fig. 6.2.

Gene regulation through alternative mRNA regulation. In this example, a single gene yields three identical pre-mRNAs that are alternatively processed into different mRNAs to alter the identity and abundance of the encoded protein. In panels (1) and (2), alternative splicing of exon 'b' yields mRNAs that encode alternative protein variants. In panels (2) and (3), alternative polyadenylation yields mRNAs that differ with respect to 3'UTR length, with the long 3'UTR variant (panel (3)) possessing regulatory elements that lead to reduced accumulation of the encoded protein