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RanBPM, a Scaffolding Protein for Gametogenesis

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Abstract

RanBPM is a multimodular scaffold protein that interacts with a great variety of molecules including nuclear, cytoplasmic, and membrane proteins. By building multiprotein complexes, RanBPM is thought to regulate various signaling pathways, especially in the immune and nervous system. However, the diversity of these interactions does not facilitate the identification of its precise mechanism of action, and therefore the physiological role of RanBPM still remains unclear. Recently, RanBPM has been shown to be critical for the fertility of both genders in mouse. Although mechanistically it is still unclear how RanBPM affects gametogenesis, the data collected so far suggest that it is a key player in this process. Here, we examine the RanBPM sterility phenotype in the context of other genetic mutations affecting mouse gametogenesis to investigate whether this scaffold protein affects the function of other known proteins whose deficiency results in similar sterility phenotypes.

1. INTRODUCTION

Multimodular scaffold proteins are crucial regulators of a great variety of physiological functions. They coordinate the physical assembly of proteins, regulating signal transduction cascades, and shaping signaling responses. RanBPM, also named RanBP9, is a scaffold protein that belongs to the Ran-binding protein family. Proteins of this family were initially identified by yeast two-hybrid as binding partners of the small Ras-like GTPase Ran. However, RanBPM is structurally and functionally unrelated to the other members of this family, as it lacks the consensus Ran-binding domain and seems devoid of any role in nuclear trafficking (Beddow, Richards, Orem, & Macara, 1995). Instead, RanBPM has been described as an adaptor protein. Its structure does not contain any known catalytic domain but includes several protein-binding domains that participate in the formation of large protein complexes. Indeed, RanBPM has been reported to interact so far with more than 45 proteins. These include structural and adhesion proteins, cytosolic kinases, cell surface tyrosine kinase receptors, nuclear receptors, and a number of proteins in the nervous system (Suresh, Ramakrishna, & Baek, 2012). RanBPM has been reported to function in the immune and nervous system (Murrin & Talbot, 2007). Yet, the *in vivo* significance of these activities needs further studies. Moreover, it is still unclear how RanBPM orchestrates the activities of such a broad spectrum of proteins that are functionally unrelated. RanBPM has a multimodular structure with “sticky” properties that makes it possible for it to associate

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nonspecifically with many other proteins *in vitro*. Indeed, many of its binding partners have been identified by yeast-two-hybrid screening and coimmunoprecipitation experiments in overexpression systems, suggesting that additional functional assays may be needed to validate the existing data. In an attempt to study the *in vivo* function of RanBPM, we recently generated and characterized mice lacking RanBPM (Puverel, Barrick, Dolci, Coppola, & Tessarollo, 2011). This loss-of-function study revealed a crucial role of RanBPM in both spermatogenesis and oogenesis. However, we still do not know how it orchestrates the signal transduction machinery required for the execution of these biological processes. In this chapter, we discuss the RanBPM-deficient phenotypes, the possible mechanism of RanBPM action in relation to its structure, spatiotemporal pattern of expression, and how it relates to other mutations affecting gametogenesis.

2. PROTEIN STRUCTURE

RanBPM comprises five main domains. These include an N-terminal proline-rich domain, a consensus SPRY protein–protein interaction domain originally identified in the SplA kinase and the ryanodine receptor (Ponting, Schultz, & Bork, 1997), a lissencephaly type-1-like homology (LisH) motif described to act as a dimerization domain (Gerlitz, Darhin, Giorgio, Franco, & Reiner, 2005; Kim et al., 2004; Mateja, Cierpicki, Paduch, Derewenda, & Otlewski, 2006), a CTLH (carboxy-terminal to LisH) domain of unknown function, and a C-terminal CRA (CT11-RanBPM) motif, reported to interact with the fragile X mental retardation protein FMRP (Menon, Gibson, & Pastore, 2004). The CRA domain has been reported to contain a nuclear localization signal (Lakshmana et al., 2010). The LisH-CTLH domain is mostly described in proteins involved in the regulation of microtubule dynamics and cell migration and is present in several proteins which associate with RanBPM to form a large protein complex (Emes & Ponting, 2001; Kobayashi et al., 2007; Nishitani et al., 2001). Thus, all these domains provide potential protein-binding sites for a great variety of molecules (Fig. 13.1).

In an initial study, RanBPM was described as a centrosomal 55-kDa protein implicated in the regulation of microtubule nucleation (Nakamura et al., 1998). Later work by the same group revealed that this was instead an N-terminal truncated protein and that the full-length RanBPM cDNA encodes a 90-kDa protein which does not localize to the microtubule-organizing center (Nishitani et al., 2001). However, it is interesting to note that in a recent study, some authors reported the occurrence of a 60-kDa-processed form (C-terminal truncation) with an enhanced stability compared to the full-length RanBPM. While the full-length protein is localized in both the nucleus and cytoplasm, the truncated form is mostly localized in the cytoplasm (Lakshmana et al., 2010). Yet, this fragment retains the capacity to form self-interacting multimeric complexes.

More recently, another protein named RanBP10 has been described as the closest homolog of RanBPM. This protein shares significant sequence similarities and, except for lacking the N-terminal proline-rich domain, it possesses all other domains and these are well conserved. So far, RanBP10 has been reported to play a role in regulating the platelet shape and function in the adult mouse (Kunert et al., 2009). The close structural similarities between

RanBPM and RanBP10 suggest the possibility of functional redundancy during the analysis of mutant mice lacking one of these proteins.

3. FUNCTION OUTSIDE THE REPRODUCTIVE SYSTEM

RanBPM is evolutionarily conserved and is ubiquitously expressed; to our knowledge, it has been found in all tissues and cell lines examined so far. It can undergo phosphorylation and ubiquitination, but the precise role of these posttranslational modifications still needs to be evaluated (Denti et al., 2004; Ideguchi et al., 2002). At the subcellular level, RanBPM has been found in the cytoplasm, nucleus, as well as associated with the cell membrane (Denti et al., 2004; Suresh et al., 2012; Valiyaveettil et al., 2008).

Among the best-known partners of RanBPM are the Met tyrosine kinase receptor for hepatocyte growth factor (HGF), whose Ras–Erk pathway is facilitated by RanBPM, and the kelch-repeat protein muskelin which acts on the regulation of cell morphology (Valiyaveettil et al., 2008; Wang, Li, Messing, & Wu, 2002). In addition to its involvement in these general cellular processes, RanBPM is known to play a role in the immune response by interacting with the β2-integrin lymphocyte function-associated antigen 1 (Denti et al., 2004). Moreover, a growing number of studies are reporting a function for RanBPM in the nervous system. For example, it has been involved in the modulation of axonal and neurite outgrowth by cooperating with the Plexin-A receptors and inhibiting the neural cell adhesion molecule L1 signaling (Cheng, Lemmon, & Lemmon, 2005; Togashi, Schmidt, & Strittmatter, 2006). Interestingly, RanBPM binds to both the low- and the high-affinity neurotrophin receptors p75NTR, TrkA, and TrkB (Bai, Chen, & Huang, 2003; Yin et al., 2010; Yuan et al., 2006), interacts with the fragile X mental retardation protein, possibly modulating its RNA-binding properties, and binds to metabotropic glutamate receptors (Menon et al., 2004; Seebahn, Rose, & Enz, 2008). RanBPM has also been found to associate with citron kinase (CITK), regulating the production of pyramidal neurons in the embryonic neocortex (Chang et al., 2010). Finally, RanBPM has been involved in Alzheimer’s disease, affecting amyloid precursor protein processing and amyloid β generation (Lakshmana et al., 2009; Woo et al., 2012).

4. PHENOTYPE OF RanBPM-DEFICIENT MICE

While RanBPM has been involved in a number of biological processes, the recent generation of RanBPM-deficient mice has provided a tool that will allow investigators to test their physiological significance *in vivo*. Homozygous mutant mice for RanBPM develop to term, suggesting that the gene is not crucial for embryonic development. However, a little more than half of the mutant animals die postnatally, while the rest survive to adulthood. Surviving *RanBPM*$^{-/-}$ mice have a normal life span but are significantly smaller than their control littermates. The major organs, including brain, heart, liver, lung, kidney, thymus, spleen, and intestine, are proportional to the body size and do not show any obvious abnormality. By contrast, mutant gonads are severely atrophic leading to sterility of both genders (Puverel et al., 2011) (Fig. 13.2). In the adult mutant testis, seminiferous tubules are decreased in size and completely devoid of germ cells, and the epididymal duct lumen does

not contain any sperm. In the mutant ovary, only a few follicles develop in young mice, but disappear in older mice.

5. EXPRESSION IN THE REPRODUCTIVE SYSTEM

RanBPM has been reported to be highly expressed in the mouse testis, especially in the maturating spermatocytes (Shibata et al., 2004). Another study has shown that the RanBPM protein is produced in spermatogonia and primary spermatocytes in the human testis, whereas in the rat testis, it appears to be expressed only in round and elongated spermatids (Tang et al., 2004). Moreover, gene expression profiling of mouse male meiotic germ cells and northern blot analysis have found RanBPM transcripts in spermatogonia and primary spermatocytes, as well as in spermatids (Rossi et al., 2004).

In order to clarify the RanBPM pattern of expression, we decided to evaluate the activity of this gene during testis development by taking advantage of a *LacZ* reporter gene inserted in the targeted *RanBPM* locus (Puverel et al., 2011). Since the *LacZ* gene product, β-galactosidase, is expressed under the control of the endogenous *RanBPM* promoter, X-gal staining constitutes a sensitive and accurate method to study the expression pattern of the *RanBPM* gene. We found that in males, *RanBPM* mRNA expression starts during embryogenesis; it is first detected in the testis of heterozygous animals at embryonic day 17.5 in clustered mouse vasa homolog (MVH)-positive germ cells. In newborns, expression is still mainly restricted to the gonocytes, while 5 days later, interstitial cells begin expressing *RanBPM*. At 2 weeks, both spermatogonia and Sertoli cells, the supporting cells of the spermatogenic process, are strongly labeled. *RanBPM* transcript is thus sequentially expressed by distinct cell populations during testis postnatal development, yet its expression is always present in the germ cell lineage. Interestingly, *RanBPM* mRNA expression is cyclically regulated during adult steady state spermatogenesis. In the adult testis, expression is detected in spermatogonia residing at the periphery of the seminiferous tubules and maintained in both primary and secondary spermatocytes. Remarkably, the highest expression is observed in primary spermatocytes undergoing the first meiotic division, although it seems to stop at the stage of differentiated tailed spermatids. *RanBPM* expression is thus dynamically regulated during the spermatogenic wave. In addition to *RanBPM* transcriptional activity, we were able to show that the protein is also expressed in the testis. Between birth and 2 weeks, RanBPM protein level is fairly constant but it increases significantly between 2 and 3 weeks, corresponding to the time when the spermatocytes of the first wave of spermatogenesis undergo the first meiotic division. By immunohistochemistry, we found that primary spermatocytes are the cells with the highest RanBPM protein expression.

The first study focusing on RanBPM function in the female reproductive system was conducted in *Drosophila* where there are two proteins (a short and a long) coded by transcripts produced by alternative splicing. The short RanBPM isoform appears to be expressed in all somatic and germline cells of the ovary, while the long isoform is specifically enriched in the germline stem cell niche where it is required for the proper arrangement of niche cells and niche cell size (Dansereau & Lasko, 2008). In mice, the *RanBPM* gene is active in both the developing and adult ovary. Interestingly, *RanBPM*

expression starts at the birth and is restricted to the germinal cell lineage, that is, oocytes reaching the dictyate arrest (Puverel et al., 2011). In the adult ovaries, *RanBPM* is present at a high level in oocytes and is maintained during follicle development, from primary to Graafian follicles. As in males, *RanBPM* transcripts are also expressed in the somatic cell lineage in the ovary since expression is detected in the theca cell layers, while granulosa cells are negative. However, whether *RanBPM* transcripts are translated into proteins in every cell type in which they have been detected is still unknown and, as suggested by the results obtained in males, *RanBPM* mRNA expression may be broader than that of the protein (Puverel et al., 2011).

6. RanBPM IS DISPENSABLE FOR PRIMORDIAL GERM CELL MIGRATION

The data available so far suggest that RanBPM is not involved in primordial germ cell (PGC) migration (Puverel et al., 2011). PGCs are derived from the epiblast from where they migrate to colonize the genital ridges by 11.5 days of embryogenesis (Bendel-Stenzel, Anderson, Heasman, & Wylie, 1998; Tres, Rosselot, & Kierszenbaum, 2004). Upon entry into the developing gonads, PGCs coalesce with somatic cells that have initiated sexual differentiation. PGCs proliferate during migration and for a short time after colonization of the gonads. Male germ cells then enter mitotic arrest while female germ cells begin to enter the first meiotic division. Several factors have been involved in this process, including the chemokine stromal cell-derived factor 1 and its receptor CXCR4, as well as stem cell factor (SCF), and the c-Kit tyrosine kinase receptor (Ara et al., 2003; Buehr, McLaren, Bartley, & Darling, 1993; Mahakali Zama, Hudson, & Bedell, 2005; Molyneaux et al., 2003; Runyan et al., 2006). X-gal staining failed to detect any RanBPM expression in E13.5 embryos, as RanBPM expression starts during late embryogenesis in males and at birth in females (Puverel et al., 2011). In addition, the lack of RanBPM does not seem to affect germ cells during embryogenesis, as gonocytes are present in the seminiferous tubules of newborn males, and the number of meiotic oocytes present in the developing ovary of mutants at day E17.5 is comparable with that of WT embryos. These observations suggest that the migration of PGCs occurs normally in mutant mice and that RanBPM does not play a role in the germ cell migration and proliferation events occurring during embryonic development.

7. FUNCTION OF RanBPM DURING POSTNATAL TESTIS DEVELOPMENT

In males, the second week of postnatal life is marked by the initiation of the spermatogenesis process with massive proliferation of spermatogonia. Macroscopically, this transition is reflected by a rapid increase in testis volume as germ cells start filling the seminiferous tubules. RanBPM mutant testes do not show any overt phenotype during the first postnatal week. However, at 2 weeks, contrary to WT testes which undergo a significant growth, they only slightly increase in size. Histological examination at 2 weeks revealed a clear decrease in the diameter of seminiferous tubules, compared to WT (Puverel et al., 2011). These tubules contain a dramatically decreased number of both spermatogonia and spermatocytes. However, the levels of apoptosis were comparable between WT and mutant at this stage, suggesting that the lack of germ cells is not the consequence of cell death events. By contrast, and detectable as early as P8, there was a clear defect in proliferation which was revealed by a dramatic decrease in the number of BrdU-positive cells, as well as

an altered organization of the proliferating cells, when compared to WT mouse testes in which these cells are typically forming a continuous ring delineating the seminiferous tubules (Puverel et al., 2011). Altogether, these data demonstrate that RanBPM is a crucial factor for spermatogonia production. Whether RanBPM acts on the differentiation of spermatogonia or on their proliferation remains to be determined.

One of the factors reported to act on spermatogonia proliferation is the tyrosine kinase receptor c-Kit. While the downregulation of its ligand, SCF affects PGCs migration, proliferation, and survival (Mahakali Zama et al., 2005; Runyan et al., 2006), c-Kit also plays a role in spermatogonia proliferation (Blume-Jensen et al., 2000; Kissel et al., 2000). RanBPM has been reported to interact with several tyrosine kinase receptors, including the HGF receptor Met and both the TrkA and TrkB neurotrophin receptors, and to possibly influence their signaling pathways. Considering that c-Kit function is critical for normal spermatogenesis, it is tempting to speculate that RanBPM may interact with this receptor and affect its signaling (Wang et al., 2002; Yin et al., 2010; Yuan et al., 2006). However, c-Kit acts on spermatogonia cell survival and entry into meiosis, and the phenotype of c-Kit mutants with a defect in the PI3-kinase pathway differs from RanBPM mutant mice in that spermatogonia are depleted by apoptosis and meiotic cells are rare or absent (Blume-Jensen et al., 2000; Kissel et al., 2000; Puverel et al., 2011). Outside the reproductive system, some studies reported a role for RanBPM in cell division. CITK is a protein that localizes to the surface of the lateral ventricles during embryonic cortex development and is essential in neurogenic mitoses (Di Cunto et al., 2000). A biochemical interaction between CITK and RanBPM has been reported recently, and the use of *in utero* RNAi to decrease RanBPM expression leads to an increased number of cells in mitosis, and a concomitant decrease in the number of cells in cytokinesis (Chang et al., 2010). Both RanBPM and RanBP10 have also been described to interact with YPEL5, a protein located at different mitosis-related subcellular structures and involved in cell cycle progression (Hosono et al., 2010). Taken together, these reports suggest that RanBPM could play a role in the proliferation of different cell types. This hypothesis is also supported by the fact that RanBPM is ubiquitously expressed and that its deletion causes partial neonatal lethality and growth retardation for reasons that are still unknown (Puverel et al., 2011).

Androgens are essential hormones for the initiation and maintenance of spermatogenesis. The androgen receptor (AR) is a nuclear receptor acting as a ligand-inducible transcription factor to modulate the expression of a number of target genes. RanBPM has been reported to associate with AR and to enhance its transcriptional activity in a ligand-dependent manner when overexpressed in prostate cancer cell lines (Rao et al., 2002). AR has been reported to be expressed in all cell types of the testis. Knockout mice for AR exhibit a severe phenotype, with an abdominal localization of the testes and an absence of epididymis formation (Wang, Yeh, Tzeng, & Chang, 2009). If RanBPM mediates AR activity in the testis, its effect is thus likely limited to specific cell types. Testicular cell-specific AR knockout mice have been described in the literature and provide a better understanding of the role of this receptor in spermatogenesis (Wang et al., 2009). Interestingly, Sertoli cell-specific and Leydig cell-specific AR mutants exhibit a decrease in testis size and seminiferous tubules development as well as an arrest in germ cell maturation, mainly at the diplotene and round spermatid stages, respectively. By contrast, germ cell-AR-deficient mice are comparable to controls,

with a normal range of spermatogenesis. If the defect in postnatal testis development observed in RanBPM-deficient mice was due to an effect of RanBPM on AR activity, one could speculate that this regulation occurs either in Sertoli or Leydig cells. However, such AR deregulation is accompanied by changes in serum hormone levels. In AR-knock-out mice and Sertoli- and Leydig-specific mutants, the levels of testosterone are decreased while LH levels are increased, whereas in RanBPM mutant mice, these hormone levels are not significantly different from those of control littermates (Puverel et al., 2011; Wang et al., 2009). In addition, RanBPM protein expression has been detected in primary spermatocytes, and we have found that RanBPM is acting in germ cells in a cell-autonomous way (see Section 10). Finally, the meiotic arrest observed in AR-deficient mice is unlikely to be due to the same defect occurring in RanBPM mice, since mutant females for AR do not exhibit any arrest in differentiation during the prophase of the first meiotic division, in contrast to RanBPM mutant females (Hu et al., 2004; Shiina et al., 2006) (Section 8).

8. MEIOTIC ARREST IN MICE DEFICIENT FOR RanBPM

Despite the reduction in spermatogonia proliferation, primary spermatocytes are still present, yet in reduced numbers, in the seminiferous tubules of *RanBPM*$^{-/-}$ male mice during the first wave of spermatogenesis. By contrast, postmeiotic cells are totally absent and no sperm is produced at any developmental stage. While somatic Sertoli and Leydig cells appear normal in the young mutant animal, spermatogonia, pachytene, and only a few diplotene spermatocytes are the most mature germ cells present in the tubules. Thus, *RanBPM*$^{-/-}$ males exhibit a stage-specific arrest of spermatogenesis, as generally observed in mutant mice in which male sterility is due to a germinal cell primary defect. The fact that mutant mice are affected at as early as 3 weeks of age shows that the first wave of germ line differentiation does not take place. In mice, the first wave of meiotic division is synchronous throughout the testis and occurs around 3 weeks, when the secondary spermatocytes and round spermatids first appear in the seminiferous tubules (Bellve et al., 1977). At this stage, RanBPM mutant testes display a high level of cell death and primary spermatocytes undergo apoptotic death instead of further developing into spermatids (Puverel et al., 2011). Meiosis is a critical step of gametogenesis. It allows genetic exchange between the paternal and maternal genomes, which occurs by recombination during the prophase of the first meiotic division. Prophase I is tightly regulated and can be divided into different stages (see also Fig. 13.3). The leptotene stage is marked by the compaction of chromatin and the programmed induction of localized double-strand breaks (DSBs) which allows the genetic exchange. Chromosome pairing and synapsis are initiated during zygotene, with full synapsis being achieved at the pachytene stage. During zygotene and pachytene stages, the DSBs are repaired by interaction with the homologs. The subsequent disjunction of homologous chromosomes is initiated at the diplotene stage; chromosomes start to separate but remain attached in the regions where crossing over has occurred (Cohen & Pollard, 2001; Zickler & Kleckner, 1999). The proper alignment and synapsis of homologous chromosomes are achieved by the formation of a large zipper-like tripartite protein complex called the synaptonemal complex, which connects the chromosomes along their entire length (Page & Hawley, 2004; Yang & Wang, 2009).

This protein complex mediates the DSBs resolution events leading to recombination. In males, the X and Y chromosomes synapse only at their pseudoautosomal region forming what is called the sex body, located in the nuclear periphery. Instead, in females, the XX chromosomes synapse along their entire length to undergo homologous recombination. In humans, incorrect assembly of the synaptonemal complex causes infertility by triggering germ cell elimination in males and the formation of aneuploid oocytes in females (Hassold & Hunt, 2001;Judis, Chan, Schwartz, Seftel, & Hassold, 2004; Miyamoto et al., 2003). The high level of apoptosis observed in *RanBPM*$^{-/-}$ seminiferous tubules is correlated to a decrease in the expression of genes that are specifically expressed at the end of Prophase I, such as Calmegin and Hox1.4 (Puverel et al., 2011; Rubin, Toth, Patel, D'Eustachio, & Nguyen-Huu, 1986; Watanabe et al., 1994). Expression of CyclinA1, which has been shown to rise dramatically in late pachytene spermatocytes and to reach a maximum in diplotene cells, is even more affected in RanBPM mutant mice (Sweeney et al., 1996). By contrast, genes expressed earlier in the differentiation process, such as A-myb, Dmc1, and Hsp70.2, are not affected (Mettus et al., 1994; Rosario, Perkins, O'Brien, Allen, & Eddy, 1992; Yoshida et al., 1998; Zakeri, Wolgemuth, & Hunt, 1988). In addition, the genes encoding the synaptonemal complex proteins SCP1 and SCP3 are expressed at normal levels in the mutant, indicative of the presence of pachytene spermatocytes (Klink, Lee, & Cooke, 1997; Meuwissen et al., 1992).

Female germ cells enter meiosis during embryonic development, around E13, and subsequently arrest at the end of Prophase I, around birth (dictyate arrest) (Speed, 1982). Oocytes remain arrested until puberty, when a pool of oocytes is recruited and completes the first meiotic division. The second meiotic round occurs only when fertilization takes place (Edson, Nagaraja, & Matzuk, 2009). Ovaries of adult *RanBPM*$^{-/-}$ females exhibit a severe dysgenesis resulting from an absence of follicles. Premature ovarian failure has many possible causes: for example, it can result from a defect in PGC migration, meiosis, and formation, survival or activation of primordial follicles (Jagarlamudi, Reddy, Adhikari, & Liu, 2010). Analysis of *RanBPM*$^{-/-}$ fetal ovaries at E17.5 revealed that the number of oocytes is comparable to that of controls. However, by postnatal day 0, there is a drastic decrease in their number (Puverel et al., 2011). Interestingly, RanBPM is first expressed at birth in the ovaries suggesting that it is essential from the very earliest stages of its expression since most germ cells are absent immediately after birth. Oocyte loss thus occurs between late embryogenesis and early postnatal development in *RanBPM*$^{-/-}$ females, and the vast majority of the oocytes fail to progress to the dictyate arrest. Early studies of meiosis in mouse fetal ovaries suggest that entry into the pachytene stage starts at E16 (Speed, 1982). Since the majority of the oocytes are between late pachytene and diplotene at the time of birth, the phenotype of RanBPM-deficient mice suggests that oocytes reach the pachytene stage unaffected but display the same meiotic arrest as observed in males. Importantly, this arrest, occurring at the same meiotic stage in both genders, suggests that RanBPM deletion affects mechanisms common to both male and female meiosis. This is even more interesting considering that the timing and synchrony of meiosis show striking differences between the two genders (Hunt & Hassold, 2002; Kolas et al., 2005). In females, the entire germ cell pool synchronously initiates meiosis *in utero* around E13.5 and meiosis transiently arrests at birth. By contrast, male meiosis is not initiated until puberty and

spermatocyte development in the adult testis is continuous and proceeds without the arrest periods occurring in females (Nebel, Amarose, & Hacket, 1961). In addition, a growing number of mouse mutants for genes involved in meiotic progression display sexual dimorphism in fertility (Fig. 13.3). These studies revealed clear differences in the stringency of the meiotic events between males and females (Cohen, Pollack, & Pollard, 2006; Handel & Eppig, 1998; Matzuk & Lamb, 2002; Morelli & Cohen, 2005). Female meiosis is more "forgiving" to errors; oocytes with meiotic defects often reach a more advanced stage than spermatocytes and can give rise to aneuploid gametes. Thus, females derived from gene-targeting experiments of genes that affect meiosis display a variety of phenotypes, ranging from subfertility with production of aneuploid embryos to complete infertility. The same meiotic defect in spermatocytes almost always leads to a complete meiotic failure, absence of progression past the zygotene- or pachytene-like stage, and apoptosis of germ cell leading to infertility (Hunt & Hassold, 2002) (see also Fig. 13.3). Although the cellular mechanisms that regulate the Prophase I events, such as homologous recombination and synapsis, appear to be largely conserved between the two genders, these processes are subjects to a different level of control (commonly defined as "checkpoints"), which is more stringent in males than in females.

In order to gain insight into the mechanism of the meiotic defect caused by RanBPM deletion, here, we compare the *RanBPM*$^{-/-}$ mouse meiotic phenotype with those of other mutants, including some of the most recently characterized (Fig. 13.3). Most mouse models lacking proteins involved in the recombination process such as Spo1, known to initiate the DSBs possibly with the cooperation of Mei1, proteins involved in DNA repair such as ATM, Dmc1, the mismatch proteins Msh4 and Msh5, and the recently identified protein Trip13, exhibit a meiotic arrest in spermatogenesis during Prophase I (Baudat et al., 2000; de Vries et al., 1999; Kneitz et al., 2000; Libby et al., 2002; Pittman et al., 1998; Roig et al., 2010; Yoshida et al., 1998). This is caused by a failure of chromosomes to synapse properly, impairing the progression of spermatocytes beyond the zygotene stage. Since, as mentioned above, the mechanisms monitoring meiosis are less stringent in females, meiosis can progress further in females but most oocytes are eliminated soon after birth as the meiotic defects become apparent during the checkpoint process. By contrast, mutant females for Tex15, a gene encoding a protein recently identified as essential for DSBs repair and synapsis, retain normal fertility (Yang et al., 2008). In mice deficient for the synaptonemal complex proteins, spermatocytes also arrest at the zygotene–pachytene transition, with mutant females either retaining fertility (Scp3, Fkbp6) or being sterile (Syce1, Tex12) (Bolcun-Filas et al., 2009; Crackower et al., 2003; Hamer et al., 2008; Yuan et al., 2002). Throughout the first meiotic prophase, the sister chromatids are held together by cohesin complex proteins. These proteins, such as SMC1β and Rec8, are essential for proper recombination, completion of synapsis, and chiasmata formation, and mutants for these genes exhibit a similar arrest at the zygotene–pachytene transition (Revenkova et al., 2004; Xu et al., 2005). By contrast, in RanBPM mutant mice, meiosis progresses apparently normally up to the pachytene stage. At this stage, there is no obvious defect in chromosome synapsis, since SCP3 immunolabeling in spermatocytes is comparable between mutants and controls (Puverel et al., 2011). The distribution of phosphorylated histone γH2AX, which marks sites of DSBs at the leptotene stage and subsequently converges around the sex

chromosomes to form the sex body, also appeared normal in *RanBPM*$^{-/-}$ spermatocytes. Thus, in *RanBPM* homozygous mutant males, meiosis still progresses slightly further than in the mouse mutants mentioned above, and the spermatocytes reach the pachytene stage with no obvious synapsis abnormalities. These data suggest that RanBPM-deficient spermatocytes are able to go through the first checkpoint, which ensures the complete synapsis of homologous chromosomes prior to entry into the pachytene stage, but may be stopped at a later checkpoint (Morelli & Cohen, 2005; Odorisio, Rodriguez, Evans, Clarke, & Burgoyne, 1998). Since recombination is required for the proper synapsis process in mice, we can also suggest that recombination is initiated properly in *RanBPM*$^{-/-}$ male mice. The mechanisms of synapsis and recombination in *RanBPM*$^{-/-}$ oocytes have not yet been elucidated, but the timing of meiotic progression in the embryo suggests that the defect is similar to that of males, making the *RanBPM*$^{-/-}$ mouse phenotype quite unique (see Fig. 13.3). A more detailed analysis of spermatocytes and oocytes using several markers of synapsis and recombination may help determine the exact nature of this defect apparently common to both genders. Nevertheless, we should note that, at this point in time, we still need to investigate whether the phenotype observed in these mutants is uniquely caused by the lack of RanBPM or whether possible compensatory effects by its homolog RanBP10 can influence its penetrance on gametogenesis.

RanBPM has been reported to interact with the MVH protein, a member of the DEAD-box family of genes encoding an ATP-dependent RNA helicase (Fujiwara et al., 1994; Shibata et al., 2004). MVH is part of a group of male-specific regulators that include Mili and Gasz; mutants for these genes display similar phenotypes, marked by a meiotic arrest at the zygotene–pachytene transition (Kuramochi-Miyagawa et al., 2004; Ma et al., 2009; Tanaka et al., 2000). These genes seem to be involved in posttranscriptional regulation during spermatogenesis via RNA processing. However, contrary to *RanBPM*$^{-/-}$ mice where both males and females are arrested at the pachytene stage, in these mutants, females are completely fertile. It is therefore difficult to link RanBPM and MVH by the phenotypes caused by the deletion of these genes.

One mutation that appears to be the closest to the RanBPM mutant phenotype in terms of timing of meiotic arrest in both genders is the one for the polyubiquitin gene, *Ubb*. *Ubb*$^{-/-}$ germ cells progress to the pachytene stage and form apparently normal synaptonemal complexes in both genders (Ryu et al., 2008). However, in males, spermatocytes do not mature beyond the pachytene stage and undergo cell death, while in females, although some oocytes reach the dictyate arrest, they are not able to complete meiosis I. Therefore, it would be of interest to determine whether the *Ubb*$^{-/-}$ phenotype can be correlated with the defects observed in *RanBPM*$^{-/-}$ mice. However, it is possible that the phenotypes caused by either Ubb or RanBPM loss have in common only the fact that they affect multiple pathways: RanBPM, by interacting with a variety of proteins through its scaffolding activity, and Ubb, in addition to its role in directing protein degradation, by affecting a great variety of other functions including membrane trafficking and transcriptional regulation.

9. ROLE OF RanBPM IN THE ADULT GONAD

As described above, in *RanBPM*$^{-/-}$ pubertal males, germ cell differentiation is arrested at Prophase I. Histological examination of mutant testes at 6 weeks of age revealed totally empty seminiferous tubules (Sertoli cell only), which is followed by tubular degeneration at later stages (Puverel et al., 2011) (Fig. 13.4). This severe phenotype is not typical of mutants with exclusive meiotic arrest. In fact, in mutant mice displaying an arrest in spermatocyte differentiation, the production of germ cells is usually maintained for a certain period despite the elimination of primary spermatocytes. For example, in mice lacking the cyclin-dependent kinase 2 gene, the alteration of chromosome synapsis leads to the elimination of spermatocytes at prophase I, yet some spermatocytes are still produced in 3-month-old mouse testes (Berthet, Aleem, Coppola, Tessarollo, & Kaldis, 2003; Ortega et al., 2003).

Instead, mice with defects in spermatogonial stem cells (SSCs) display a phenotype that more closely resembles the one observed in the RanBPM-deficient mice. SSCs provide differentiating cells by a self-renewal mechanism, thus keeping the spermatogenesis going (Oatley & Brinster, 2008). Some authors have suggested that SSCs are not involved in the first round of mouse spermatogenesis since the first wave of germline differentiation initiates directly from gonocytes (Yoshida et al., 2006). By using a transgenic system, these authors provided evidence that germ cells originating from gonocytes specifically contribute to the leading edge of pubertal spermatogenesis, generating the first spermatozoa that are released at around P35. Several factors have been shown to be involved in the stem cell renewal process, including the Glial cell line-derived neurotrophic factor Gdnf (Meng et al., 2000), the transcription factor Plzf (Buaas et al., 2004; Costoya et al., 2004), the ERM protein, produced by Sertoli cells (Chen et al., 2005), and the RNA-binding protein NANOS2 (Sada, Suzuki, Suzuki, & Saga, 2009). For example, null mice for GDNF and ERM show an arrest of germ cell production between 4 and 6 weeks (Chen et al., 2005; Meng et al., 2000). Therefore, the similarity of this phenotype with the one displayed by *RanBPM*$^{-/-}$ mutant animals, which are severely depleted of all germ cell types including transit-amplifying spermatogonia as early as 1 month of age, suggests that RanBPM may cause a defect in the spermatogonia self-renewal process as well (Puverel et al., 2011). However, the lack of germ cell production could also be related to the defect in spermatogonia proliferation observed in juvenile animals (Section 7). Taken together, the mutant phenotype displayed by *RanBPM*$^{-/-}$ males shows distinct features, suggesting that this scaffold protein may be critical in more than one mechanism in the control of spermatogenesis.

As outlined in females, RanBPM is crucial for normal oocyte development. Mutant females display a premature ovarian failure and only young adults still retain some follicles in their ovaries (Fig. 13.5). The measure of vaginal impedance in *RanBPM*$^{-/-}$ pubertal females indicates normal estrus cycles, suggesting that follicle and stromal cell functions are intact (Puverel et al., 2011). By contrast, mutant females older than 5 months are not cycling anymore, in accordance with the absence of follicles at this age. Thus, although mutant ovaries contain decreased numbers of follicles, these are sufficient to support normal estrus cycles. The finding that pubertal mutant females are cycling strongly suggests that the sterility of these mice is not caused by hormonal deficiencies and argues in favor of a cell-autonomous role of RanBPM in the oocyte. Oocytes orchestrate and coordinate the

development of the mammalian ovarian follicles (Eppig, Wigglesworth, & Pendola, 2002). Following the superovulation treatment (PMSG, pregnantmare serumgonadotropin injection), *RanBPM*$^{-/-}$ females developed morphologically normal antral follicles, suggesting that follicles are able to mature in absence of RanBPM (Puverel et al., 2011). Whether *RanBPM*$^{-/-}$ females are able to ovulate and if oocytes are meiotically competent to give rise to normal embryos is still an open question.

10. CELL-AUTONOMOUS FUNCTION OF RanBPM

RanBPM transcripts are present in both the somatic and germinal cell lineages of the testis (Section 5), suggesting that the spermatogenesis defect displayed by *RanBPM*$^{-/-}$ mutant males could be either due to a defect in the Sertoli cell nursing function or to a defect intrinsic to the germ cells. We were able to address this issue by generating chimeric mice with homozygous mutant ES cells to study the extent of the ES cell contribution to the chimeras (Puverel et al., 2011). These experiments showed that while control cells could contribute to all cell types in the male testis, mutant ES cells failed to produce any germ cell while producing other cell types. Importantly, mice with testicular supporting cells produced by mutant ES cells had normal spermatogenesis. Together, these data suggest that RanBPM functions in a cell-autonomous fashion in male germ cells and that it is dispensable from supporting cells for spermatogenesis completion.

11. CONCLUSION

Since its first characterization, RanBPM has been the object of a growing interest from the scientific community. Devoid of any known functional domain, it has been suggested that RanBPM is a scaffolding protein important to assemble high molecular weight protein complexes that shape the activity of various signal transduction pathways. Functionally, RanBPM has been implicated in diverse biological roles, especially in the immune and nervous systems. However, with the recent generation and characterization of a mouse lacking this scaffolding protein, it has become apparent that RanBPM plays a crucial role in spermatogenesis and oogenesis. The main features of this mutant are (1) a defect in postnatal testis development suggesting that RanBPM is essential for spermatogonia production in the young male and (2) a meiotic arrest in both genders during the prophase of the first meiotic division, showing that RanBPM is required for normal progression of meiosis. Even though the exact mechanism of action of RanBPM in the gonad remains to be determined, the results from chimera experiments strongly suggest a cell-autonomous function of RanBPM in germ cells. Importantly, the unique phenotype of RanBPM mutant mice compared to mice lacking other genes critical for normal mouse reproduction (Fig. 13.3) suggests that this scaffold protein may influence the activity of multiple pathways or interact with other, yet unknown, proteins. Regardless, the characterization of RanBPM function in mouse gonad development provides new insight into some key events regulating mammalian gametogenesis.

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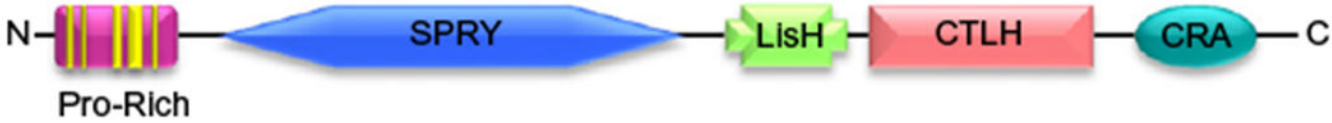


Figure 13.1.

Structure of RanBPM protein. RanBPM is composed of five protein–protein interaction domains, including a proline-rich domain (Pro-Rich), a SPRY domain, the functionally related LisH and CTLH domains, and a C-terminal CRA domain. *Adapted from* Suresh et al. (2012).

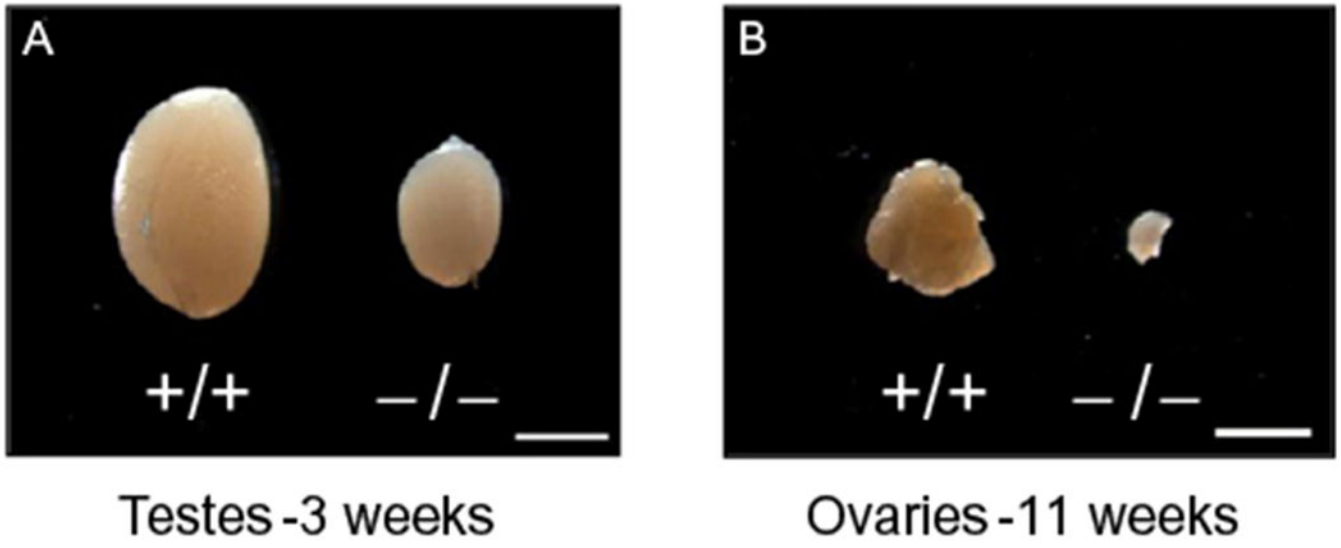
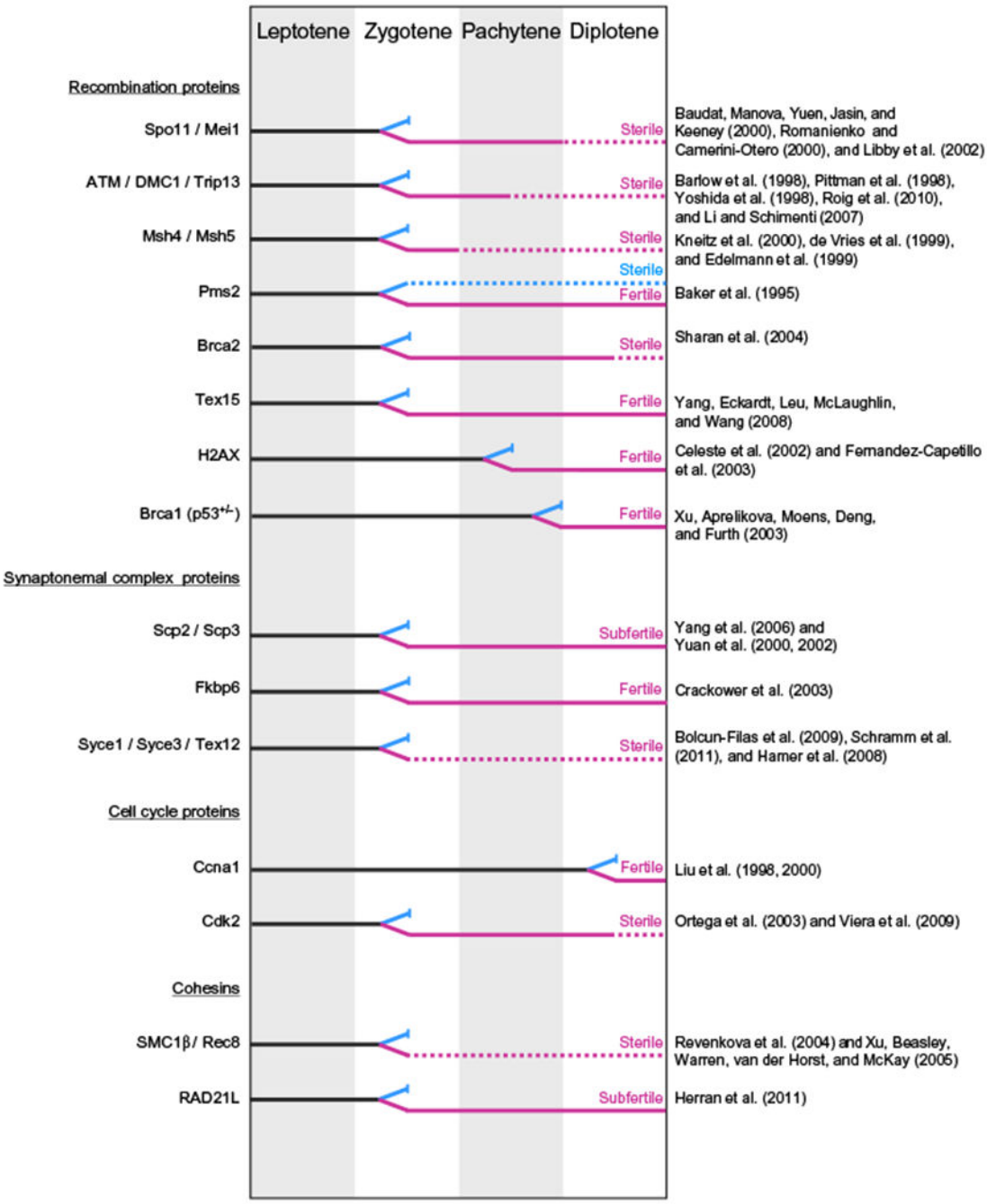


Figure 13.2.

Representative pictures showing the gonad atrophy in RanBPM homozygous mutant mice. Mutant and wild-type mouse testes (A) and ovaries (B) from 3-week-old males and 11-week-old females. Scale bars: 2 mm.



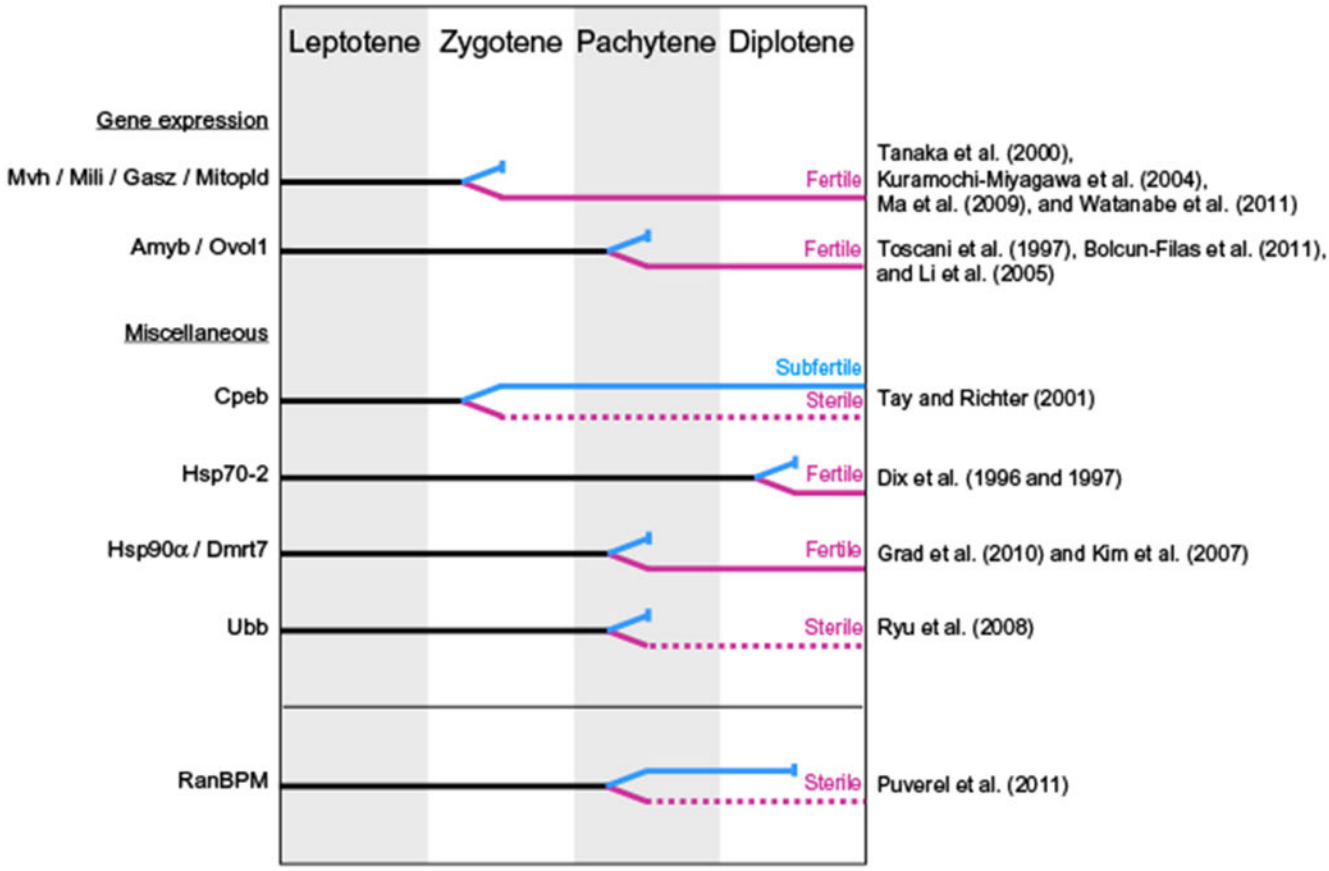


Figure 13.3.

Diagram comparing known mouse mutants exhibiting an arrest during the prophase of the first meiotic division. A black solid line indicates stages during which male and female meioses are similar. When meiotic progression is different between the genders, a blue line indicates male progression and a purple line female progression. A meiotic arrest is represented by a solid vertical bar and dotted lines indicate that only a proportion of germ cells persists. Only genes for which information is available for both genders have been included. *Adapted from* Hunt and Hassold (2002).

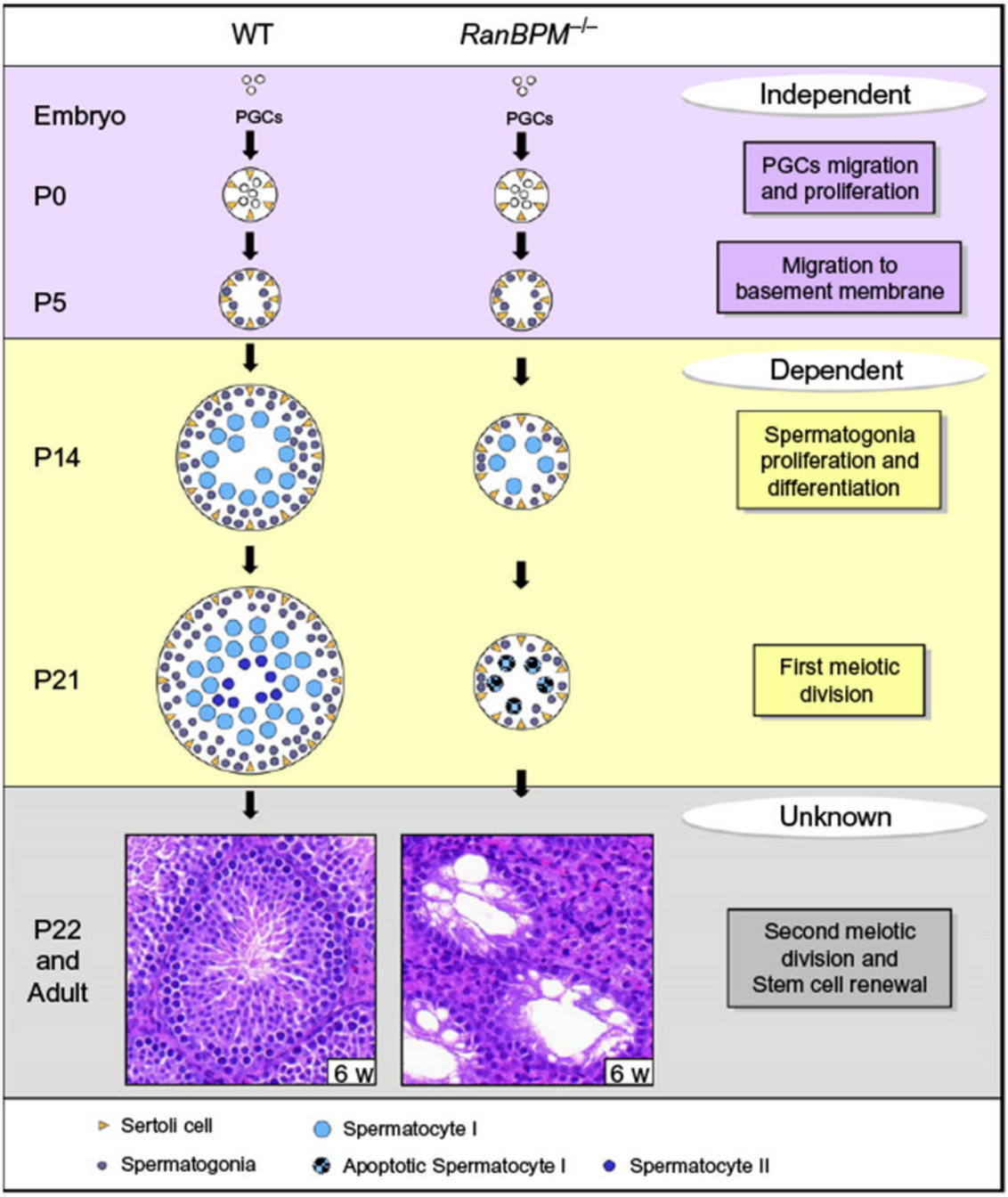


Figure 13.4.

Model illustrating the role of RanBPM in spermatogenesis. Schematic representing the stages at which RanBPM plays a role in spermatogenesis. RanBPM is not required for primordial germ cell migration and proliferation and gonocyte migration to the basement membrane of developing seminiferous tubules in the newborn (purple box). However, it is required in the pubertal male at the crucial step of spermatogonia production and for the meiotic progression of primary spermatocytes (yellow box). Whether RanBPM is involved in the second meiotic division and the maintenance of adult spermatogenesis is still

unknown, since the elimination of all germ cells in the young mutant does not allow the study of any time point past the meiotic arrest occurring during the prophase of the first meiotic division (gray box). P: postnatal day.

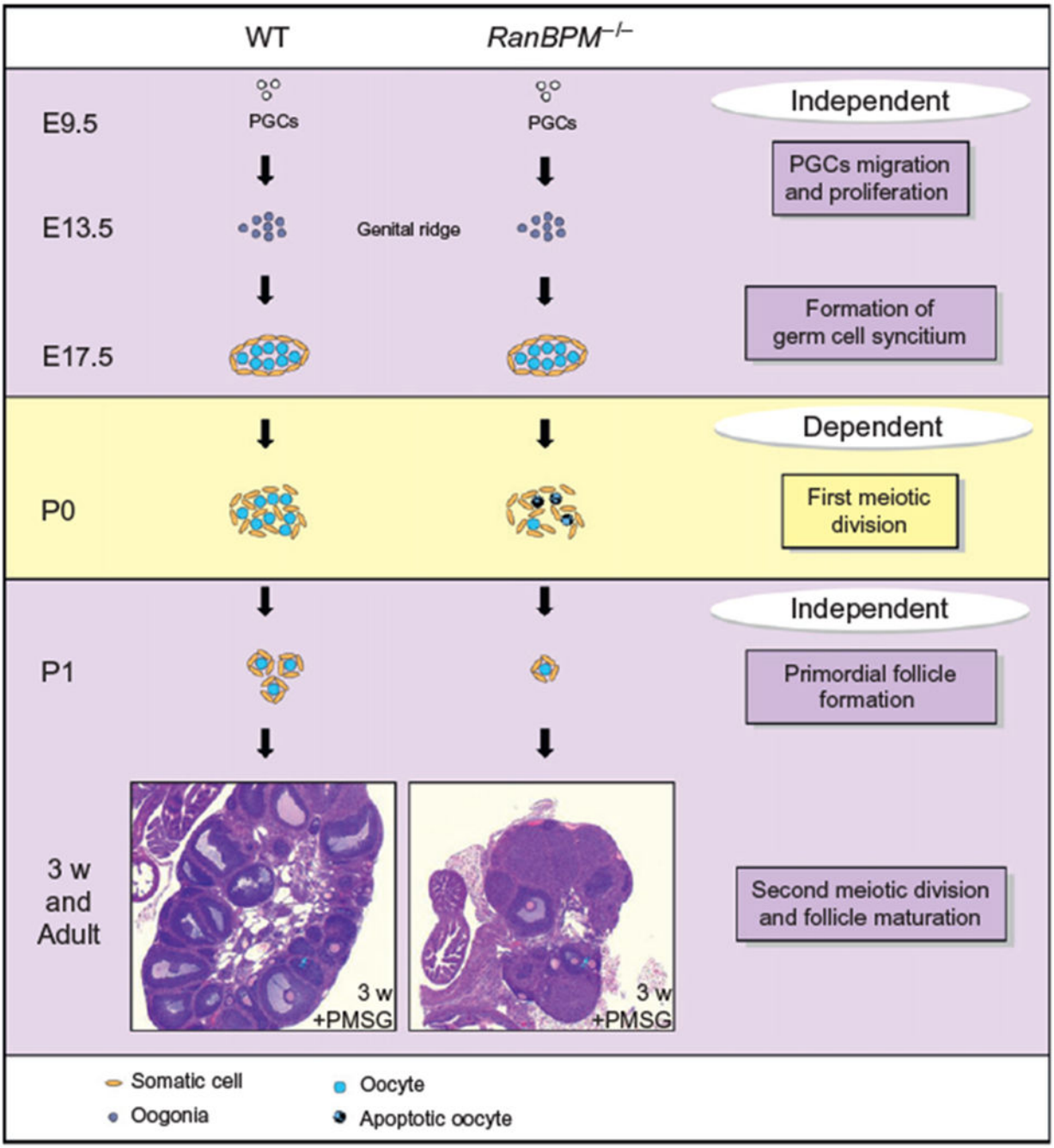


Figure 13.5.

Model illustrating the role of RanBPM in oogenesis. Schematic representing the stages at which RanBPM plays a role in oogenesis. As in males, RanBPM is not essential for primordial germ cell proliferation and migration toward the developing gonad (top purple box). However, RanBPM is crucial for the meiotic progression of oocytes, since germ cells exhibit a meiotic arrest during the prophase of the first meiotic division, as in males (yellow box). The observation that some oocytes survive to form primordial follicles and that these follicles can mature upon PMSG stimulation suggests that RanBPM is not required for the

maturation of germ cells in pubertal and adult mice, although the ability of these oocytes to produce embryos has not been assessed (bottom purple box). E: embryonic day and P: postnatal day.