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Review

An overview on the diversity of cellular organelles during the germ cell cycle

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Summary. In mammals, germ cells undergo a long journey from specification until sexual maturation. During this journey, which takes place during the entire life cycle of mammals, the germ cells dynamically change their morphology, their expression profile and also the number and character of their cellular bodies. The focus of this review will be the diversity of cellular organelles present in the nucleus and cytoplasm at the different phases of germ cell development. We discuss how these organelles associate and behave to form a multitude of bodies that have long been observed by scientists, and how their presence or absence is used to characterize different stages of germ cell development. These organelles include the female Barr body, polar bodies and Balbiani body; and the male sex body and chromatoid body. It is concluded that compartmentalization of organelles and molecules in the cytoplasm (in particular of mitochondria and RNAs) and of the sex chromosomes in the nucleus seems to be important for regulating germ cell development throughout the life cycle.

Key words: Germ cells, Embryo, Organelle, Oogenesis, Spermatogenesis

Prominent organelles from zygote to specification to sex differentiation

In mice, the primordial germ cells (PGCs) are specified around embryonic day (E)7.25 in the proximal posterior part of the embryo at the base of the allantois (Fig. 1A-D). This event marks the definitive separation between the germline and the somatic lineages. Despite constant advances in the field, the mechanism that regulates this separation is not well understood. The specified PGCs are not easily recognised just on a morphological basis, even though the PGCs have a high nucleo-cytoplasmic volume ratio, often a horseshoe-shaped nucleus, and exhibit an oval form compared to the surrounding more cuboidal- and columnar-shaped cells. In addition, PGCs have a relatively dense cytoplasm packed with free ribosomes and polysomes and have pseudopodia (Fig. 1E,F) during migration to the genital ridges (Spiegelman and Bennett, 1973; Clark and Eddy, 1975). Some cellular organelles display characteristic features that help to recognize PGCs as such.

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P-granules (perinuclear germ granules) and P-bodies (RNA processing bodies)

As opposed to many other organisms, including the frog *X. laevis*, the zebrafish *D. rerio*, the nematode worm *C. elegans* and the fruitfly *D. melanogaster*, the mouse zygote does not seem to have an accumulation of germ plasm determinants ("nuage") (Nieuwkoop and Sutasurya, 1979). The presence of germ plasm (P-granules in *C. elegans*, polar granules in *D. melanogaster* or germinal granules in *X. laevis*) is characteristic of organisms with a "preformation" or "inheritance" mode of germ cell formation. Germ plasm is an accumulation of maternal mRNAs and RNA-binding proteins in the oocyte. For example in *D. melanogaster*, it includes Oskar, Vasa, Tudor, Nanos and Piwi (Jin and Xie, 2006). As a consequence, when the zygote undergoes cleavage to form the multicellular embryo, the cells that physically inherit the germ plasm become the PGCs. The presence of germ plasm is therefore both sufficient and necessary for the formation of germ cells in organisms showing an "inheritance" mode of germ cell formation. This mode of germ cell formation releases the body plan from morphological constrains (the germ line is immediately set aside), allowing a high degree of morphological variation (Johnson et al., 2003).

In the mouse, germ plasm/polar granules/P-granules have not been described as such during preimplantation development, nor in PGCs until sex determination. Indeed, formation of PGCs in mice occurs by inductive

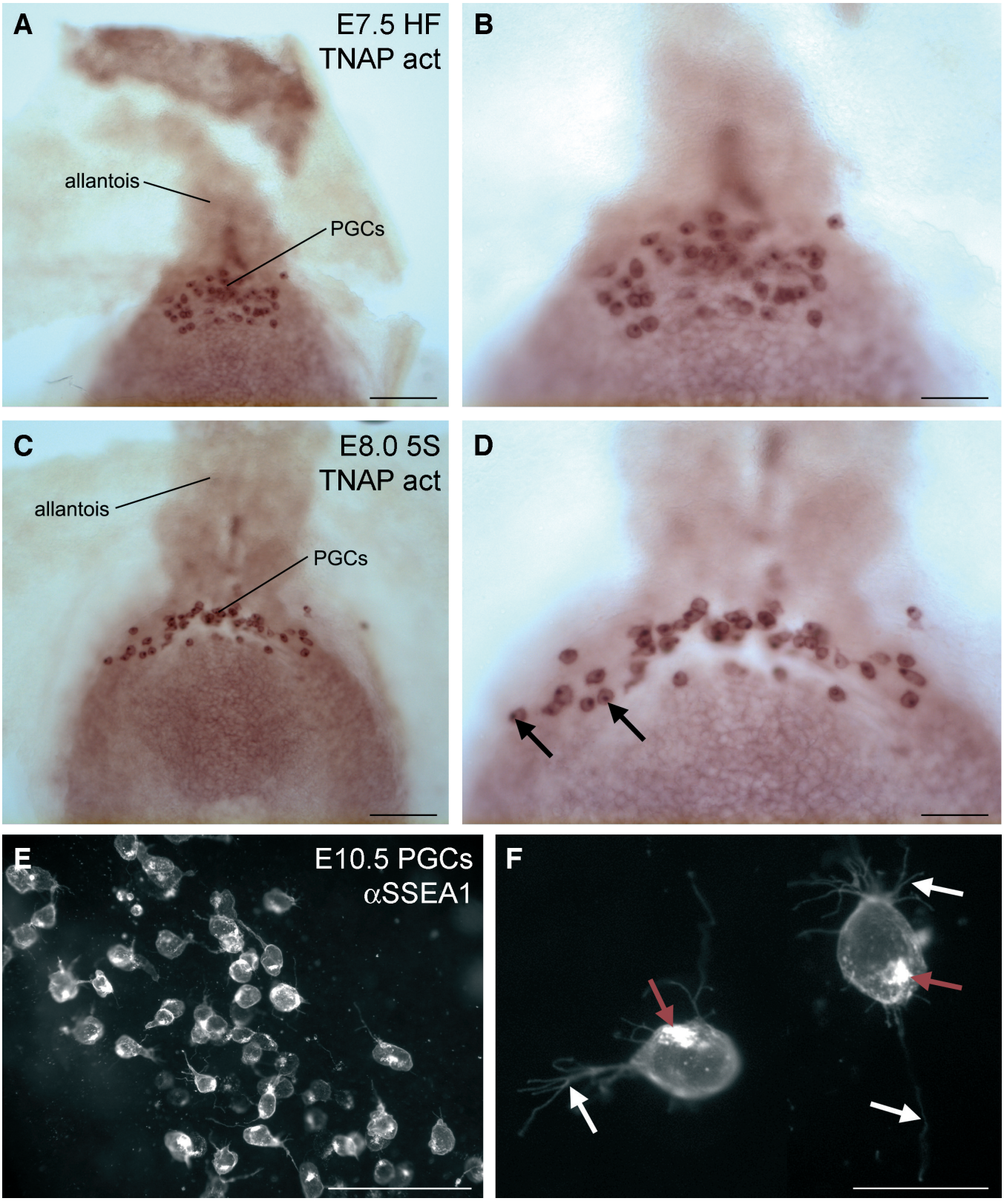

Fig. 1. Primordial germ cells (PGCs) between specification and arrival to the genital ridges. **A-D.** Posterior view of a mouse embryo at embryonic day (E)7.5 at the headfold stage (HF) **(A, B)** and an E8.0 embryo with 5 somites (5S) **(C, D)** stained for alkaline phosphatase activity (TNAP act) showing the PGCs (red staining). Note the characteristic "dot" staining the Golgi apparatus (black arrows in **D**) and the cell membrane of the PGCs. PGCs are formed at the base of the allantois, but rapidly become part of the endoderm. **E, F.** Genital ridges of an E10.5 mouse embryo immunostained for SSEA1, showing the Golgi apparatus (brown arrows in **F**) and the cell membrane, including the extensive network of pseudopodia (white arrows in **F**). PGCs, primordial germ cells. Scale bars: A, C, E, 100 μm; B, D, 50 μm; F, 25 μm.

interactions ("inductive", "regulative" or "epigenetic" mode) between the epiblast and the extra-embryonic ectoderm and visceral endoderm in early post-implantation embryos around E7.25. The inductive role has been attributed to the bone morphogenetic protein (BMP) signalling pathway (Lawson et al., 1999; Ying et al., 2000; Ying and Zhao, 2001; Hayashi et al., 2002; de Sousa Lopes et al., 2004). In addition, Vasa and Piwi homologues have not been observed in just specified PGCs, but their expression is instead only detected later in development, when the PGCs are about to enter the genital ridges (Toyooka et al., 2000; Kuramochi-Miyagawa et al., 2001).

The germline specificity of germ plasm/P-granules contrasts with the widely spread presence of other mRNA/protein containing particles known as the P-bodies. The P-bodies, reported from yeast to man, are discrete cytoplasmic foci responsible for the decapping and degradation of mRNAs (Sheth and Parker, 2003), including mRNAs that have been targeted by microRNAs (miRNAs). In mammalian cells, miRNA incorporated by the RNA-induced silencing complex RISC binds target mRNAs and directs them to P-bodies (Liu et al., 2005; Sen and Blau, 2005). The P-bodies repress mRNA expression either by inhibiting translation or by promoting mRNA degradation. P-bodies also resemble "stress-granules" that harbour untranslated mRNAs which accumulate during stress (Kedersha et al., 2005). The relationship between P-bodies and P-granules is still unclear. However, P-bodies may be heterogeneous particles and different cell types may use different types of P-bodies to regulate different aspects of RNA storage and metabolism. Further analysis of these two particles may reveal a possible relationship.

The mitochondria

Mitochondria are pivotal cytoplasmic organelles present in almost all eukaryotic cells and their role in adenosine triphosphate (ATP) production through oxidative phosphorylation is well understood. Mitochondria harbour genetic material in the form of one or more copies of circular double-stranded mitochondrial DNA (mtDNA; approximately 16.5 Kb in mammals) and they vary considerably in number, size, shape and distribution in the cell. In normal fertilization, there is little contribution of the sperm cell to the mitochondria of the newly formed zygote and any paternal mtDNA is degraded early during preimplantation development by still uncharacterized mechanisms (Kaneda et al., 1995). Since mitochondria and mtDNA are not made *de novo*, all mitochondria in the adult mammal are derived from those in the oocyte (maternal origin).

The mtDNA sequences in mammalian cells, including oocytes, are mostly of a single variant (homoplasmy). Genotyping of the mtDNA of heteroplasmic farm animals demonstrated that these genotypes can change rapidly and return to homoplasmy within several generations (Upholt and Dawid, 1977; Olivo et al., 1983; Ashley et al., 1989). This has led to the hypothesis that somewhere in the development of the germ line there should be a mitochondrial 'bottleneck' and various hypotheses have subsequently been put forward on when and how this bottleneck should occur. In mammalian maturing oocytes the number of mitochondria increases rapidly, but mitochondrial replication is halted in early embryonic development during the cleavage stages and only resumes after implantation. This results in a decreasing number of mitochondria (and therefore of mtDNA) per cell, as the mitochondria are simply distributed among the cells of the embryo (Dumollard et al., 2007; May-Panloup et al., 2007; Shoubridge and Wai, 2007 for review). Human PGCs in the yolk sac endoderm only contain about 10 mitochondria per cell, and this number increases rapidly to about 100 mitochondria in germ cells in the ovaries and close to 10,000 mitochondria in primordial follicle oocytes (Jansen and de Boer, 1998).

Intriguingly, it has recently been concluded that in the mouse the bottleneck occurs without a reduction in the mtDNA content (Cao et al., 2007), whereas others have demonstrated a significant reduction in mtDNA in early PGCs (Cree et al., 2008; Wai et al., 2008). All groups agree that there is a physical bottleneck, as the actual number of mitochondria is lowest in early PGCs (about 200 mitochondria/PGC). This physical bottleneck would allow selection against severe mtDNA mutations. Wai and colleagues (2008) have suggested that the physical bottleneck does not coincide with the genetic bottleneck, but that the genetic bottleneck occurs only during oocyte maturation due to the preferential replication of a small set of mtDNA, providing selection against less deleterious mtDNA mutations. Further research will clarify the nature and mechanisms that lead to the genetic bottleneck.

The Golgi apparatus

The Golgi apparatus is a dynamic cytoplasmic organelle with multiple functions as "biosynthetic centre" and "sorting centre" involved both in endocytic and exocytic trafficking in the cell. It plays a particularly important role directing proteins to different destinations, including lysosomes, secretory vesicles and the plasma membrane (reviewed by De Matteis and Luini, 2008). The Golgi apparatus is not a prominent feature of early PGCs, but some of the proteins that are expressed specifically in the cell membrane of PGCs, including TNAP/Akp52 (Fig. 1A-D), Fragillis/Mil1/Iftmit3 and, somewhat later in development, the SSEA1-antigen (Fig. 1E,F), are observed in the Golgi apparatus. The Golgi apparatus has therefore become a convenient morphological marker to identify (and quantify) PGCs from the moment of specification at E7.25 until sex differentiation at E12.5.

The Barr body (or sex chromatin)

The Barr body (Fig. 2A), named after Murray Barr

who described it for the first time (Barr and Bertram, 1949), is a chromatin dense structure located perinuclearly and is present in interphase female, but not male, somatic cells. The Barr body corresponds to the physically inactive X chromosome, and the number of Barr bodies is always the number of X chromosomes minus one, also in individuals with more than two X chromosomes (Barr and Carr, 1960).

The Barr body is a dynamic structure that is not always present during embryonic development. At the morula stage, all cells of the female mouse embryo contain a silent paternal X chromosome, but at the blastocyst stage, the cells of the inner cell mass reactivate the silent X chromosome. Shortly after implantation however, the Barr body is again present in all cells of the mouse embryo. In contrast to the cells of the trophectoderm that retain a silent paternal X chromosome (imprinted X inactivation), the cells of the inner cell mass of the embryo show a random pattern of X inactivation. The mechanism leading to imprinted or random X chromosome inactivation is still not completely understood (for a review see Payer and Lee, 2008). Directly involved in the initiation mechanism that leads to X chromosome silencing during mouse early development is the non-coding mRNA *Xist*, the polycomb protein complex PRC2 containing Ezh2/Eed, and the histone H3 modification H3K27me3 (Fig. 2A).

Female PGCs contain a Barr body just after specification (Fig. 2A), however during migration to the genital ridges, the PGCs lose the Barr body and in fact start reactivating the inactive X chromosome (de Napoles et al., 2007; Sugimoto and Abe, 2007; Chuva de Sousa Lopes et al., 2008). In the genital ridges and before meiosis entry, female germ cells show transcription and translation products from both active X chromosomes.

Prominent organelles and structures during oogenesis

Mitochondria and the formation of cysts in the female genital ridges

In mouse embryos, PGCs become incorporated into the hindgut from which they migrate to the developing gonads starting around E9.5. The first PGCs reach the genital ridges at about E10.5. During migration, the number of PGCs increases by normal mitosis from less than 100 to tens of thousands at E13.5 (Tam and Snow, 1981).

In the female gonads, germ cells do not undergo classical mitosis, but the daughter cells show incomplete cytokinesis, remaining in close contact with each other (Fig. 2B) via cytoplasmic bridges ("ring canals"). The germ cells forming each cyst divide synchronously (Pepling and Spradling, 1998). The cysts are reminiscent of those observed in the ovaries of other species such as the fruitfly *D. melanogaster* or the frog *X. laevis* (Pepling et al., 1999). The purpose of the cysts is not well understood, but signalling molecules may pass through the ring canals and certain cell organelles, including mitochondria and endoplasmic reticulum, have been observed in the ring canals just prior to cyst breakdown.

In mouse ovaries, the number of germ cells declines rapidly at birth between E20.5 and E22.5 and this coincides exactly with the period of cyst breakdown (Pepling and Spradling, 2001). Possibly, germ cell apoptosis takes place to remove cells with chromosomal abnormalities, but this would implicate removal of all

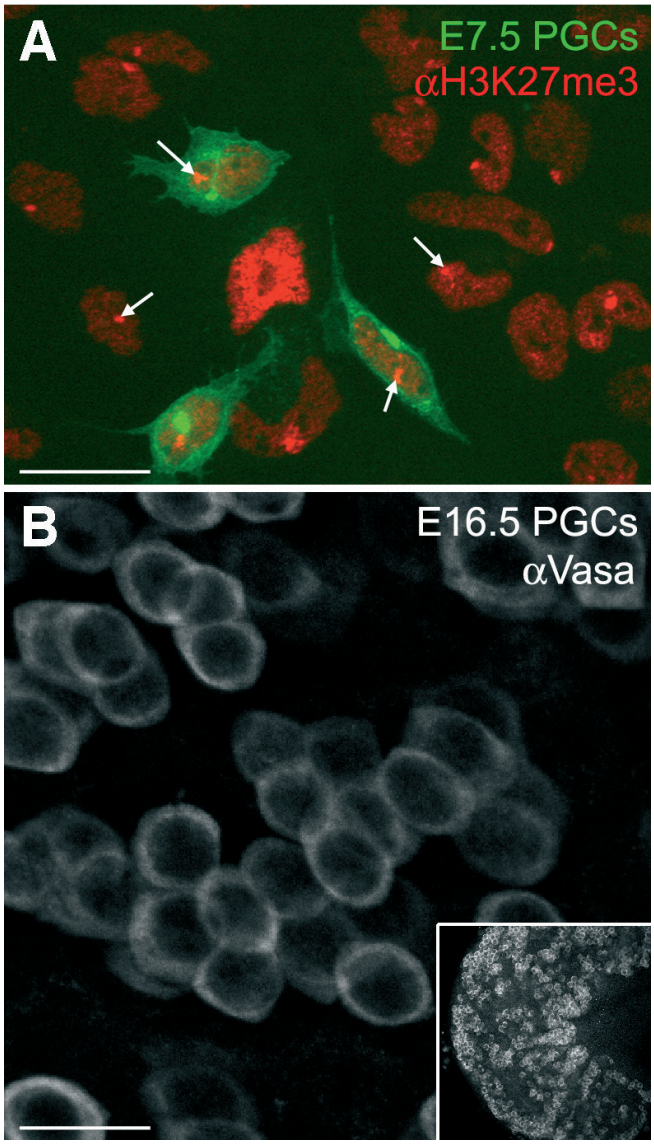

Fig. 2. Characteristics of female primordial germ cells. **A.** Murine female germ cells (in green) at E7.5, as well as female somatic cells, exhibit a prominent Barr body in their nucleus (white arrows), corresponding to the inactive X chromosome. The Barr body is visible as an accumulation of H3K27me3 (in red). **B.** Murine female germ cells in the genital ridges undergo mitosis with incomplete cytokinesis, resulting in the formation of germ cell cysts clearly visible at E16.5. See overview of a female E16.5 ovary in the insert. The germ cells are immunostained with Vasa. Scale bars: A, B, 20 μm.

cells originating from one abnormal germ cell (the whole cyst). However, instead of all germ cells clustered together entering apoptosis, it has been demonstrated that apoptosis occurs in a subset of oocytes within individual cysts (Pepling and Spradling, 2001). There is increasing evidence that also in mammals cyst formation participates in oocyte selection, and that from each cyst only one or two cells become definitive oocytes, whereas the other cells of the cyst may primarily function as nurse cells, such as in *Drosophila*. Interestingly, Pepling and Spradling (2001) suggest that cyst formation and breakdown may ensure that definitive oocytes acquire high-quality mitochondria and that this corresponds with the mitochondrial genetic bottleneck. A possible relationship between the model proposed by Pepling and Spradling (2001) of mitochondria transfer between sister oocytes and the model proposed by Wai and colleagues (2008) of preferential replication (see section the mitochondria) remains to be demonstrated.

The Balbiani body (or mitochondrial cloud)

The Balbiani body (Henneguy, 1887) is a cytoplasmic structure present in young immature oocytes in mammals and many other species, for example spiders and myriapods, where it was extensively described by E. G. Balbiani (reviewed by Kloc et al., 2004). The Balbiani body is a transient structure composed of a large number of mitochondria, Golgi complexes, endoplasmic reticulum. The Balbiany body also contains ill-defined electron-dense granulofibrillar material that congregates at a perinuclear location, but as the oocyte matures it moves to a peripheral location in the oocyte cortex (in some species corresponding to the future vegetal pole) and disperses (reviewed by Kloc et al., 2004). The composition of the Balbiani body also appears to be dynamic. The mechanism regulating the migration of the Balbiani body from the perinuclear location to the oocyte cortex (Fig. 3A) and its dispersion is unknown.

The function of the Balbiani body in organisms with the "preformation" or "inheritance" mode of germ cell formation seems to be that of contributing to the assembly and transportation of the germ plasm determinants to the cortex of the mature oocyte (P-granules in *C. elegans*; polar granules in *D. melanogaster*; germinal granules in *X. laevis*). In those organisms, the composition of the electron-dense material is probably similar between the Balbiani body and the germ granules, and includes Vasa, Dazl and Nanos. In mammals, which show an "inductive", "regulative" or "epigenetic" mode of germ cell formation and therefore do not have germ plasm determinants, the function of the Balbiani body remains largely obscure, but may include the controlled amplification of mitochondria or the creation of an asymmetry or polarity in the oocyte. In fact, the cytoplasm of developing rat oocytes has been demonstrated to contain a distinct polar basophilic zone that could correspond to remnants of the dispersed Balbiani body. This polar basophilic zone has only been identified in oocytes from healthy follicles, and has not been observed in atretic oocytes (Young et al., 1999). Possibly, a functional link exists between the Balbiani body, mitochondria, polarity and oocyte atresia.

Interestingly, in mice the Balbiani body is a very transient structure only recently described in young primordial follicles (Pepling et al., 2007). In contrast to human oocytes and those of other non-mammalian organisms, the mouse Balbiani body does not contain Vasa, but is enriched for the protein Trailer Hitch, Tral (Castrillon et al., 2000; Pepling et al., 2007). In *Drosophila*, Tral is associated with the Balbiani body and is important for correct mRNA localisation of, for instance, *Gurken*, possibly by regulating normal endoplasmic reticulum exit site distribution (Wilhelm et al., 2005). However, the function of Tral in mouse oocytes is as yet unknown.

The germinal vesicle and the nucleolus

The germinal vesicle (GV) is the name given to the nucleus of an oocyte arrested in the diplotene stage of prophase I. A germinal vesicle stage oocyte is tetraploid (4N) and still has a nuclear envelope, containing a duplicated set of chromosomes and a large round nucleolus surrounded by chromatin (Fig. 3B,C). Two types of GV oocytes have been acknowledged based on the presence or absence of a defined rim of chromatin: the SN configuration (surrounded nucleolus, Fig. 3B,C) and NSN configuration (non surrounded nucleolus).

In general, the nucleolus is a prominent nuclear structure where the ribosomal precursors are synthesised and assembled. It contains ribosomal RNAs (rRNAs) and about 500 different proteins (Andersen et al., 2005). Ribosomes are essential for protein synthesis in the cell and therefore absolutely necessary for cell growth and proliferation.

In mammals, primordial oocytes arrested in development have an inactive nucleolus, although during folliculogenesis the nucleolus becomes transiently active until the end of the growth phase in the antral follicle (Maddox-Hyttel et al., 2007). This is then followed by the germinal vesicle break down (GVBD), characterized by the disappearance of the nucleolus and the resumption of meiosis I leading to the extrusion of the first polar body. It has been demonstrated that components of the maternal nucleolus are essential for successful early embryonic development in mammals (Ogushi et al., 2008).

Apart from the nuclear changes, oocyte maturation is characterized by extensive reorganization of cell organelles. In oocytes of various mammalian species the mitochondria translocate again from a cortical to a perinucelar area shortly before GVBD and continue to aggregate in the perinuclear region at metaphase I (Van Blerkom and Runner, 1984; Sun et al., 2001). The endoplasmic reticulum also reorganizes during oocyte maturation, placing a calcium storage compartment, necessary for oocyte activation after fertilisation,

beneath the plasma membrane (Kline, 2000). Although much is still unknown, it is clear that local cytoplasmic events that are essential for proper oocyte maturation occur before GVBD (Hölzenspies et al., 2009).

The first and second polar body

The formation of the first and second polar bodies (Fig. 3D) results from the extreme asymmetric meiotic divisions (meiosis I and meiosis II, respectively) that take place in the maturing oocyte to reduce the maternal DNA content from 4N to 1N, with minimal reduction of cytoplasm (and organelles). The first polar body is diploid containing two complete sets of chromosomes (2N) and the second polar body is haploid (1N). Occasionally the first polar body also undergoes a second meiotic division. The polar bodies are located inside the zona pellucida (Fig. 3D) as tiny cells and eventually degenerate. In principle, the genetic material of the polar bodies is identical to that of the remaining oocyte, and it appears that only a lack of cytoplasm and organelles prevents the developmental capacity of these cells. Indeed, when the chromosomes of the first polar body were transplanted to an enucleated oocyte, the cell underwent a second meiotic division and led to a healthy animal after fertilization (Wakayama and Yanagimachi,

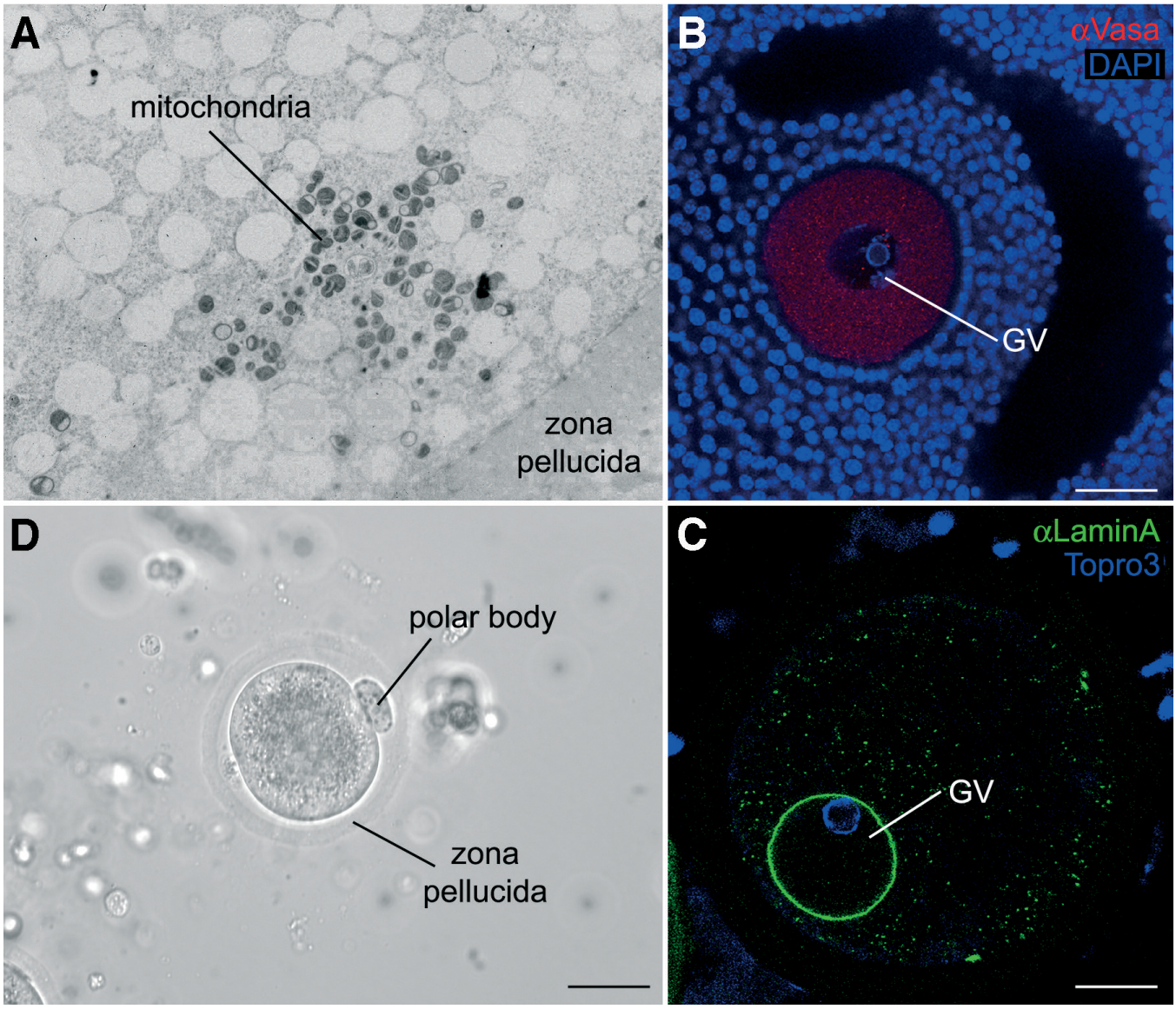

Fig. 3. Characteristics of female oocytes. **A.** Electron microscopy view of an agglomeration of mitochondria in the cortex of a bovine oocyte, near the zona pellucida. **B, C.** Germinal vesicle (GV) oocytes of the mouse **(B)** and pig **(C)** stained by immunofluorescence for Vasa (cytoplasm) and LaminA (nuclear envelope), respectively. Note the condensed chromatin (DAPI and Topro3 staining) forming the characteristic rim around the nucleolus. **D.** Mouse MI oocyte together with a polar body inside the zona pellucida. Scale bars: B, 40 µm; C, 20 µm; D, 50 µm.

1998). Similarly, the chromosomes of the second polar body have full potential to participate in normal embryonic development (Wakayama et al., 1997). The first and second meiotic divisions have to be carefully orchestrated, not only in the proper segregation of genetic material, but also in the correct distribution of cytoplasmic components.

In mammals, the position of the polar bodies, in particular the second polar body, and the asymmetry portrayed in the oocyte and zygote, has been related to polarization of the embryo during preimplantation and a matter of intense debate throughout the years. However, studying the highly "regulative" mouse embryo has proven difficult to pin-down. The latest view (Rossant and Tam, 2009) suggests that it is the inadvertently physical constraint posed by the ellipsoidal shape of the zona pellucida that causes a bias in the development of polarity in the mammalian embryo, and that existing asymmetries in the mammalian oocyte/zygote are an "evolutionary relic" that can influence axis formation but are easily overruled.

Prominent organelles and structures during spermatogenesis

The sex body (or XY body)

The sex body is a dense transient nuclear compartment characteristic of mammalian spermatocytes in meiotic prophase I and consists of the transcriptionally silenced X and Y chromosomes and associated proteins (Lenhossek, 1898; Solari, 1974). The sex body resembles the Barr body, which consists of the transcriptionally silent X chromosome present in female somatic cells. However, in contrast to the Barr body, the sex body is visible only when meiotic synapsis between the autosomal chromosomes is complete at the pachytene stage. The sex chromosomes X and Y, which are largely nonhomologous (Fig. 4A) showing only limited synapsis on their pseudoautosomal regions (Burgoyne, 1982), are silenced by a process known as meiotic sex chromosome inactivation (MSCI) in the sex body. In contrast to the Barr body, the silencing of the sex chromosomes in the sex body is not *Xist* dependent (McCarrey et al., 2002; Turner et al., 2002), even though *Xist* RNA is found to coat the sex body.

Meiotic silencing of unsynapsed chromatin is actually a widely spread mechanism observed from nematodes to mammals (both in males and females) to prevent regions of chromosomes without a homologous counterpart from undergoing recombination (Kelly and Aramayo, 2007). What recognizes the unsynapsed regions of sex chromosomes during meiosis in mouse spermatocytes is unclear, but at least the meiotic DNA double-strand breaks (DSBs) in the parts of chromosomes that fail to synapse accumulate BRCA1 and ATR at the pachytene stage. This is followed by the accumulation of H2AX phosphorylation (γH2AX) resulting in transcriptional silencing by MSCI (for a review see Turner, 2007). At the pachytene stage, the sex body is visible at a perinuclear location (Fig. 4A) and translocates to a central position in the nucleus at the diplotene stage. After the completion of meiosis, the X and Y chromosomes are only partially reactivated in the haploid spermatids, and this post-meiotic sex chromosome repression may have evolved to suppress genomic conflicts and sex ration distortion (Ellis et al., 2005).

In mice, MCSI is not a prerequisite for imprinted (paternal) X chromosome inactivation observed in preimplantation female embryos (Okamoto et al., 2005), even though the paternal X is probably inherited in an epigenetically inactive state (Huynh and Lee, 2003).

The chromatoid body

The chromatoid body is a spherical cytoplasmic granule visible in pachytene spermatocytes and spermatids (von Brunn, 1876; Schreiner and Schreiner, 1905). Its number in spermatocytes is variable, but it is usually a single granule in mouse and rat spermatids (Fig. 4B). In round spermatids, the chromatoid body is a dynamic granule that moves actively in the vicinity of the nuclear pores, but during spermiogenesis the chromatoid body moves caudally to the base of the flagellum at the opposite site of the forming acrosome and disperses or is degraded. The chromatoid body does not have an encircling membrane, but it is surrounded by a number of small vesicles.

A detailed analysis of the chromatoid body proteome and transcriptome has not yet been performed, but some of the known components of the chromatoid body include the proteins Vasa, Dicer, Tudor, and mRNAs and miRNAs. In mice, the chromatoid body also contains Piwi homologues (Miwi, Mili and Miwi2) and Maelstrom, proteins that when genetically deleted give rise to specific post-natal spermatogenesis defects. Moreover, a recent class of small RNAs, the Piwi-interacting RNAs (piRNAs), has been isolated from mouse testes and further studies on their function may help to understand the role of the chromatoid body during spermiogenesis (for a comprehensive review see Yokota, 2008). Interestingly, the chromatoid body shares similarities with the embryonic P-granules and the somatic P-bodies, like the presence of mRNAs, RNA decapping enzymes, exonucleases and Argonaut proteins, and all these granules seem to regulate aspects of RNA storage, metabolism and be involved in the microRNA pathway.

The sperm: acrosome, centrosome, tail and mitochondial neck

Spermiogenesis is the process by which spermatids differentiate to mature sperm and this is accompanied by characteristic morphological changes and the development of specific structures. One of these structures is the acrosome, a specialized lysosome that develops from the Golgi apparatus to form a flattened vesicle visible as a cap-like structure on the anterior half

of the sperm nucleus when sperm maturation is completed (Fig. 4C). The acrosome contains digestive enzymes (hyaluronidase and acrosin) that are expelled after fusion of the inner and outer leaflets of the acrosome membranes, when the sperm cell contacts the zona pellucida of the oocyte. The acrosome enzymes are responsible for the digestion of a hole in the zona pellucida that enables proximity with the oocyte followed by fertilization.

Inheritance of the centrosome (centriole) in most mammals, including humans, obeys Boveri's rule of exclusive paternal inheritance. Mice are an exception to this rule as they exhibit maternal inheritance. The centrosome consists of a pair of centrioles, each a small cylindrical organelle made of 9 groups of 3 fused microtubes, perpendicular to one another. The presence of the centrosome in the zygote is necessary for spindle formation and cell division. The sperm tail flagellum (Fig. 4C) originates from one of the centrioles and forms the axoneme, a 9+2 structure of microtubules plus associated dynein arms, which are responsible for the sperm tail movement. During spermiogenesis, a packed sheath of mitochondria deposits around the anterior part of the axoneme (Fig. 4C) and will be responsible for the energy supply needed to propel the sperm.

Concluding remarks

Germ cells are probably the most dynamic cells in the body during the life cycle. It is therefore not surprising that compartmentalization of organelles and molecules and the formation and degradation of

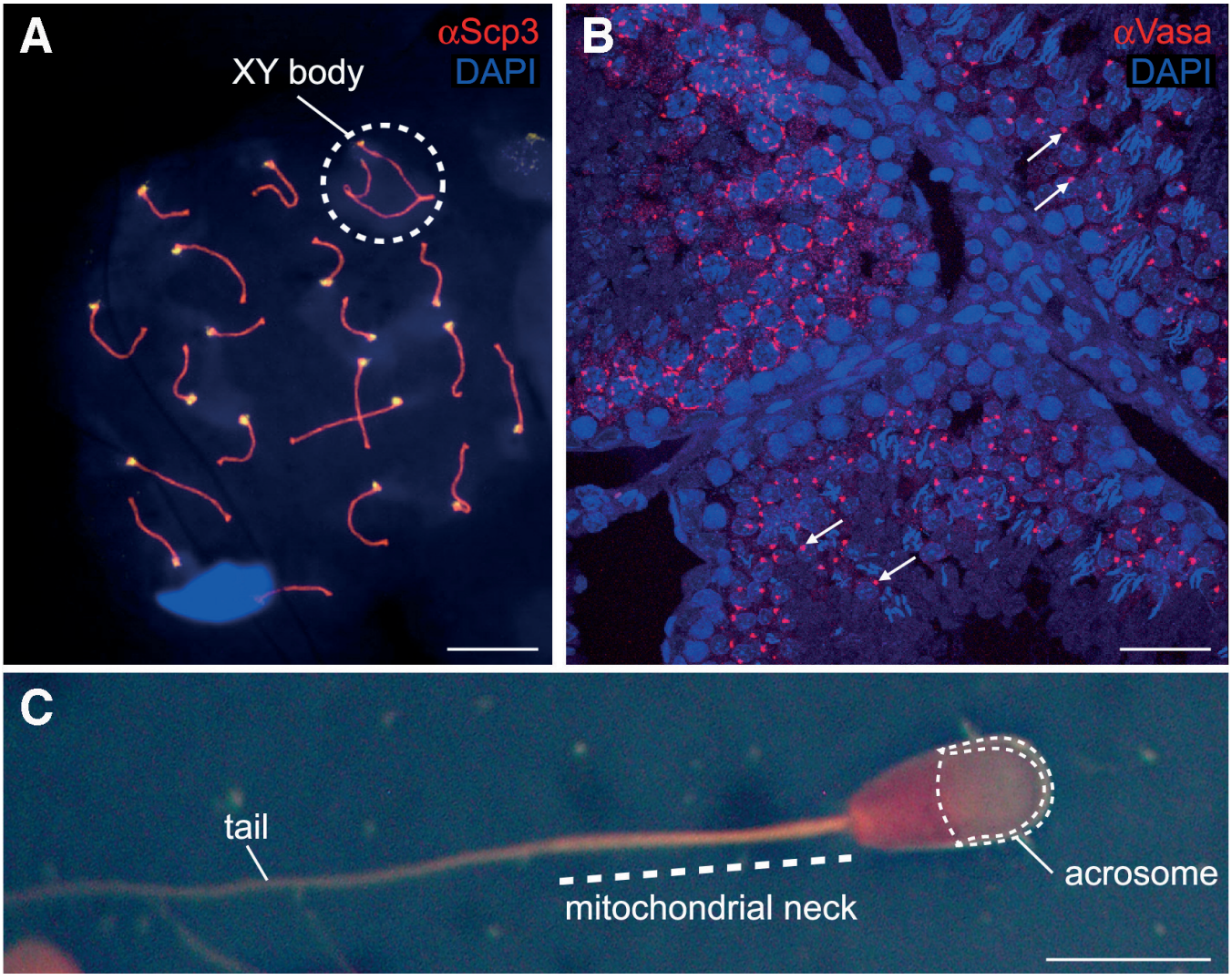

Fig. 4. Characteristics of male germ cells during spermatogenesis. **A.** Meiotic spread of a mouse pachytene spermatocyte immunostained for Scp3 (in red), showing all the synapsed autosomal chromosomes, but only partially synapsed sex chromosomes forming the XY body. **B.** Section through the testis of an adult male rat stained by immunofluorescence for Vasa, which accumulates in the chromatoid bodies of pachytene spermatocytes and spermatids (white arrows). **C.** Mature bovine sperm cell stained with heamatoxilin and eosin. The tail, mitochondria neck and acrosome are clearly visible. Scale bars: A, 7 µm; B, 50 µm; C, 5 µm.

"bodies" is ever changing. Paradoxically, the germ cells are subjected to strict rules during development, not only to ensure that the next generation can be formed normally, but also to prevent them from going astray and becoming tumorigenic. Germ cells naturally follow two fates, depending on their gonadal environment, and are induced to become either male or female germ cells independently of their chromosomal constitution.

In the cytoplasm, the germ cells, both female and male, pay continuous special attention to the regulation and localization of mitochondria and RNAs. These two key cellular components play a major role developing the next generation, both in organisms with "epigenetic" and "inheritance" modes of germ cell formation.

In the nucleus, differences in sex chromosome constitution are a source of imbalances in gene expression and chromosome pairing and therefore germ cells (and somatic cells) had to devise mechanisms to rapidly inactivate and reactivate the sex chromosomes circumventing possible problems.

Most germ cells cellular bodies have been identified by classical cytological analysis, and it is only now that we are starting to discover their molecular components and understand their function during the germ cell cycle.

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