RNA-binding proteins, RNA granules, and gametes: is unity strength?

Mai Nguyen-Chi and Dominique Morello¹

Université Montpellier 2, UMR 5235, Dynamique des Interactions Membranaires Normales et Pathologiques, Place Eugène Bataillon, Bât 24 CC 107, 34095 Montpellier Cedex 5, France and ¹Université Paul Sabatier, CNRS-UMR 5547, IFR 109, 118 Route de Narbonne, 31062 Toulouse Cedex 04, France

Correspondence should be addressed to D Morello; Email: morello@cict.fr

Abstract

Changes in mRNA translation and degradation represent post-transcriptional processes operating during gametogenesis and early embryogenesis to ensure regulated protein synthesis. Numerous mRNA-binding proteins (RBPs) have been described in multiple animal models that contribute to the control of mRNA translation and decay during oogenesis and spermatogenesis. An emerging view from studies performed in germ cells and somatic cells is that RBPs associate with their target mRNAs in RNA–protein (or ribonucleoprotein) complexes (mRNPs) that assemble in various cytoplasmic RNA granules that communicate with the translation machinery and control mRNA storage, triage, and degradation. In comparison with *Xenopus*, *Caenorhabditis elegans*, or *Drosophila*, the composition and role of cytoplasmic RNA-containing granules in mammalian germ cells are still poorly understood. However, regained interest for these structures has emerged with the recent discovery of their role in small RNA synthesis and transposon silencing through DNA methylation. In this review, we will briefly summarize our current knowledge on cytoplasmic RNA granules in murine germ cells and describe the role of some of the RBPs they contain in regulating mRNA metabolism and small RNA processing during gametogenesis.

Reproduction (2011) 142 803–817

Introduction

Gametogenesis is a complex process by which primordial germ cells (PGCs) proliferate during embryonic development and differentiate into diploid precursor cells, oogonia or spermatogonia in females or males respectively. These precursors undergo cell division and differentiation to produce haploid germ cells, the oocyte and sperm, that ultimately fuse to give rise to a totipotent zygote that further develops into an embryo (Zhao & Garbers 2002, Hemberger *et al.* 2009; see Figs 1 and 2 for detailed steps of male and female germ cell development respectively). A hallmark of germ line cells throughout the animal kingdom is that they contain cytoplasmic germinal granules that are not delimited by a membrane and correspond to aggregates of mRNAs, small RNAs, and proteins, including many RNA-binding proteins (RBPs; Eddy 1975, Saffman & Lasko 1999, Houston & King 2000). In *Drosophila*, *Caenorhabditis elegans* (*C. elegans*), or *Xenopus*, those granules, also called germinal granules or nuage (for ‘cloud’ in French) because they form electron-dense structures of various shapes and numbers (Eddy 1975), were shown to play a seminal role for the establishment of germ line, as they favor asymmetric assembly of germ cell determining components in the oocyte cytoplasm (Ikenishi 1998).

In mammals, germ cell specification relies on signals from extraembryonic tissues (Zhao & Garbers 2002) and is not supposed to involve germinal granules. However, the existence of different RNA-containing granules at various stages of mammalian male and female germ cell development is suggestive of their role in germ cell maintenance and differentiation. Recent advances in the identification of their components combined with functional studies using knockout models give support to this hypothesis, showing that the impairment of germinal granule formation is detrimental for germ cell differentiation. In this review, we will briefly summarize our current knowledge on cytoplasmic RNA granules in murine germ cells and describe the role of some of their RBPs in post-transcriptional regulation during spermatogenesis, oogenesis, and early development.

RNA granules in mammalian germ cells

Although collectively referred to as nuage, mammalian germ cells contain RNA or ribonucleoprotein (mRNP) granules in their cytoplasm, which appear distinct by their morphology, as visualized by electron microscopy (EM), and by their RNA and protein content, as observed by immunofluorescence (IF) microscopy. Schematically, there are three different types of germ line RNA granule,

Figure 1 IMC, PBs, and CBs in mouse male germ cells. (A) During mouse development, primordial germ cells (PGCs) are first identified at embryonic day 6.25 (E6.25) and are specified at E7.25, forming a group of ~40 cells located in the extraembryonic region posterior to the primitive streak (Hemberger *et al.* 2009). They proliferate and migrate to the developing gonads at E11.5. In males, PGCs become arrested at the G1 stage as prospermatogonia (pro-SG) until further development in juvenile gonads. Spermatogenesis takes place in seminiferous tubules of the testis. At the basement membrane, spermatogonia (SG) mitotically divide either to regenerate stem cells or to differentiate into primary spermatocytes that will enter meiosis. Through the first meiotic division, each primary spermatocyte through leptotene (L), zygotene (Z), pachytene (P) and finally diplotene (D) stages yields a pair of secondary spermatocytes (Spc II), which complete the second meiotic division, forming haploid cells called spermatids. Through a process called spermiogenesis, round (RS) and subsequently elongated (ES) spermatids differentiate into sperm (Spz) that enter the lumen of the tubule. One of the major changes in the haploid nucleus is the replacement of histones by protamines that facilitate the compaction of chromatin and causes a complete shutdown of transcription several days before the end of differentiation. In the mouse, the entire development from stem cell to spermatozoon takes 34 days, meiosis and spermiogenesis lasting both roughly the same time (nearly 2 weeks). Expression periods of the proteins contained in germinal RNA granules and described in this review are indicated. (B) Electron microscopic images of germinal granules in murine male germ cells. In prospermatogonia (left), intermitochondrial cement (IMC; arrows) is observed as small electron-dense material without limiting membranes among mitochondrial clusters. In meiotic spermatocytes (center), the size and frequency of IMC increase (particularly at the pachytene stage, as shown by the arrow) compared with prospermatogonia. In the meantime, chromatoid bodies (CBs) emerge independently of IMC and are observed as prominent, amorphous aggregates in the free cytoplasm (arrowhead). In haploid spermatids (right), IMC is no longer seen, whereas CBs (arrowhead) become more massive and are aggregated as a solitary architecture of submicron sizes. Chromatoid bodies at this stage occasionally show a close association with intracellular membrane vesicles (reproduced, with the permission of Chuma, from Tanaka T, Hosokawa M, Vagin VV, Reuter M, Hayashi E, Mochizuki AL, Kitamura K, Yamanaka H, Kondoh G, Okawa K *et al.* 2011 Tudor domain containing 7 (*Tdrd7*) is essential for dynamic ribonucleoprotein (RNP) remodeling of chromatoid bodies during spermatogenesis. *PNAS* **108** 10579–10584. © 2011 The authors).

Figure 2 IMC and PBs in mouse female germ cells and early embryogenesis. During mouse development, primordial germ cells (PGCs) are first identified at embryonic day 6.25 (E6.25) and are specified at E7.25, forming a group of ~ 40 cells located in the extraembryonic region posterior to the primitive streak (Hemberger *et al.* 2009). They proliferate and migrate to the developing gonads at E11.5. In females, oocytes enter and progress through the first meiotic prophase (leptotene (L), zygotene (Z) and pachytene (P) stages) and become arrested in the diplotene stage of the first meiotic prophase I. They remain at this stage until puberty. During and after puberty, upon hormonal stimulation (LH, luteinizing hormone), a few primary oocytes are released from cell cycle arrest and resume meiosis. The nuclear membranes of competent oocytes break down (GVBD, germinal vesicle breakdown), chromatin condenses, the spindle apparatus forms, and the chromosomes separate and segregate. Oocytes progress through telophase and the disproportionate cytokinesis leads to extrusion of the first polar body that signals meiosis I completion and egg ovulation. The secondary oocyte starts meiosis II without DNA replication and stops at metaphase II. Fertilization leads to completion of meiosis II with extrusion of the second polar body. The mature fertilized egg subsequently reaches the maternal to zygotic transition and develops through successive mitotic divisions into a blastocyst that implants in uterine endometrium. The localization of different RNA granules throughout oocyte development and early embryogenesis is indicated as well as the time of maternal transcriptional shutoff and zygotic transcriptional activation.

P body-like granules, intermitochondrial cement (IMC), and chromatoid bodies (CBs). First, the processing bodies (P bodies or PBs) have been named after the PBs described in somatic cells where they appear as small ($\sim 0.3 \mu\text{m}$), non-membranous cytoplasmic foci that concentrate mRNAs, small RNAs, and proteins involved in mRNA decay (such as the decapping complex DCP1/DCP2 and the exonuclease XRN1), mRNA translation repression, and RNA interference (RNAi; Boxes 1 and 2; Eulalio *et al.* 2007, Parker & Sheth 2007, Aizer & Shav-Tal 2008, Kulkarni *et al.* 2010). In the female germ line, PB-like granules, i.e. structures that contain most markers of the somatic PBs, are encountered in meiotically incompetent oocytes (see below; Flemr *et al.* 2010). In males, PB-like granules are found in the prospermatogonia of fetal gonads (also referred to as gonocytes during the period in which germ cells are mitotically arrested into G0) and, later on, in spermatogonia and meiotic spermatocytes of juvenile and adult testes (Aravin *et al.* 2009, Suzuki *et al.* 2010). The second type of RNA granule is the IMC. It displays an infrastructure that is not described in somatic cells and contains unique proteins. IMC appears upon EM analysis as electron-dense amorphous material located among clusters of mitochondria. In female germ line, IMC is clearly visible in oocytes during perinatal period and disappears when oocytes initiate growth

(Fig. 2 and Chuma *et al.* 2009). In males, IMC is first visible in gonocytes at E15.5. Then, it is found in spermatogonia and pachytene spermatocytes and disappears at the early steps of spermiogenesis (Fawcett *et al.* 1970, Meikar *et al.* 2011; Fig. 1B). The third type of germinal RNA granule is the CB, which is found only in the male germ cells. It is first visible in the cytoplasm of mid-to-late meiotic spermatocytes (Parvinen 2005, Tanaka *et al.* 2011), using EM, as a perinuclear sponge-like network of moderately electron-dense material interspersed with numerous pores (Aravin *et al.* 2009; Fig. 1B). This diffuse material concentrates progressively in a single perinuclear granule with a diameter of ~ 1 – $1.5 \mu\text{m}$ in the cytoplasm of round spermatids (Fig. 1B). As spermiogenesis proceeds, the CB decreases in size and finally disappears, a part of it being discarded with the residual body (Fawcett *et al.* 1970, Parvinen 2005, Kotaja *et al.* 2006a, Meikar *et al.* 2011). The CB is a dynamic structure (Kotaja *et al.* 2006a, Meikar *et al.* 2011) that makes frequent contact with the nuclear envelope and communicates with adjacent haploid cells through the intercellular bridges that interconnect germ cells of the same clone (Huckins 1978), suggesting that the CB can provide a mean to homogenize the cellular content of genetically different haploid cells (Parvinen 2005). CB assembly and movements rely on intact microtubule network (Kotaja *et al.* 2006b), a property that they share

Box 1 P-bodies and RNA granules in mammalian somatic cells

Mammalian cells house various microscopically visible cytoplasmic granules that contain untranslated mRNAs and ensure their regulated translation and decay. The most intensely studied examples concern the processing bodies (P bodies or PBs) that are present in all cell types, including germ cells (see the main text) and the stress granules (SGs) that appear transiently in response to various stresses. Other mRNA-containing granules that are observed in a more restricted category of cells have also been described. For instance, the neuronal mRNA granules are present in neurons. They are distinct from PBs and SGs, although they can share some components. The list of components shared with SGs includes SMN, Staufen, Smaug, fragile X mental retardation protein, Pumilio, ZBP1, and CPEB (reviewed in [Thomas et al. \(2011\)](#)). Another example is illustrated by ALK granules (AGs) that are formed in transformed cells expressing the oncogenic ALK tyrosine kinase. AGs exhibit similarities with PBs and SGs ([Fawal et al. 2011](#)). In all cases, RNA granules are non-membranous structures that exchange components with the surrounding cytosol. They are highly dynamic, using the cytoskeleton to move, assemble, and disassemble ([Aizer & Shav-Tal 2008](#), [Fawal et al. 2011](#)). Although studied by many laboratories, the function of these somatic granules in mRNA metabolism is still under debate. PBs have long been considered as active sites of mRNA degradation, since they concentrate proteins involved in mRNA decay, such as the DCP1 decapping enzyme, the XRN1 5'–3' exonuclease, various components of the CCR4–NOT deadenylation complex, and the RCK/p54 RNA helicase (for a recent and more exhaustive list, see [Kulkarni et al. \(2010\)](#)). PBs also contain the four Argonaute proteins and GW182, showing their potential implication in translational repression, nonsense-mediated mRNA decay, and RNAi-mediated repression ([Eulalio et al. 2007](#), [Parker & Sheth 2007](#), [Kulkarni et al. 2010](#)). However, the formal proof that PBs function in mRNA degradation remains to be obtained since degradation takes place independently of PBs and recruitment of ribonucleoprotein particles (mRNPs) to PBs might be a consequence of translational repression but not a requirement ([Eulalio et al. 2007](#)). In addition, the observation that mRNAs trapped in PBs can return to translation in hepatocarcinoma cells subjected to stress has led to the suggestion that PBs might also behave as storage sites for translationally arrested mRNAs ([Bhattacharyya et al. 2006](#)). In contrast with PBs, SGs concentrate many translational components, including translation initiation or elongation factors as well as ribosomal proteins. This suggests that the polyadenylated mRNAs they contain are protected from degradation during the stress and ready for translation upon stress removal, a hypothesis that remains to be demonstrated. SGs also contain proteins regulating the translation and decay of mRNAs containing AU-rich sequences in their 3'-UTR (ARE-mRNAs). Two of these proteins, TIA1/TIAR and HuR, are also present in the chromatoid body of round spermatids ([Nguyen-Chi et al. 2009](#), [Tanaka et al. 2011](#) respectively) together with numerous other RBPs (see main text). The analogy between SGs that associate with PBs in somatic cells and CBs that fuse with PBs in spermatogenesis led [Chuma et al. \(2006, 2009\)](#) to propose that CBs may have a function analogous to somatic SGs ([Tanaka et al. 2011](#)).

with RNA granules found in somatic cells, such as PBs, stress granules (SGs), or the neuronal granules that are specifically encountered in neurons ([Kiebler & Bassell 2006](#); [Box 1](#)). CBs contain numerous RBPs, including proteins that are i) expressed specifically in germ cells such as mouse VASA homolog (MVH) and its protein partners involved in the biogenesis of P-element-induced wimpy testis (Piwi)-interacting RNAs (piRNAs; see below) or ii) also found in somatic PBs, such as components of the RNA-induced silencing complex (RISC) or of mRNA decay machinery. CBs have therefore been proposed to constitute important centers of post-transcriptional processing and mRNA storage or degradation ([Meikar et al. 2011](#)).

RNA granules, transposon silencing, mRNA stability/translation in murine male germ cells

RNA granules of mammalian male germ cells contain RBPs that regulate mRNA stability and translation and

are thus crucial both for the maintenance of progenitor cells in fetal gonads and for the final steps of differentiation during adult spermiogenesis. Many studies have been performed in the last 4 years on RBPs and their associated non-RNA-binding proteins that have been summarized in different reviews. Here, we will restrict our analysis to proteins that have been shown to concentrate in RNA granules and focus mainly on NANOS, PIWI family members, and MVH.

mRNA storage and degradation in the PBs of fetal spermatogonia: the amazing case of Nanos2

NANOS proteins belong to a family of evolutionarily conserved zinc-finger motif-containing RBPs that play crucial role in germ cell maintenance and differentiation ([Wang & Lehmann 1991](#) and review in [Shen & Xie \(2010\)](#)). Three NANOS homologs, NANOS1–3, exist in the mouse, NANOS2 and NANOS3 expression being restricted to the germ cell lineage. NANOS3 expression

Box 2 miRNAs and endo-small-interfering RNAs

miRNAs are subdivided into two classes: canonical and non-canonical miRNAs. Most canonical miRNAs are transcribed by RNA PolIII polymerase from long endogenous primary single-strand transcripts (pri-miRNAs) that contain hairpins. In the nucleus, hairpins are recognized by the microprocessor, a complex of two RBPs, DGCR8, and the RNase III enzyme DROSHA that cleaves the pri-mRNAs at the base of the hairpin. The resulting hairpin containing pre-miRNA is transported to the cytoplasm where it is cleaved by DICER, another RNase III enzyme, leading to a single short ~23 nt duplex RNAs. By contrast, the production of non-canonical miRNAs does not rely on the microprocessor. Their precursors that contain short hairpin (sh) or are embedded in introns bypass the microprocessor step and are directly processed by DICER to produce shRNAs or mirtrons. On the other hand, small-interfering RNAs (siRNAs), are produced from long double-stranded RNAs (dsRNAs) resulting from opposing strand transcription, inverted repeats, or pseudogenes. dsRNAs are directly processed by DICER to produce multiple 20–24 nt siRNAs. Subsequently, one strand of the mature small RNAs (miRNAs, siRNAs, or shRNAs) associates with one member of Argonaute family to form the active RNA-induced silencing complex in which the small RNAs serve as guide for identifying mRNA that will be post-transcriptionally silenced (miRNA mediated) or degraded (siRNA mediated). Schematically, if the match between the small RNA and its target is perfect, the target is cleaved and degraded; if the match is imperfect, the translation is inhibited. In mammalian cells, Argonaute 2 (AGO2) is the only member of the four AGO proteins to display RNase III endonucleolytic ('slicer') activity on target RNA, meaning that the four AGO proteins function in miRNA-mediated translational repression while only AGO2 functions in siRNA-mediated destabilization. DGCR8 mutant cells fail to produce canonical miRNAs, but non-canonical miRNAs and siRNAs are produced normally. In mammalian cells, only one *Dicer* gene exists. Its inactivation compromises siRNA and miRNA production and functions. AGO2 inactivation inhibits siRNA activity (among numerous recent reviews (Kim *et al.* 2009, Suh & Blelloch 2011)).

starts as soon as PGCs are formed in embryos (E7.25) and its inactivation in mice results in the germ cell-less phenotype in both sexes (review in Yamaji *et al.* (2010)). However, NANOS2 can substitute for NANOS3 during early PGC development since *Nanos3*-deficient germ cells can be rescued by NANOS2 ectopic expression. By contrast, the loss of NANOS2, which is exclusively expressed in male PGCs after their entry into the genital ridge, results in a male germ cell deficiency, despite the presence of NANOS3 in these cells, indicating that NANOS2 exerts a specific function during male germ cell development (Suzuki *et al.* 2007). Indeed, *Nanos2*^{-/-} fetal gonads overexpress meiotic genes, leading to premature meiosis initiation, and are feminized. Thus, NANOS2 plays a key role by suppressing meiosis and promoting male-type differentiation in the embryonic male gonads. In the adult, it is required to maintain spermatogonial stem cells (Sada *et al.* 2009).

In a recent study, Suzuki *et al.* (2010) shed light on the molecular mechanisms underlying NANOS2 function in gonocytes. They discovered that NANOS2 localizes in cytoplasmic foci that include DCP2 and XRN1 and thus correspond to PBs. They demonstrated that NANOS2 is required both for the formation of PBs and for their gradual increase in male gonocytes after mid-gestation, from E13.5 to E17.5. During this short window of expression, NANOS2 interacts physically with the deadenylation complex CCR4–NOT and is necessary to drive this complex to PBs. In turn, NANOS2 associates with meiotic mRNAs such as *Sycp3*, *Stra8*, or *Dazl* *in vitro* and *in vivo* and is able to trigger their degradation

when associated with the CCR4–NOT complex. Hence, NANOS2 may prevent the precocious activity of meiotic genes in the embryonic male gonads (Suzuki *et al.* 2010). Together, these experiments suggest that NANOS2 mediates transient accumulation of specific mRNAs in PBs where they are stored in a translationally silent state. Alternatively, due to the presence of CCR4–NOT complex, they might be deadenylated and subsequently degraded in this compartment. After birth, once NANOS2 expression begins to disappear, transcripts could be released from PBs and translated to promote germ cell differentiation.

Interestingly, experiments in zebra fish and mice have revealed that *Nanos* expression itself is regulated at the post-transcriptional level. Indeed, in mice, a knock-in strategy revealed that *Nanos2* 3'-UTR is required to enhance the production of NANOS2 protein in the male gonads (Tsuda *et al.* 2006). In zebra fish, where there is only one NANOS member, *Nanos* U-rich 3'-UTR interacts with Dead end (DND), an RBP expressed in PGCs whose binding to mRNAs inhibits micro-RNA (miRNA) access and hence prevents translation repression during PGC development (Tsuda *et al.* 2006, Kedde *et al.* 2007). In mice, the *DND1* mutation leads to depletion of PGCs, teratoma development, and sterility (Cook *et al.* 2011). These defects were shown to be due to a strong downregulation of male differentiation genes, including *Nanos2*, and upregulation of the meiotic markers *Stra8* and *Sycp3*. Whether the increased expression of *Stra8* and *Sycp3* in *Dnd*^{-/-} germ cells is a consequence of *Nanos2* or other DND1 target

misregulation remains to be clarified. Indeed, the phenotype might be contributed by the intrinsic ability of DND1 to bind transcripts that regulate negatively the cell cycle, such as $p21^{Cip1}$ and $p27^{Kip1}$, in such a way that $Dnd1^{-/-}$ germ cells fail to enter mitotic arrest at G0 (Cook *et al.* 2011). This in turn might be responsible for the development of teratomas. Finally, it remains to be investigated whether DND1 exhibits a precise localization in the cytoplasm of fetal germ cells where it could associate and concentrate with its target mRNAs. Altogether, these data highlight the complex network of post-transcriptional regulation allowing timely regulated expression of proteins crucial for maintaining and promoting the male germ cell state and illustrate the potential role of PBs in this process.

PBs and IMC in fetal testes: a role in PIWI pathway

MIWI proteins control spermatogenesis

The Argonaute (AGO) family is divided into two distinct subfamilies: the AGO proteins and the PIWI proteins. While AGO proteins bind to small-interfering RNAs (siRNAs) and miRNAs and play important role in siRNA and miRNA pathways in many different tissues, PIWI proteins are mostly restricted to the germ line. With their small ~26–31 nt long piRNAs partners, they play fundamental and evolutionarily conserved roles in germ line development and gametogenesis (reviewed

in Thomson & Lin (2009)). In mice, three PIWI genes have been described: *Miwi* (Piwil1), *Mili* (Piwil2), and *Miwi2* (Piwil4). MILI and MIWI2 associate with the highly repetitive piRNAs derived from repetitive sequences that are expressed in immature testes. In later stages of spermatogenesis, MILI and MIWI associate to a second class of piRNAs that correspond to non-repetitive elements and become abundant during the pachytene stage (reviewed in Suh & Blelloch (2011) and Box 3). Mice bearing target mutations in *Miwi* (Deng & Lin 2002), *Mili* (Kuramochi-Miyagawa *et al.* 2004), or *Miwi2* (Carmell *et al.* 2007) have been generated. In the three cases, homozygote-deficient males but not females are sterile (Table 1). While the deletion of *Mili* and *Miwi2* results in early arrest of meiosis I (at the zygotene–pachytene transition; Table 1), the deletion of *Miwi* results in later defects that appear in the beginning of the haploid phase (round spermatids; Deng & Lin 2002), showing that MILI/MIWI2 and MIWI have distinct functions. The production of repetitive piRNAs is impaired in *Miwi2*- and *Mili*-deficient testes, expression of retrotransposons (TEs) such as LINE-1 is enhanced, and the TE DNA methylation that normally ensures TE silencing is lost (Kuramochi-Miyagawa *et al.* 2008). These data show that MILI and MIWI2 proteins are involved in the production of pre-pachytene piRNAs and strongly suggest that these repetitive piRNAs promote *de novo* methylation of transposons and their silencing.

Box 3 piRNAs

Among the three major classes of small RNAs that have been described so far, P-element-induced wimpy testis (PIWI)-associated RNAs, piRNAs, about 30 nt long, are mostly germ line specific. They are generated from long single-stranded RNA precursors by a process that is DICER independent and requires PIWI proteins. In mouse, piRNAs have been separated into two classes based on the timing of their expression, their repetitive vs non-repetitive nature, and the PIWI proteins with which they are associated. The highly repetitive piRNAs are encoded by complex and repetitive intergenic sequences. They are expressed before meiotic pachytene stage and are associated with MILI and MIWI2. In contrast, the non-repetitive piRNAs become abundant during the pachytene stage and are associated with MILI and MIWI proteins. Based on the experiments performed in *Drosophila*, a model for biogenesis of a mammalian repetitive piRNAs in the early stages of spermatogenesis has been proposed. In this so called ‘ping-pong mechanism’, the sense-strand piRNA precursor transcripts are exported from the nucleus and directed to cytoplasmic RNA granules where they are recognized by antisense-strand piRNAs (whose production through primary processing is not yet understood) that are bound to MIWI2. MIWI2 cleavage of the sense transposon mRNA generates sense piRNAs that are recognized by MILI. In turn, these sense piRNAs anneal with the antisense strand of the precursor transcript that is subsequently cleaved by MILI, generating a new antisense piRNA that can then bind to MIWI2 (Kim *et al.* 2009). This forms a post-transcriptional amplification loop that leads to more mature piRNAs. Besides MIWI proteins, repetitive piRNA production requires many other proteins that have not yet been identified in mice. In murine germ cells, several recent studies suggest that the two major steps of the amplification loop take place in close but distinct subcellular compartments, which correspond to intermitochondrial cement and processing bodies in fetal prospermatogonia (see Fig. 3 and the main text for references). The functions of piRNAs that are produced in vast amounts remain to be fully investigated. However, several lines of evidence show that in fetal spermatogonia, PIWI proteins and their associated piRNAs lead to epigenetic repression of transposon encoding regions through DNA methylation, the piRNAs working in the nucleus as guides to initiate specific *de novo* methylation of actively transcribed transposons with which they share complementarities (reviewed recently in Kim *et al.* (2009), Meikar *et al.* (2011), and Suh & Blelloch, (2011)).

Table 1 RBPs and some of their partners involved in the biogenesis of small RNAs and/or found in RNA granules of male and female germ cells.

Gene deleted/ phenotype in	Spermatogenesis	Oogenesis	Early embryogenesis	References
<i>Dazl</i>	Loss of germ cells; absence of gamete production; no meiotic initiation; no sex-specific cellular differentiation events (in C57BL/6 background)	No meiotic initiation;	ND	Ruggiu <i>et al.</i> (1997) and Gill <i>et al.</i> (2011)
<i>Cpeb</i>	Arrest at the pachytene stage; impaired formation of the synaptonemal complex; absence of gamete production	absent	ND	Tay & Richter (2001)
<i>Dgcr8</i>	ND	No phenotype	Arrest shortly after implantation	Wang <i>et al.</i> (2007) and Suh <i>et al.</i> (2010)
<i>Dicer</i>	Subfertility; post-meiotic arrest (round-elongating spermatid block); reduced number of PGCs	Early meiotic arrest; massive mRNA misregulation	Arrest shortly after implantation	Bernstein <i>et al.</i> (2003), Murchison <i>et al.</i> (2007), Tang <i>et al.</i> (2007), Hayashi <i>et al.</i> (2008), Maatouk <i>et al.</i> (2008), Spruce <i>et al.</i> (2010) and Suh <i>et al.</i> (2010)
<i>Ago2</i>	No phenotype	Early meiotic arrest (reduced miRNA and siRNA expression; conditional deletion)	No pre-implantation embryos; arrest shortly after implantation (gene trap)	Morita <i>et al.</i> (2007), Hayashi <i>et al.</i> (2008) and Kaneda <i>et al.</i> (2009)
<i>Dnd1</i>	PGC loss (no mitotic arrest at G0) Teratomas on the 129/SvJ background	PGC loss, but not precisely determined	Partial embryonic lethality in the 129/SvJ background; precise stage of lethality ND	Bhattacharya <i>et al.</i> (2007) and Cook <i>et al.</i> (2011)
<i>Miw12</i>	Arrest in meiosis (zygotene–pachytene block)	No phenotype	No phenotype	Carmell <i>et al.</i> (2007), Aravin <i>et al.</i> (2008) and Kuramochi-Miyagawa <i>et al.</i> (2008)
<i>Mili</i>	Arrest in meiosis (zygotene–pachytene block)	No phenotype	No phenotype	Kuramochi-Miyagawa <i>et al.</i> (2004, 2008), Aravin <i>et al.</i> (2006, 2007, 2008) and Unhavaithaya <i>et al.</i> (2009)
<i>MVH/DDX4</i>	Arrest in meiosis (leptotene to zygotene block); loss of IMC; defective piRNA production	No phenotype	No phenotype	Tanaka <i>et al.</i> (2000) and Kuramochi-Miyagawa <i>et al.</i> (2010)
<i>Mael</i>	Arrest in meiosis (zygotene apoptosis); normal pre-pachytene (repetitive) piRNA production	No phenotype	No phenotype	Soper <i>et al.</i> (2008)
<i>TDRD9</i>	Arrest in meiosis (zygotene stage); weak effect on piRNA biogenesis	No phenotype	No phenotype	Shoji <i>et al.</i> (2009)
<i>TDRD1/MTR-1</i>	Arrest in meiosis (zygotene–pachytene block); weak effect on piRNA biogenesis; disruption of IMC; impaired CB integrity in round spermatids	Fertility even though absence of IMC	No phenotype	Chuma <i>et al.</i> (2006), Reuter <i>et al.</i> (2009) and Wang <i>et al.</i> (2009)
<i>Gas2</i>	Arrest in meiosis (zygotene–pachytene block)	No phenotype	No phenotype	Ma <i>et al.</i> (2009)
<i>HuR</i>	Meiotic block at cell divisions and post-meiotic arrest (conditional KO)	No phenotype	Mid-gestation lethality	Ghosh <i>et al.</i> (2009), Katsanou <i>et al.</i> (2009) and Nguyen-Chi <i>et al.</i> (2011)
<i>Miw1</i>	Post-meiotic arrest	No phenotype	No phenotype	Deng & Lin (2002)
<i>TDRD6</i>	Post-meiotic arrest (at elongating (13–14 stage) spermatids); fragmented CBs in round spermatids; increased miRNA expression	No phenotype	No phenotype	Vasileva <i>et al.</i> (2009) and Tanaka <i>et al.</i> (2011)
<i>TDRD7</i>	Post-meiotic arrest (at round spermatids, before 4–6 stages); IMC intact; impaired CB architecture in round spermatids; not required for piRNA biogenesis	No phenotype	No phenotype	Vasileva <i>et al.</i> (2009) and Tanaka <i>et al.</i> (2011)

Some indications on the consequences of their deletion in oogenesis, spermatogenesis, and early embryogenesis are mentioned. ND, not determined.

MIWI2/MAEL/TDRD9 and MILI/TDRD1/GASZ exhibit different subcellular localizations that impact on piRNA production

Examination of *Miwi2* and *Mili* expression in embryonic germ cells (from E18.5 male embryos) brought remarkable information concerning the cytoplasmic localization of proteins involved in the fetal piRNA pathway (Aravin *et al.* 2009, Shoji *et al.* 2009, Wang *et al.* 2009). Using a combination of IF, EM, and immuno-EM, it was shown that MILI and MIWI2 localize to distinct types of germinal cytoplasmic granule. On the one hand, MIWI2 colocalizes with two other proteins MAEL and TDRD9 in granules that are enriched in markers for somatic PBs, such as DDX6 and GW182 (Shoji *et al.* 2009), and were thus called 'piP-bodies' (piPBs) to indicate simultaneous presence of piRNA pathway and P-body components (Fig. 1; Aravin *et al.* 2009). MAEL is the murine homolog of the *Drosophila* protein Maelstrom that is required for the production of piRNAs during *Drosophila* oogenesis (review in Klattenhoff & Theurkauf (2008)). MAEL contains neither Piwi/Argonaute/Zwille (PAZ) or PIWI domain characteristic of AGO proteins nor any other known RNA-binding domain but is required for TE silencing (Soper *et al.* 2008). In mice, its inactivation gives rise to a spermatogenic defect similar to that observed in mutants lacking *Mili* and *Miwi2* (O'Donnell *et al.* 2008, Soper *et al.* 2008). TDRD9 is an ATPase/DEXH-type helicase that contains a TUDOR domain that targets methylated proteins and whose mutation causes male sterility due to a meiotic failure similar to that observed in *Miwi2* mutants (Shoji *et al.* 2009; Table 1). MAEL is not only required for transposon silencing but also required for the organization of piPBs since its deletion leads to ultrastructural changes of piPBs and impaired localization of MIWI2 and TDRD9 that stay diffuse in the cytoplasm (Aravin *et al.* 2009).

On the other hand, MILI accumulates with TDRD1, another germ line TUDOR domain containing protein (Reuter *et al.* 2009) into a cytoplasmic structure, called the IMC or pi-bodies or piBs, that constitutes a type of germinal cytoplasmic granules distinct from piPBs as visualized by EM (Fig. 1; Tanaka *et al.* 2011) and also because they do not contain components of PBs (Aravin *et al.* 2009). At this developmental stage, IMC also contains germ cell-specific protein with four Ankyrin Repeats, a Sterile alpha motif and a basic leucine Zipper domain (GASZ) that lacks known RNA-interacting domain but whose deletion phenocopies the male sterility defect observed in *Mili* mutant testes (Ma *et al.* 2009). IMC/piBs are lost in the absence of *Tdrd1*, *Mili* (Kuramochi-Miyagawa *et al.* 2010), or *Gasz*, the later one leading to a progressive absence of MILI and TDRD1 expression in germ cells (Ma *et al.* 2009). Remarkably, MILI, GASZ, and TDRD1 not only mutually control their localization but also regulate the localization of

MIWI2–MAEL–TDRD9 in piPBs. This is illustrated by *Mili* deletion that leads to a complete loss of accumulation of MAEL or TDRD9 in piPBs and a relocalization of MIWI2 – whose expression is normally prominent in the nucleus – to the cytoplasm (Shoji *et al.* 2009). The same situation was observed in *Tdrd1* mutant fetal germ cells, where TDRD9 and MIWI2 were dispersed in the cytoplasm and granules were not assembled (Reuter *et al.* 2009, Shoji *et al.* 2009). Also, in *Gasz* mutant germ cells, there is a strong reduction in the expression of MILI, MAEL, TDRD1, MVH, and TDRD6 (see below; Ma *et al.* 2009). This pinpoints the dynamic interactions between piPBs and IMC/pi-bodies, reflecting most probably at the molecular level, the requirement of both structures to produce repetitive piRNAs that are required to suppress TE expression (Kim *et al.* 2009, Thomson & Lin 2009, Suh & Blelloch 2011; Box 3 and Fig. 3). However, it remains to be determined whether the meiotic prophase defects observed in MIWI2, MILI, MAEL, and GASZ mutants are exclusively caused by TE activation or if these proteins exert other specific roles during meiosis, such as chromosome synapsis, meiotic recombination, or control of mRNA translation (Ma *et al.* 2009).

piRNA processing and gene silencing of TEs also require MVH

The complexity of piRNA processing and gene silencing of TEs in murine germ cells has been recently illustrated by the study of another crucial component of nuage, MVH. MVH belongs to the family of DEAD-box RNA helicases and is expressed exclusively in germ lineage. In *Drosophila*, its homolog VASA is essential for germ cell development and oogenesis, while in mice MVH is not required for oogenesis but is crucial for spermatogenesis where it is expressed from the middle of gestation (E10.5) to round spermatid (reviewed in Kuramochi-Miyagawa *et al.* (2010)). Indeed, *Mvh* deletion leads to male germ cell differentiation arrest during meiotic prophase with anomalies comparable to that observed in *Miwi2*- and *Mili*-deficient testes, including defects in piRNA processing and gene silencing of transposons (Tanaka *et al.* 2000, Kuramochi-Miyagawa *et al.* 2010). In fetal germ cells, similar to *Tdrd1* or *Mili* mutation, *Mvh* deficiency induces the loss of IMC, and thus mislocalization of TDRD1 and MILI. However, it does not affect piPB formation, as seen at the molecular level using TDRD9 as a marker of piPBs and at the ultrastructure level by EM (Kuramochi-Miyagawa *et al.* 2010). Unexpectedly, MIWI2 is not present in those piPBs but remains in the nucleus. Since MIWI2 localization has been suggested to depend on piRNA expression, its mislocalization in *Mvh* mutant cells may be due to the decreased piRNA levels (Kuramochi-Miyagawa *et al.* 2010). Since in *Miwi2* mutants piRNA production is significantly reduced but IMC is intact, it is suggested that MVH rather than piRNAs is required for the construction of IMC (Kuramochi-Miyagawa *et al.* 2010).

Figure 3 Components of 'nuage' structures and remodeling of chromatoid bodies during spermatogenesis. (Left) In the cytoplasm of prospermatogonia that populate fetal testes, two RNA containing structures have been described that seem to play important roles in repetitive piRNA biosynthesis and mRNA storage/degradation. On the one hand, IMC or pi-body concentrates MILI, MVH, GASZ, and two members of the TUDOR family, TDRD1 and 7 (Chuma *et al.* 2006, 2009). On the other hand, pi-P Body (piPB) includes MIWI2, MAEL, and TDRD9, which are required for their integrity, as well as recognized components of somatic PBs, such as DCP1a, DDX6, GW182, DCP2, XRN1, and p54/RCK (Aravin *et al.* 2009, Shoji *et al.* 2009, Kuramochi-Miyagawa *et al.* 2010, Suzuki *et al.* 2010, Tanaka *et al.* 2011). The RBP NANOS2 also belongs to PBs where it brings components of the CCR4–NOT deadenylation complex (Suzuki *et al.* 2010). Interplay between IMC and piPBs appears necessary to generate repetitive piRNAs through a positive amplification loop (the ping-pong cycle described in Box 3 and in the recent review by Suh & Blelloch (2011)). (Right) In the cytoplasm of post-meiotic haploid round spermatids, a unique RNA containing structure, the chromatoid body (CB) is present that concentrates numerous components, some of which were already present in pre-meiotic prospermatogonia or at meiotic spermatocyte steps. This led to the proposal that the CB represents a hybrid structure formed from the pre-existing IMC and pi-PB granules (Tanaka *et al.* 2011). CBs contain miRNAs and polyadenylated mRNAs as well as many proteins involved in various RNA pathways: piRNA biosynthesis (MVH, TDRD1, 6, 7, 9, MAEL, MILI, and MIWI), miRNA pathway (AGO2–3, DICER), mRNA decay (DDX6, GW182, and AGO2), or translation (HuR, TIAR, PABP, eIF4E, and eIF3; Nguyen-Chi *et al.* 2009, Meikar *et al.* 2011, Tanaka *et al.* 2011). Whether all of them are simultaneously active or only after reception of timely regulated signals and in which RNA metabolism pathway are they involved remain important questions for the future.

Whatever the case, this study highlights the essential role of MVH in the formation of IMC and in the communication between IMC and pi-PBs for piRNA biogenesis and subsequent TE silencing in fetal testes.

CBs in adult spermatids: a center for multiple small RNA pathways

miRNA pathway

At later stages during male germ cell differentiation, MVH, MAEL, MIWI, MILI, TDRD1, and TDRD9 localize within the CB of round spermatids (reviewed in Vasileva *et al.* (2009)). CBs also harbor components of somatic PBs, such as DCP1a, XRN1, and GW182 (reviewed in Meikar *et al.* (2011); Fig. 3). Their presence suggests that mRNA decay is at work in CBs (Kotaja *et al.* 2006a), but to date, there is no experimental evidence sustaining this hypothesis. In addition, the CB concentrates many components of the miRNA pathway, including DICER, which interacts with MVH, AGO2, AGO3, miRNAs, and polyadenylated mRNAs (Box 2), strongly suggesting that functional miRNA-mediated mRNA control takes place

in this compartment (Kotaja *et al.* 2006a, Kotaja & Sassone-Corsi 2007). Testing this hypothesis requires the generation of sophisticated mice in which a given CB component (DICER or AGO2, for instance) would be specifically inactivated at the onset of spermiogenesis. Such mice are not yet available. However, the study of mice with specific loss of *Dicer* in the germ line has already brought important information concerning the requirement of miRNA pathway for the completion of spermatogenesis (Hayashi *et al.* 2008, Maatouk *et al.* 2008). Indeed, *Dicer*-deleted PGCs and spermatogonia exhibit poor proliferation (Hayashi *et al.* 2008) and male fertility is severely reduced with spermiogenesis defects similar to those observed in *Miwi* mutants (Deng & Lin 2002). piRNA production is not altered in *Dicer1* mutant germ cells, suggesting that the phenotype is due to a loss of endo-siRNAs or miRNAs (Vasileva *et al.* 2009). However, normal spermatogenesis is observed in *Ago2* mutant testes where this protein is required for endo-siRNA production, showing that endo-siRNAs are not necessary to fulfill male germ cell differentiation (Hayashi *et al.* 2008). The miRNA pathway is not

affected in *Ago2* mutant cells possibly due to strong redundancy of mammalian AGO proteins in terms of miRNA activity (Su *et al.* 2009). All together, these studies show that the function of DICER in spermatogenesis is independent of AGO2 and strongly suggest that an altered miRNA pathway is responsible for the phenotype observed in *Dicer* mutants (reviewed in Suh & Blelloch (2011)).

Involvement of miRNAs in spermatogenesis is strengthened by the study of another TUDOR family member, TDRD6. By contrast to other family members, TDRD6 is not expressed in male embryonic gonads but appears first in primary spermatocytes (17.5 days *post partum* (dpp)) where it physically interacts with MVH, MILI, and MIWI (Vasileva *et al.* 2009). In *Tdrd6*^{-/-}, mutant testes, post-meiotic round spermatids do not elongate, a phenotype similar to *Dicer* mutants (Vasileva *et al.* 2009, Tanaka *et al.* 2011). CB architecture is disrupted in these cells, most probably because MAEL, MIWI, and MVH are mislocalized in the absence of TDRD6. TEs are not activated, showing that the piRNA pathway takes place normally, but many miRNAs and their precursors are upregulated. Altogether, these data demonstrate that TDRD6 is involved in CB formation and regulates miRNA expression (Vasileva *et al.* 2009).

Understanding the relationship between CBs and PB-like granules

Last but not least, TDRD6 does not act alone but in concert with another member of *Tdrd* gene family TDRD7 that also concentrates in the CB of round spermatids but is expressed earlier in fetal gonocytes (Tanaka *et al.* 2011). *Tdrd7*^{-/-} germ cell differentiation stops at the round spermatid stage (Table 1). Careful EM examination of *Tdrd7*^{-/-} spermatocytes and spermatids revealed that their CBs harbor a very peculiar architecture that is not observed in other *Tdrd* mutants. In particular, the CB and PB components that are expressed in the same 'hybrid' structure in wild type (WT) round spermatids do not merge in *Tdrd7* mutant spermatids (Tanaka *et al.* 2011). These findings and other observations made in pachytene spermatocytes where TDRD7 concentrates in IMC helped to build a model for the dynamics of CB formation during spermatogenesis. In this model, TDRD7 and TDRD6 sequentially contribute to structure the CB: TDRD7 first allowing PB and CB coalescence in a unique hybrid structure and TDRD6 maintaining the hybrid CB architecture at later phases of spermiogenesis (Tanaka *et al.* 2011; Fig. 3). Importantly, the piRNA production and DNA methylation of TEs were normal in *Tdrd7*^{-/-} testes, showing that the structural integrity of CBs is not a prerequisite for piRNA biogenesis and function.

Many other proteins in the CB: the case of HuR

Among the numerous RBPs accumulating in the CB and awaiting for further characterization and functional

analyses (Meikar *et al.* 2011), we have recently carefully analyzed HuR/ELAVL1. HuR was first described for its ability to control stability and translation of U- and AU-rich mRNAs in somatic cells (reviewed in Hinman & Lou (2008)). More recently, HuR was also shown to counteract miRNA activity by inhibiting their ability to direct their associated mRNAs to PBs. Thus, HuR can suppress the inhibitory effect of miRNAs by redirecting mRNAs to polysomes (Meisner & Filipowicz 2010). In the germ cells, HuR is mainly localized in the nucleus but transiently concentrates in the CB of early round spermatids where it interacts with MVH (Nguyen-Chi *et al.* 2009). In later stages of spermatid differentiation, it exits the CB and travels to polysomes, together with its target mRNAs, suggesting that this RBP participates to mRNA storage/translation in round spermatids (Nguyen-Chi *et al.* 2009). Conditional deletion of HuR has no effect on oogenesis but severely compromises spermatogenesis, leading to extensive death of spermatocytes during meiotic divisions (Nguyen-Chi *et al.* 2011). In addition, the few mutant spermatocytes that survive beyond that meiotic stage fail to proceed through spermatid elongation, a phenotype similar to *Tdrd6*, *Tdrd7*, and *Miwi* mutant testes (Nguyen-Chi *et al.* 2011). At the molecular level, the defects observed in *HuR*^{-/-} germ cells were correlated with decreased translation of *Hspa2*, a member of heat-shock protein family that is crucial for spermatogenesis (Nguyen-Chi *et al.* 2011 and references therein). Absence of HuR could lead to misexpression of other ARE- or U-rich-mRNAs whose translation is directly controlled by HuR, like *Hspa2*, or indirectly through its interplay with miRNAs. Further sophisticated studies are necessary to address the mechanisms of action of HuR in germ cells.

All together, these data show that CBs are dynamic RNP structures that are remodeled during spermatogenesis. CBs contain actors of mRNA degradation, miRNA and piRNA pathways and thus might represent crucial centers of post-transcriptional processing and storage of mRNAs that are maintained translationally silent. Release of mRNAs from this structure could be a mean to activate their translation once the genome of spermatids becomes transcriptionally inactive (Parvinen 2005, Kotaja *et al.* 2006a, Nguyen-Chi *et al.* 2009).

RNA granules and mRNA stability/translation in mouse oocytes

In mice, the final stages of oocyte maturation, fertilization, and early embryonic development occur in the absence of gene transcription. Thus, the post-transcriptional regulation of resident maternal mRNAs, including storage, regulated mRNA translation, and degradation, is a fundamental process to permit oocyte maturation and early embryonic divisions until the zygote genome becomes activated at the two-cell stage (Fig. 2; Kang &

Han 2011). In parallel with what we have learnt from mouse male germ lineage and what we know from oocytes of *Drosophila*, *Xenopus*, and *C. elegans* (Aizer *et al.* 2008, Noble *et al.* 2008, Radford *et al.* 2008, Standart & Minshall 2008), it is tempting to speculate that RNA granules might also participate in the control of gene expression during mouse oocyte growth, maturation, and early embryo development. Although information on RNA granules, their dynamics, their protein, and RNA content in mouse oocyte is still scarce, several recent studies give support to this assertion, showing that PBs and IMC are present at different steps of oogenesis and that some of their RBPs play fundamental role in the control of mRNA translation.

PBs and miRNAs

In a recent paper, Flemr *et al.* (2010) have shown using IF confocal analysis and a battery of antibodies recognizing components of PBs in somatic cells that PBs are present in meiotically incompetent oocytes (Fig. 2). Subsequently, typical PBs disappear in fully grown germinal vesicle (GV) oocytes. A subset of PB components, but not the RNA-decapping enzyme DCP1A, accumulates instead in subcortical aggregates (SCAs). These structures also contain untranslated polyadenylated maternal mRNAs and several RBPs, including CPEB, a protein that binds the cytoplasmic polyadenylation element (CPE) motif present in various 3'-UTR and controls their polyadenylation (see below; Richter 2007; Fig. 2). Upon resumption of meiosis, SCAs disintegrated, thus releasing maternal mRNAs that become available for translation or degradation. The first wave of mRNA degradation takes place concomitantly owing in part to modifications of the length of their polyA tail (Richter 2007, Brook *et al.* 2009, Zuccotti *et al.* 2011) and to the upregulation of DCP1A expression (Flemr *et al.* 2010). PBs remain absent in pre-implantation embryos until the morula stage.

Because PB formation in somatic cells relies on miRNA-mediated mRNA silencing (Eulalio *et al.* 2007), it was hypothesized that the absence of PBs could reflect inefficient miRNA activity in oocytes. Indeed, it was shown that maternal miRNAs poorly repress endogenous or transgenic target mRNAs (Ma *et al.* 2010). In addition, oocytes lacking the maternal DGCR8 RBP, which is required for the miRNA but not the endo-siRNA-mediated pathway (Box 2; Suh & Blelloch (2011)), develop normally and can be fertilized. *Dgcr8*^{-/-} pre-implantation embryos progress normally even when both maternal and zygotic *Dgcr8* alleles have been deleted, showing that neither maternal nor zygotic DGCR8 is needed for oogenesis and early embryonic development (Suh *et al.* 2010; Table 1). In contrast, the loss of DICER or AGO2, both of which are required for endo-siRNA biosynthesis, leads to meiotic defects (Table 1), showing that endo-siRNAs regulate meiosis (Hayashi *et al.* 2008, Maatouk *et al.* 2008) and reviewed

in Suh & Blelloch (2011). These results demonstrate that despite the fact that miRNAs are abundant in the oocyte (Tang *et al.* 2007), miRNA activity – at least from canonical miRNAs (Box 2) – is not required during oocyte growth and oocyte-to-zygote transition. Together, these data leave open the exciting hypothesis that suppression of miRNA function is a pre-requisite for the major genome reprogramming that takes place at the very beginning of mammalian development and that most probably requires active endo-siRNA pathway (Svoboda 2010).

RBPs and translational control

The translation of maternal mRNAs in the oocyte is regulated by various mechanisms, including the control of mRNA polyA tail length and recruitment to polysomes. Inhibition or activation of translation relies on *cis*-acting elements usually located in the 3'-UTR of mRNAs and the small RNAs and RBPs with which they interact (reviewed in Vasudevan *et al.* (2006), Brook *et al.* (2009) and MacNicol & MacNicol (2010)). The roles of specific RBPs in these processes, in particular CPEB, PABP, Pumilio2, or Musashi, have been well studied in *Xenopus* (reviewed in Vasudevan *et al.* (2006) and MacNicol & MacNicol (2010)). Yet, more limited information is available in mice as far as their mechanisms of action and their subcellular localization are concerned. In mice, CPEB mediates cytoplasmic polyadenylation of many mRNAs, a process that is required at multiple steps during the female meiotic program (reviewed in Belloc *et al.* (2008)). The requirement of CPEB during oogenesis was demonstrated by the loss of oocytes at the pachytene stage in *Cpeb1* mutant females (Tay & Richter 2001). Similarly, absence of *Dazl* results in a complete loss of female germ cells before birth (Ruggiu *et al.* 1997; Table 1). Among DAZ (deleted in azoospermia) family of RBPs that were extensively studied in males (Lee *et al.* 2006), DAZL is the only family member detected in the cytoplasm of mouse oocytes at all oogenic stages (Ruggiu *et al.* 1997). In male germ cells, DAZL binds to a subset of mRNAs via a specific DAZL-binding motif and regulates their translation (Lee *et al.* 2006). In addition, it interacts with the dynein–dynactin complex suggesting that it controls their transport and recruitment to polysomes. In females, a recent large-scale analysis of mRNAs either not translated or associated with polysomes at various stages of oocyte maturation *in vivo* sheds light on CPEB and DAZL interplay and function. Analysis of the 3'-UTRs of polysome-associated mRNAs revealed that a large part of them contain CPEB1 and DAZL consensus binding motifs. Specific inactivation of DAZL or CPEB expression by injection of morpholino oligonucleotides into GV oocytes significantly decreases oocyte progression through meiosis. However, DAZL and CPEB are not expressed simultaneously but rather show opposite

pattern of expression, CPEB1 expression being high in GV oocytes and decreasing rapidly following oocyte maturation, while DAZL expression increases in the mean time (up to meiosis II (MII) and zygote). Interestingly, *Dazl* mRNA not only contains CPE but also numerous DAZL consensus motifs, leading to the hypothesis that *Dazl* expression might be sequentially controlled by CPEB1 and DAZL. In the proposed model, CPEB1 initially promotes *Dazl* polyadenylation and translation during the GV–MI transition. Then, the newly synthesized DAZL protein increases the translation of its own mRNA. This post-transcriptional regulatory cascade leads to a progressive accumulation of DAZL, which in turn promotes the translation of DAZL target mRNAs, including *Tpx2*, *Tex19.1*, and other regulators of the cell cycle necessary for spindle assembly, the MI–MII transition, and early embryonic development (Chen *et al.* 2011). In addition, DAZL is known to interact with several other proteins, including Pumilio2 that may act as a translational repressor (Vasudevan *et al.* 2006, Brook *et al.* 2009, MacNicol & MacNicol 2010). Understanding more precisely how these proteins interplay and whether their activity is compartmentalized in the cytoplasm remains to be investigated, even though some data are already available. As mentioned above, CPEB1 expression is dynamic during oogenesis (Flemr *et al.* 2010). On the other hand, DAZL has been shown in various human tumoral cell lines to exhibit a cytoplasmic granular expression (<http://www.proteinatlas.org/ENSG00000092345/subcellular>). In addition, when over-expressed in HeLa cells, DAZL concentrates in cytoplasmic RNA granules that most probably correspond to SGs because they contain TIA1, a characteristic marker of SGs (Lee *et al.* 2006; Box 1). These data strongly suggest that in somatic cells, DAZL participates to the storage of its target mRNAs at specific cytoplasmic sites. In oocyte, DAZL seems to be diffusely distributed throughout the oocyte cytoplasm but is also enriched at the poles of both MI and MII metaphase spindles (Chen *et al.* 2011). A careful simultaneous analysis of DAZL and CPEB1 localization throughout oogenesis remains to be undertaken.

IMCs and piRNPs

Most RBPs that belong to specialized RNA structures, IMC, piPBs, or CBs in male germ cells, are also expressed during mouse oogenesis. Although a detailed study of their precise subcellular localization at various steps of oogenesis has not yet been undertaken, it is striking to observe that their inactivation has no detrimental consequences on oogenesis, while they all lead to male sterility (Table 1). For instance, the deletion of *Miwi*, *Mili*, *Miwi2*, *Mvh*, *Tdrd1*, or *Tdrd9* that control IMC or piPB formation and contribute to piRNA production and TE silencing in male gonocytes does not alter oogenesis. Chuma *et al.* (2006, 2009) analyzed in detail the localization of MVH and TDRD1 and found that

they concentrate in IMC of both male and female germ cells. Despite the fact that *Tdrd1* mutants lack, and *Mvh* mutants show great reduction of, IMC in spermatogenic cells and oocytes, the males were sterile and the females fully fertile, showing that IMC formation is not a prerequisite for oocyte development and female fertility (Chuma *et al.* 2006). These results are particularly surprising in view of the fact that in *Drosophila*, the mutations in *Piwi*, *Aubergine* (*Aub*), *Ago3*, and other piRNA pathway genes all lead to severe defects in oogenesis, including loss of germ line stem cells, accompanied by a loss of piRNA production and a loss of TE silencing (Klattenhoff & Theurkauf 2008). Therefore, it remains to be understood how TE silencing takes place in mouse oogenesis and why meiosis appears normal in the females that do not express one of the major actors of piRNA pathway. However, if these proteins act redundantly, it might be necessary to generate double or triple mutants to reveal the role of piRNAs in the mouse female germ line.

Concluding remarks and perspectives

Since their first discovery, at the end of the 19th century (Arkov & Ramos 2010), germ granules have delivered many of their secrets. EM, fluorescence imagery, and biochemical experiments have been particularly useful to describe the RNA and protein content of those cytoplasmic non-membranous and fibrous organelles, appreciate their diversity and dynamics, and understand their formation during germ cell differentiation. More recently, the discovery of their implication in small RNAs biosynthesis has reinforced emphasis on their study. Numerous mouse knockout (KO) models have been generated to elucidate the physiological function(s) of a given RBP, alone or associated with its partners, in oogenesis or spermatogenesis. From their analysis and other studies briefly mentioned in this review, it appears that numerous RBPs involved in germ cell differentiation and fertility are not diffuse in the cytosol. Instead, they exhibit a timely regulated subcellular cytoplasmic compartmentalization that is essential for their activity through mechanisms conserved in distant organisms. However, beyond this paradigm, there are pending questions that remained to be addressed specifically with the mouse model. In particular:

Why do mouse female germ cells seem more refractory to impaired assembly of mRNPs than male ones whose differentiation is arrested when RNA granules cannot form? Is there any relationship with the peculiar sexual differentiation of PGCs in mammalian cells? Is it because miRNAs or piRNAs are not playing any role during oogenesis, a striking observation that remains to be confirmed?

There are also several questions concerning the subtitle of this review, ‘is unity strength?’ What is the purpose of

concentrating factors in discrete RNA-containing foci? Is it to deplete the cytoplasm from adverse effects of certain enzymes or is it to concentrate proteins whose activity requires otherwise non-abundant cofactors, facilitating their specific post-translational modifications, if required?

What are the signals that orchestrate the timely regulated assembly of proteins and their RNA partners in a given RNA structure? Are all the proteins belonging to a given RNA structure active simultaneously or active at all?

Refined approaches based on the generation of timely programmed mutations using sophisticated KO strategies and production of antibodies recognizing specifically an RBP or one of its post-translation modifications constitute necessary but doable tools to address these questions in the near future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Funding

This review did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

We apologize to colleagues whose work could not be cited due to space limitation. We thank Dr E Christians and N Vanzo for stimulating discussion and critical reading of the manuscript.

References

- Aizer A & Shav-Tal Y 2008 Intracellular trafficking and dynamics of P bodies. *Prion* **2** 131–134. (doi:10.4161/pri.2.4.7773)
- Aizer A, Brody Y, Ler LW, Sonenberg N, Singer RH & Shav-Tal Y 2008 The dynamics of mammalian P body transport, assembly, and disassembly in vivo. *Molecular Biology of the Cell* **19** 4154–4166. (doi:10.1091/mbc.E08-05-0513)
- Aravin A, Gaidatzis D, Pfeffer S, Lagos-Quintana M, Landgraf P, Iovino N, Morris P, Brownstein MJ, Kuramochi-Miyagawa S, Nakano T *et al.* 2006 A novel class of small RNAs bind to MILI protein in mouse testes. *Nature* **442** 203–207. (doi:10.1038/nature04916)
- Aravin AA, Sachidanandam R, Girard A, Fejes-Toth K & Hannon GJ 2007 Developmentally regulated piRNA clusters implicate MILI in transposon control. *Science* **316** 744–747. (doi:10.1126/science.1142612)
- Aravin AA, Sachidanandam R, Bourc'his D, Schaefer C, Pezic D, Toth KF, Bestor T & Hannon GJ 2008 A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. *Molecular Cell* **31** 785–799. (doi:10.1016/j.molcel.2008.09.003)
- Aravin AA, van der Heijden GW, Castaneda J, Vagin VV, Hannon GJ & Bortvin A 2009 Cytoplasmic compartmentalization of the fetal piRNA pathway in mice. *PLoS Genetics* **5** e1000764. (doi:10.1371/journal.pgen.1000764)
- Arkov AL & Ramos A 2010 Building RNA-protein granules: insight from the germline. *Trends in Cell Biology* **20** 482–490. (doi:10.1016/j.tcb.2010.05.004)
- Belloc E, Pique M & Mendez R 2008 Sequential waves of polyadenylation and deadenylation define a translation circuit that drives meiotic progression. *Biochemical Society Transactions* **36** 665–670. (doi:10.1042/BST0360665)
- Bernstein E, Kim SY, Carmell MA, Murchison EP, Alcorn H, Li MZ, Mills AA, Elledge SJ, Anderson KV & Hannon GJ 2003 Dicer is essential for mouse development. *Nature Genetics* **35** 215–217. (doi:10.1038/ng1253)
- Bhattacharyya SN, Habermacher R, Martine U, Closs EI & Filipowicz W 2006 Relief of microRNA-mediated translational repression in human cells subjected to stress. *Cell* **125** 1111–1124. (doi:10.1016/j.cell.2006.04.031)
- Bhattacharya C, Aggarwal S, Zhu R, Kumar M, Zhao M, Meistrich ML & Matin A 2007 The mouse dead-end gene isoform alpha is necessary for germ cell and embryonic viability. *Biochemical and Biophysical Research Communications* **355** 194–199. (doi:10.1016/j.bbrc.2007.01.138)
- Brook M, Smith JW & Gray NK 2009 The DAZL and PABP families: RNA-binding proteins with interrelated roles in translational control in oocytes. *Reproduction* **137** 595–617. (doi:10.1530/REP-08-0524)
- Carmell MA, Girard A, van de Kant HJ, Bourc'his D, Bestor TH, de Rooij DG & Hannon GJ 2007 Miwi2 is essential for spermatogenesis and repression of transposons in the mouse male germline. *Developmental Cell* **12** 503–514. (doi:10.1016/j.devcel.2007.03.001)
- Chen J, Melton C, Suh N, Oh JS, Horner K, Xie F, Blueloch R & Conti M 2011 Genome-wide analysis of translation reveals a critical role for deleted in azoospermia-like (*Dazl*) at the oocyte-to-zygote transition. *Genes and Development* **25** 755–766. (doi:10.1101/gad.2028911)
- Chuma S, Hosokawa M, Kitamura K, Kasai S, Fujioka M, Hiyoshi M, Takamune K, Noce T & Nakatsuji N 2006 Tdrd1/Mtr-1, a tudor-related gene, is essential for male germ-cell differentiation and nuage/germinal granule formation in mice. *PNAS* **103** 15894–15899. (doi:10.1073/pnas.0601878103)
- Chuma S, Hosokawa M, Tanaka T & Nakatsuji N 2009 Ultrastructural characterization of spermatogenesis and its evolutionary conservation in the germline: germinal granules in mammals. *Molecular and Cellular Endocrinology* **306** 17–23. (doi:10.1016/j.mce.2008.11.009)
- Cook MS, Munger SC, Nadeau JH & Capel B 2011 Regulation of male germ cell cycle arrest and differentiation by DND1 is modulated by genetic background. *Development* **138** 23–32. (doi:10.1242/dev.057000)
- Deng W & Lin H 2002 Miwi, a murine homolog of piwi, encodes a cytoplasmic protein essential for spermatogenesis. *Developmental Cell* **2** 819–830. (doi:10.1016/S1534-5807(02)00165-X)
- Eddy EM 1975 Germ plasm and the differentiation of the germ cell line. *International Review of Cytology* **43** 229–280. (doi:10.1016/S0074-7696(08)60070-4)
- Eulalio A, Rehwinkel J, Stricker M, Huntzinger E, Yang SF, Doerks T, Dörner S, Bork P, Boutros M & Izaurralde E 2007 Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. *Genes and Development* **21** 2558–2570. (doi:10.1101/gad.443107)
- Fawal M, Jean-Jean O, Vanzo N & Morello D 2011 Novel mRNA-containing cytoplasmic granules in ALK-transformed cells. *Molecular Biology of the Cell* **22** 726–735. (doi:10.1091/mbc.E10-07-0569)
- Fawcett DW, Eddy EM & Phillips DM 1970 Observations on the fine structure and relationships of the chromatoid body in mammalian spermatogenesis. *Biology of Reproduction* **2** 129–153. (doi:10.1095/biolreprod.2.1.129)
- Flemr M, Ma J, Schultz RM & Svoboda P 2010 P-body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. *Biology of Reproduction* **82** 1008–1017. (doi:10.1095/biolreprod.109.082057)
- Ghosh M, Aguila HL, Michaud J, Ai Y, Wu MT, Hemmes A, Ristimäki A, Guo C, Furneaux H & Hla T 2009 Essential role of the RNA-binding protein HuR in progenitor cell survival in mice. *Journal of Clinical Investigation* **119** 3530–3543. (doi:10.1172/JCI38263)
- Gill ME, Hu YC, Lin Y & Page DC 2011 Licensing of gametogenesis, dependent on RNA binding protein DAZL, as a gateway to sexual differentiation of fetal germ cells. *PNAS* **108** 7443–7448. (doi:10.1073/pnas.1104501108)
- Hayashi K, Chuva de Sousa Lopes SM, Kaneda M, Tang F, Hajkova P, Lao K, O'Carroll D, Das PP, Tarakhovskiy A, Miska EA *et al.* 2008 MicroRNA

- biogenesis is required for mouse primordial germ cell development and spermatogenesis. *PLoS ONE* **3** e1738. (doi:10.1371/journal.pone.0001738)
- Hemberger M, Dean W & Reik W 2009 Epigenetic dynamics of stem cells and cell lineage commitment: digging Waddington's canal. *Nature Reviews. Molecular Cell Biology* **10** 526–537. (doi:10.1038/nrm2727)
- Hinman MN & Lou H 2008 Diverse molecular functions of Hu proteins. *Cellular and Molecular Life Sciences* **65** 3168–3181. (doi:10.1007/s00018-008-8252-6)
- Houston DW & King ML 2000 Germ plasm and molecular determinants of germ cell fate. *Current Topics in Developmental Biology* **50** 155–181.
- Huckins C 1978 Spermatogonial intercellular bridges in whole-mounted seminiferous tubules from normal and irradiated rodent testes. *American Journal of Anatomy* **153** 97–121. (doi:10.1002/aja.1001530107)
- Ikenishi K 1998 Germ plasm in *Caenorhabditis elegans*, *Drosophila* and *Xenopus*. *Development, Growth & Differentiation* **40** 1–10. (doi:10.1046/j.1440-169X.1998.t014-00001.x)
- Kaneda M, Tang F, O'Carroll D, Lao K & Surani MA 2009 Essential role for Argonaute2 protein in mouse oogenesis. *Epigenetics & Chromatin* **2** 9. (doi:10.1186/1756-8935-2-9)
- Kang MK & Han SJ 2011 Post-transcriptional and post-translational regulation during mouse oocyte maturation. *BMB Reports* **44** 147–157. (doi:10.5483/BMBRep.2011.44.3.147)
- Katsanou V, Milatos S, Yiakouvakis A, Sgantzis N, Kotsoni A, Alexiou M, Harokopos V, Aidinis V, Hemberger M & Kontoyiannis DL 2009 The RNA binding protein Elavl1/HuR is essential for placental branching morphogenesis and embryonic development. *Molecular and Cellular Biology* **29** 2762–2776. (doi:10.1128/MCB.01393-08)
- Kedde M, Strasser MJ, Boldajipour B, Oude Vrielink JA, Slanchev K, le Sage C, Nagel R, Voorhoeve PM, van Duijse J, Orom UA *et al.* 2007 RNA-binding protein Dnd1 inhibits microRNA access to target mRNA. *Cell* **131** 1273–1286. (doi:10.1016/j.cell.2007.11.034)
- Kiebler MA & Bassell GJ 2006 Neuronal RNA granules: movers and makers. *Neuron* **51** 685–690. (doi:10.1016/j.neuron.2006.08.021)
- Kim VN, Han J & Siomi MC 2009 Biogenesis of small RNAs in animals. *Nature Reviews. Molecular Cell Biology* **10** 126–139. (doi:10.1038/nrm2632)
- Klattenhoff C & Theurkauf W 2008 Biogenesis and germline functions of piRNAs. *Development* **135** 3–9. (doi:10.1242/dev.006486)
- Kotaja N & Sassone-Corsi P 2007 The chromatoid body: a germ-cell-specific RNA-processing centre. *Nature Reviews. Molecular Cell Biology* **8** 85–90. (doi:10.1038/nrm2081)
- Kotaja N, Bhattacharyya SN, Jaskiewicz L, Kimmins S, Parvinen M, Filipowicz W & Sassone-Corsi P 2006a The chromatoid body of male germ cells: similarity with processing bodies and presence of Dicer and microRNA pathway components. *PNAS* **103** 2647–2652. (doi:10.1073/pnas.0509333103)
- Kotaja N, Lin H, Parvinen M & Sassone-Corsi P 2006b Interplay of PIWI/Argonaute protein MIWI and kinesin KIF17b in chromatoid bodies of male germ cells. *Journal of Cell Science* **119** 2819–2825. (doi:10.1242/jcs.03022)
- Kulkarni M, Ozgur S & Stoecklin G 2010 On track with P-bodies. *Biochemical Society Transactions* **38** 242–251. (doi:10.1042/BST0380242)
- Kuramochi-Miyagawa S, Kimura T, Ijiri TW, Isobe T, Asada N, Fujita Y, Ikawa M, Iwai N, Okabe M, Deng W *et al.* 2004 Mili, a mammalian member of piwi family gene, is essential for spermatogenesis. *Development* **131** 839–849. (doi:10.1242/dev.00973)
- Kuramochi-Miyagawa S, Watanabe T, Gotoh K, Totoki Y, Toyoda A, Ikawa M, Asada N, Kojima K, Yamaguchi Y, Ijiri TW *et al.* 2008 DNA methylation of retrotransposon genes is regulated by Piwi family members MILI and MIWI2 in murine fetal testes. *Genes and Development* **22** 908–917. (doi:10.1101/gad.1640708)
- Kuramochi-Miyagawa S, Watanabe T, Gotoh K, Takamatsu K, Chuma S, Kojima-Kita K, Shiromoto Y, Asada N, Toyoda A, Fujiyama A *et al.* 2010 MVH in piRNA processing and gene silencing of retrotransposons. *Genes and Development* **24** 887–892. (doi:10.1101/gad.1902110)
- Lee KH, Lee S, Kim B, Chang S, Kim SW, Paick JS & Rhee K 2006 Dazl can bind to dynein motor complex and may play a role in transport of specific mRNAs. *EMBO Journal* **25** 4263–4270. (doi:10.1038/sj.emboj.7601304)
- Ma L, Buchold GM, Greenbaum MP, Roy A, Burns KH, Zhu H, Han DY, Harris RA, Coarfa C, Gunaratne PH *et al.* 2009 GASZ is essential for male meiosis and suppression of retrotransposon expression in the male germline. *PLoS Genetics* **5** e1000635. (doi:10.1371/journal.pgen.1000635)
- Ma J, Flemr M, Stein P, Berninger P, Malik R, Zavolan M, Svoboda P & Schultz RM 2010 MicroRNA activity is suppressed in mouse oocytes. *Current Biology* **20** 265–270. (doi:10.1016/j.cub.2009.12.042)
- Maatouk DM, Loveland KL, McManus MT, Moore K & Harfe BD 2008 Dicer1 is required for differentiation of the mouse male germline. *Biology of Reproduction* **79** 696–703. (doi:10.1095/biolreprod.108.067827)
- MacNicol MC & MacNicol AM 2010 Developmental timing of mRNA translation – integration of distinct regulatory elements. *Molecular Reproduction and Development* **77** 662–669. (doi:10.1002/mrd.21191)
- Meikar O, Da Ros M, Korhonen H & Kotaja N 2011 Chromatoid body and small RNAs in male germ cells. *Reproduction* **142** 195–209. (doi:10.1530/REP-11-0057)
- Meisner NC & Filipowicz W 2010 Properties of the regulatory RNA-binding protein HuR and its role in controlling miRNA repression. *Advances in Experimental Medicine and Biology* **700** 106–123.
- Morita S, Horii T, Kimura M, Goto Y, Ochiya T & Hatada I 2007 One Argonaute family member, Eif2c2 (Ago2), is essential for development and appears not to be involved in DNA methylation. *Genomics* **89** 687–696. (doi:10.1016/j.ygeno.2007.01.004)
- Murchison EJ, Stein P, Xuan Z, Pan H, Zhang MQ, Schultz RM & Hannon GP 2007 Critical roles for Dicer in the female germline. *Genes and Development* **21** 682–693. (doi:10.1101/gad.1521307)
- Nguyen-Chi M, Chalmel F, Agius E, Vanzo N, Khabar KS, Jegou B & Morello D 2009 Temporally regulated traffic of HuR and its associated ARE-containing mRNAs from the chromatoid body to polysomes during mouse spermatogenesis. *PLoS ONE* **4** e4900. (doi:10.1371/journal.pone.0004900)
- Nguyen-Chi M, Auriol J, Jégou B, Kontoyiannis D, Turner J, de Rooij DJ & Morello D 2011 The RNA-binding ELAVL1/HUR is essential for mouse spermatogenesis, acting both at meiotic and post-meiotic stages. *Molecular Biology of the Cell* **16** 2875–2885. (doi:10.1091/mbc.E11-03-0212)
- Noble SL, Allen BL, Goh LK, Nordick K & Evans TC 2008 Maternal mRNAs are regulated by diverse P body-related mRNP granules during early *Caenorhabditis elegans* development. *Journal of Cell Biology* **182** 559–572. (doi:10.1083/jcb.200802128)
- O'Donnell KA, Burns KH & Boeke JD 2008 A descent into the nuage: the maelstrom of transposon control. *Developmental Cell* **15** 179–181. (doi:10.1016/j.devcel.2008.07.016)
- Parker R & Sheth U 2007 P bodies and the control of mRNA translation and degradation. *Molecular Cell* **25** 635–646. (doi:10.1016/j.molcel.2007.02.011)
- Parvinen M 2005 The chromatoid body in spermatogenesis. *International Journal of Andrology* **28** 189–201. (doi:10.1111/j.1365-2605.2005.00542.x)
- Radford HE, Meijer HA & de Moor CH 2008 Translational control by cytoplasmic polyadenylation in *Xenopus* oocytes. *Biochimica et Biophysica Acta* **1779** 217–229. (doi:10.1016/j.bbagr.2008.02.002)
- Reuter M, Chuma S, Tanaka T, Franz T, Stark A & Pillai RS 2009 Loss of the Mili-interacting Tudor domain-containing protein-1 activates transposons and alters the Mili-associated small RNA profile. *Nature Structural & Molecular Biology* **16** 639–646. (doi:10.1038/nsmb.1615)
- Richter JD 2007 CPEB: a life in translation. *Trends in Biochemical Sciences* **32** 279–285. (doi:10.1016/j.tibs.2007.04.004)
- Ruggiu M, Speed R, Taggart M, McKay SJ, Kilanowski F, Saunders P, Dorin J & Cooke HJ 1997 The mouse *Dazl* gene encodes a cytoplasmic protein essential for gametogenesis. *Nature* **389** 73–77. (doi:10.1038/37987)
- Sada A, Suzuki A, Suzuki H & Saga Y 2009 The RNA-binding protein NANOS2 is required to maintain murine spermatogonial stem cells. *Science* **325** 1394–1398. (doi:10.1126/science.1172645)
- Saffman EE & Lasko P 1999 Germline development in vertebrates and invertebrates. *Cellular and Molecular Life Sciences* **55** 1141–1163. (doi:10.1007/s000180050363)
- Shen R & Xie T 2010 NANOS: a germline stem cell's guardian angel. *Journal of Molecular Cell Biology* **2** 76–77. (doi:10.1093/jmcb/mjp043)
- Shoji M, Tanaka T, Hosokawa M, Reuter M, Stark A, Kato Y, Kondoh G, Okawa K, Chujo T, Suzuki T *et al.* 2009 The TDRD9–MIWI2 complex is

- essential for piRNA-mediated retrotransposon silencing in the mouse male germline. *Developmental Cell* **17** 775–787. (doi:10.1016/j.devcel.2009.10.012)
- Soper SF, van der Heijden GW, Hardiman TC, Goodheart M, Martin SL, de Boer P & Bortvin A 2008 Mouse maelstrom, a component of nuage, is essential for spermatogenesis and transposon repression in meiosis. *Developmental Cell* **15** 285–297. (doi:10.1016/j.devcel.2008.05.015)
- Spruce T, Pernaute B, Di-Gregorio A, Cobb BS, Merckenschlager M, Manzanares M & Rodríguez TA 2010 An early developmental role for miRNAs in the maintenance of extraembryonic stem cells in the mouse embryo. *Developmental Cell* **19** 207–219. (doi:10.1016/j.devcel.2010.07.014)
- Standart N & Minshall N 2008 Translational control in early development: CPEB, P-bodies and germinal granules. *Biochemical Society Transactions* **36** 671–676. (doi:10.1042/BST0360671)
- Su H, Trombly MI, Chen J & Wang X 2009 Essential and overlapping functions for mammalian Argonautes in microRNA silencing. *Genes and Development* **23** 304–317. (doi:10.1101/gad.1749809)
- Suh N & Blelloch R 2011 Small RNAs in early mammalian development: from gametes to gastrulation. *Development* **138** 1653–1661. (doi:10.1242/dev.056234)
- Suh N, Baehner L, Moltzahn F, Melton C, Shenoy A, Chen J & Blelloch R 2010 MicroRNA function is globally suppressed in mouse oocytes and early embryos. *Current Biology* **20** 271–277. (doi:10.1016/j.cub.2009.12.044)
- Suzuki A, Tsuda M & Saga Y 2007 Functional redundancy among Nanos proteins and a distinct role of Nanos2 during male germ cell development. *Development* **134** 77–83. (doi:10.1242/dev.02697)
- Suzuki A, Igarashi K, Aisaki K, Kanno J & Saga Y 2010 NANOS2 interacts with the CCR4–NOT deadenylation complex and leads to suppression of specific RNAs. *PNAS* **107** 3594–3599. (doi:10.1073/pnas.0908664107)
- Svoboda P 2010 Why mouse oocytes and early embryos ignore miRNAs? *RNA Biology* **7** 559–563. (doi:10.4161/rna.7.5.12940)
- Tanaka SS, Toyooka Y, Akasu R, Katoh-Fukui Y, Nakahara Y, Suzuki R, Yokoyama M & Noce T 2000 The mouse homolog of *Drosophila* Vasa is required for the development of male germ cells. *Genes and Development* **14** 841–853. (doi:10.1101/gad.14.7.841)
- Tanaka T, Hosokawa M, Vagin VV, Reuter M, Hayashi E, Mochizuki AL, Kitamura K, Yamanaka H, Kondoh G, Okawa K *et al.* 2011 Tudor domain containing 7 (Tdrd7) is essential for dynamic ribonucleoprotein (RNP) remodeling of chromatoid bodies during spermatogenesis. *PNAS* **108** 10579–10584. (doi:10.1073/pnas.1015447108)
- Tang F, Kaneda M, O'Carroll D, Hajkova P, Barton SC, Sun YA, Lee C, Tarakhovsky A, Lao K & Surani MA 2007 Maternal microRNAs are essential for mouse zygotic development. *Genes and Development* **21** 644–648. (doi:10.1101/gad.418707)
- Tay J & Richter JD 2001 Germ cell differentiation and synaptonemal complex formation are disrupted in CPEB knockout mice. *Developmental Cell* **1** 201–213. (doi:10.1016/S1534-5807(01)00025-9)
- Thomas MG, Loschi M, Desbats MA & Boccaccio GL 2011 RNA granules: the good, the bad and the ugly. *Cellular Signalling* **23** 324–334. (doi:10.1016/j.cellsig.2010.08.011)
- Thomson T & Lin H 2009 The biogenesis and function of PIWI proteins and piRNAs: progress and prospect. *Annual Review of Cell and Developmental Biology* **25** 355–376. (doi:10.1146/annurev.cellbio.24.110707.175327)
- Tsuda M, Kiso M & Saga Y 2006 Implication of nanos2–3'UTR in the expression and function of nanos2. *Mechanisms of Development* **123** 440–449. (doi:10.1016/j.mod.2006.04.002)
- Unhavaithaya Y, Hao Y, Beyret E, Yin H, Kuramochi-Miyagawa S, Nakano T & Lin H 2009 MILI, a PIWI-interacting RNA-binding protein, is required for germ line stem cell self-renewal and appears to positively regulate translation. *Journal of Biological Chemistry* **284** 6507–6519. (doi:10.1074/jbc.M809104200)
- Vasileva A, Tiedau D, Firooznia A, Muller-Reichert T & Jessberger R 2009 Tdrd6 is required for spermiogenesis, chromatoid body architecture, and regulation of miRNA expression. *Current Biology* **19** 630–639. (doi:10.1016/j.cub.2009.02.047)
- Vasudevan S, Seli E & Steitz JA 2006 Metazoan oocyte and early embryo development program: a progression through translation regulatory cascades. *Genes and Development* **20** 138–146. (doi:10.1101/gad.1398906)
- Wang C & Lehmann R 1991 Nanos is the localized posterior determinant in *Drosophila*. *Cell* **66** 637–647. (doi:10.1016/0092-8674(91)90110-K)
- Wang Y, Medvid R, Melton C, Jaenisch R & Blelloch R 2007 DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nature Genetics* **39** 380–385. (doi:10.1038/ng1969)
- Wang J, Saxe JP, Tanaka T, Chuma S & Lin H 2009 Mili interacts with tudor domain-containing protein 1 in regulating spermatogenesis. *Current Biology* **19** 640–644. (doi:10.1016/j.cub.2009.02.061)
- Yamaji M, Tanaka T, Shigeta M, Chuma S, Saga Y & Saitou M 2010 Functional reconstruction of NANOS3 expression in the germ cell lineage by a novel transgenic reporter reveals distinct subcellular localizations of NANOS3. *Reproduction* **139** 381–393. (doi:10.1530/REP-09-0373)
- Zhao GQ & Garbers DL 2002 Male germ cell specification and differentiation. *Developmental Cell* **2** 537–547. (doi:10.1016/S1534-5807(02)00173-9)
- Zuccotti M, Merico V, Cecconi S, Redi CA & Garagna S 2011 What does it take to make a developmentally competent mammalian egg? *Human Reproduction Update* **17** 525–540. (doi:10.1093/humupd/dmr009)

Received 16 July 2011

First decision 5 September 2011

Accepted 5 October 2011