Introduction

The biology of spermatogenesis: the past, present and future

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The physiological function of spermatogenesis in *Caenorhabditis elegans*, *Drosophila melanogaster* and mammals is to produce spermatozoa (1n, haploid) that contain only half of the genetic material of spermatogonia (2n, diploid). This half number of chromosomes from a spermatozoon will then be reconstituted to become a diploid cell upon fertilization with an egg, which is also haploid. Thus, genetic information from two parental individuals can be passed onto their offspring. Spermatogenesis takes place in the seminiferous epithelium of the seminiferous tubule, the functional unit of the mammalian testis. In mammals, particularly in rodents, the fascinating morphological changes that occur during spermatogenesis involving cellular differentiation and transformation, mitosis, meiosis, germ cell movement, spermiogenesis and spermiation have been well documented from the 1950s through the 1980s. During this time, however, the regulation of, as well as the biochemical and molecular mechanisms underlying these diverse cellular events occurring throughout spermatogenesis, have remained largely unexplored. In the past two decades, important advancements have been made using new biochemical, cell and molecular biology techniques to understand how different genes, proteins and signalling pathways regulate various aspects of spermatogenesis. These include studies on the differentiation of spermatogonia from gonocytes; regulation of spermatogonial stem cells; regulation of spermatogonial mitosis; regulation of meiosis, spermiogenesis and spermiation; role of hormones (e.g. oestrogens, androgens) in spermatogenesis; transcriptional regulation of spermatogenesis; regulation of apoptosis; cell–cell interactions; and the biology of junction dynamics during spermatogenesis. The impact of environmental toxicants on spermatogenesis has also become an urgent issue in the field in light of declining fertility levels in males. Many of these studies have helped investigators to understand important similarities, differences and evolutionary relationships between *C. elegans*, *D. melanogaster* and mammals relating to spermatogenesis. In this Special Issue of the *Philosophical Transactions of the Royal Society B: Biological Sciences*, we have covered many of these areas, and in this *Introduction*, we highlight the topic of spermatogenesis by examining its past, present and future.

Keywords: spermatogenesis; seminiferous; mitosis; meiosis; differentiation

1. SPERMATOGENESIS: THE PAST

Perhaps, two of the most important discoveries in the past century in the field of spermatogenesis were the identification of the seminiferous epithelial cycle in mammals (LeBlond & Clermont 1952; Clermont 1972; De Kretser & Kerr 1988; Clermont *et al.* 1993), as well as the hypothalamic–pituitary–testicular axis that regulates spermatogenesis (Sharpe 1994). Without a doubt, these two findings have helped investigators to understand different cellular events that occur during spermatogenesis and to identify critical regulatory biomolecules and/or pathways with the use of staged seminiferous tubules isolated by transillumination stereomicroscopy (Parvinen 1982).

Indeed, a recent study using this 6-decade-old technique has helped investigators to identify different sets of genes that can potentially regulate different cellular events during spermatogenesis (Johnston *et al.* 2008). On the other hand, this technique, powerful as it is, still has its inherent limitations. For instance, at stage VIII of the seminiferous epithelial cycle, spermiation and blood–testis barrier (BTB) restructuring are known to take place simultaneously (LeBlond & Clermont 1952; Hess & de Franca 2008). Thus, stage VIII tubules isolated from the testis would be useful to identify genes and/or proteins that regulate these two cellular events. It is worth noting, however, that other important cellular events also occur in the seminiferous epithelium at stage VIII that are unrelated to spermiation and BTB restructuring. These include germ cell cycle progression associated with the differentiation of preleptotene to leptotene spermatocytes, preparation of genetic material in pachytene
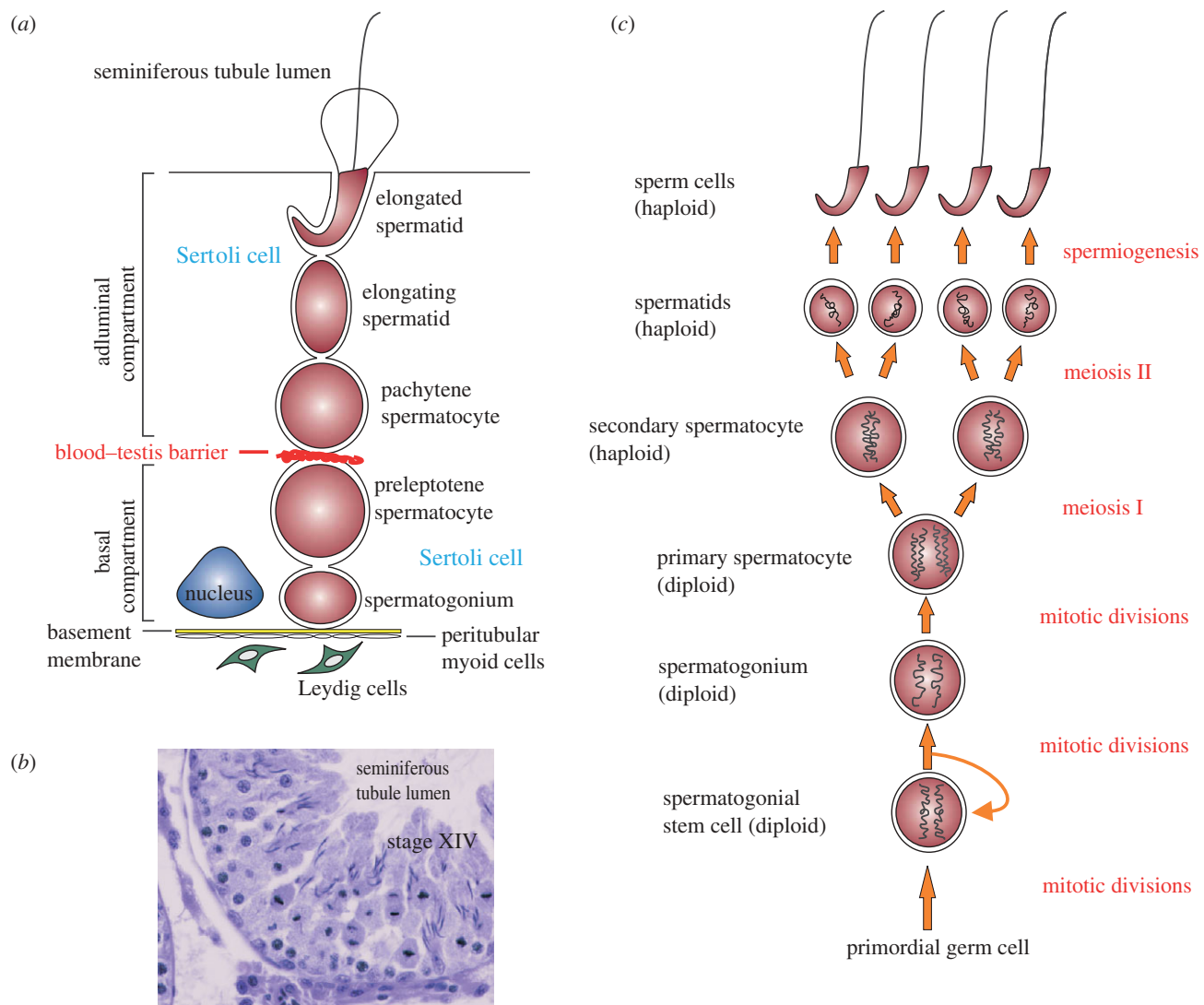
Figure 1. The biology of spermatogenesis in the rat. (*a*) Schematic drawing of the seminiferous epithelium from a seminiferous tubule in the adult rat testis, illustrating the morphological features of different germ cells during development and their intimate relationship with the Sertoli cell. Leydig cells that produce testosterone and oestradiol-17β via steroidogenesis are restricted to the interstitium. The blood–testis barrier (BTB) comprising tight junctions, basal ectoplasmic specializations and desmosome-gap junctions physically divides the seminiferous epithelium into a basal and an apical (adluminal) compartment. (*b*) Cross-section of a stage XIV tubule from an adult rat testis during which time meiosis occurs. Several newly formed spermatids from secondary spermatocytes at anaphase are clearly visible in this stage. (*c*) Drawing depicting the process that occurs in male germ cells during spermatogenesis.

spermatocytes for differentiation into diplotene spermatocytes at stage XIII and the eventual transformation of step 8 spermatids into step 9 spermatids. This is in addition to the occurrence of other Sertoli–Sertoli and Sertoli–germ cell interactions, as well as other paracrine and autocrine events (De Kretser & Kerr 1988; De Kretser 1990).

Figure 1 depicts key cellular events occurring during spermatogenesis in the seminiferous epithelium of the rat testis. Figure 1*a* is a schematic drawing illustrating the relative location of germ cells at different stages of their development where Leydig cells, under the influence of LH, undergo steroidogenesis to produce testosterone and oestradiol-17β that play a critical role in the regulation of spermatogenesis. The BTB situated near the basement membrane segregates the epithelium into a basal and an apical (adluminal) compartment. As such, meiosis which occurs at stage XIV of the seminiferous epithelial

cycle in the rat (figure 1*b*), together with the subsequent steps of post-meiotic germ cell development, are all sequestered from the systemic circulation, that is, they take place in a specialized microenvironment behind the BTB known as the adluminal compartment (figure 1*a*). Figure 1*c* depicts some of the key cellular events of spermatogenesis in rats, and these events are also applicable to other species including *Caenorhabditis elegans* and *Drosophila melanogaster*.

2. SPERMATOGENESIS: THE PRESENT
In this Special Issue, we have compiled several articles with the aim of providing up-to-date reviews in the field, especially in light of recent technological advancements that have helped us to better understand spermatogenesis. These include the use of proteomics and genomics (Calvel *et al.* 2010) and mouse genetic models (Verhoeven *et al.* 2010) to

examine different aspects of spermatogenesis. There are also other facets of spermatogenesis that have gained momentum in recent years such as the evolution of spermatogenesis in *C. elegans*, *D. melanogaster* and mammals (White-Cooper & Bausek 2010); regulation of apoptosis (Shaha *et al.* 2010); roles of oestrogens (Carreau *et al.* 2010; Carreau & Hess 2010), aromatase (Carreau *et al.* 2010) and androgens (Verhoeven *et al.* 2010; Walker 2010) in spermatogenesis; non-genomic action of testosterone and its impact on spermatogenesis (Walker 2010); regulation of mitosis and meiosis during spermatogenesis (Wolgemuth & Roberts 2010); impact of environmental toxicants and lifestyle effects on spermatogenesis (Sharpe 2010); spermatogonial stem cell biology (Phillips *et al.* 2010); transcriptional regulation of spermatogenesis (Bettegowda & Wilkinson 2010); and the roles of actin (Lie *et al.* 2010), gap junctions (Pointis *et al.* 2010), tight junctions (Morrow *et al.* 2010; Mruk & Cheng 2010) and anchoring junctions (Kopera *et al.* 2010; Mruk & Cheng 2010) in spermatogenesis.

As depicted in figure 1*c* which summarizes different cellular events that occur during spermatogenesis, it is obvious that some of the events listed on the right panel, such as spermiogenesis and its related cellular processes (e.g. spermatid adhesion to Sertoli cells) can become prime targets to disrupt spermatogenesis, leading to male infertility. Indeed, in recent years there has been an interest to develop new approaches for male contraception in order to provide additional means to reduce overpopulation and/or control population growth, particularly in developing countries, so that limited global resources in the globe can sustain the world's population. Herein, we wish to use a recent example to illustrate the importance of studying spermatogenesis, which can lead to exciting developments in a related field, such as male contraception. In the 1970s, there was an interest to develop male contraceptive drugs based on the core structure of indazole-3-carboxylic acid, such as lonidamine [1-(2,4-dichlorobenzyl)-indazole-3-carboxylic acid] (Palazzo *et al.* 1966; Corsi & Palazzo 1976). In rodents, lonidamine and its analogues were shown to be potent compounds that induced spermatid loss from the seminiferous epithelium without affecting cell adhesion in other organs (Silvestrini 1981; Silvestrini *et al.* 1984). In the late 1980s, after much experimentation, we noticed that this class of compounds primarily affected a unique ultrastructure in the seminiferous epithelium known as the apical ectoplasmic specialization (apical ES), a testis-specific atypical adherens junction (AJ) type (Russell & Peterson 1985; Cheng & Mruk 2002; Mruk & Cheng 2008; Vogl *et al.* 2008; Wong *et al.* 2008; Wong & Cheng 2009). However, lonidamine was subsequently shown to be toxic for use as a male contraceptive. Instead, it was developed into an anti-cancer drug whose mechanism of action is to inhibit hexokinase activity during mitochondrial respiration in cancer cells that were first sensitized with X-irradiation, which caused mitochondria to condense (note: condensed mitochondria are also present abundantly in elongating/elongated spermatids) (Silvestrini

1981; Silvestrini *et al.* 1984). We subsequently screened several dozen new analogues of lonidamine by investigating whether these compounds could induce the steady-state mRNA and/or protein level of testin, a protein localizing to the apical ES whose expression is tightly coupled to the integrity of this cell junction (Cheng & Bardin 1987; Cheng *et al.* 1989; Grima *et al.* 1997, 1998). Based on a series of studies that spanned almost 10 years, we selected adjudin (formerly called AF-2364) [1-(2,4-dichlorobenzyl)-1*H*-indazole-3-carbohydrazide] as our candidate compound, which was shown to induce testin expression by as much as 10- to 50-fold (Cheng *et al.* 2001), and this finding of testin induction by adjudin was subsequently confirmed in a gene profiling study (Xia *et al.* 2007). Our goal was to identify a new compound that would specifically affect Sertoli–germ cell adhesion, thereby defoliating developing spermatids from the epithelium (Cheng *et al.* 2001). Indeed, in a study using ^{3}[H]-adjudin to assess its tissue distribution, this drug distributed evenly among all organs in the adult rat. While ^{3}[H]-adjudin was not specifically uptaken by the testis, it only perturbed cell adhesion in the testis, particularly at the apical ES, and induced premature release of elongating/elongated spermatids from the seminiferous epithelium (Chen *et al.* 2003; Cheng *et al.* 2005). These findings were subsequently confirmed by licensed toxicologists in different toxicity studies (Mruk *et al.* 2006), as well as in recent studies from other laboratories (Tash *et al.* 2008*a*; Sarkar & Mathur 2009). In essence, adjudin is the hydrazide form of lonidamine. The similarity between adjudin and lonidamine is somewhat analogous to aspirin and its natural compound, salicyclic acid. Salicyclic acid, the active ingredient in extracts of willow bark or leaves is highly toxic; but aspirin, the acetylated form of salicyclic acid which was prepared by Felix Hoffman at the Bayer company in Germany in 1897 and became one of the most widely used non-steroid anti-inflammatory drugs, is not toxic (Vane & Botting 2003). In recent years, another analogue of indazole-carboxylic acid, known as gamendazole, has also emerged as a candidate for male contraception (Tash *et al.* 2008*a*,*b*). Gamendazole is significantly more potent than adjudin because it has an additional trifluoro group in the indazole ring (Tash *et al.* 2008*a*), thus making it more biologically active versus lonidamine, the free acid derivative of indazole-carboxylic acid and adjudin, the hydrazide derivative of lonidamine (Silvestrini *et al.* 1984; Cheng *et al.* 2001). While this trifluoro group increases gamendazole's potency by as much as approximately two- to eightfold ($6-25$ mg kg^{-1} b.w. for gamendazole versus $37.5-50$ mg kg^{-1} b.w. for adjudin in order for these compounds to effectively deplete germ cells from the seminiferous epithelium in virtually all seminiferous tubules in the testis) (Cheng *et al.* 2001, 2005; Tash *et al.* 2008*a*), mortality (i.e. three out of five deaths representing a 60% mortality rate) was observed in rats treated with gamendazole at 200 mg kg^{-1} b.w. but not with adjudin in the same study (Tash *et al.* 2008*a*). In the study on adjudin, two acute toxicity studies performed by licensed toxicologists using

mice ($n = 3$) and rats ($n = 10$) at doses of up to 1000 and 2000 mg kg^{-1} b.w., respectively, reported that there were no fatalities in both studies (Mruk *et al.* 2006). Additionally, a subchronic toxicity study performed by licensed toxicologists at 50 mg kg^{-1} b.w. for 29 days in male ($n = 10$) and female ($n = 10$) rats also reported no mortality (Mruk *et al.* 2006). These findings also illustrate that much research is needed to correlate results based on structure–activity studies in order to identify the best candidate compounds for human use. It is our belief that different formulations of either one of these two compounds will emerge as a drug that can induce reversible fertility in humans. At the very least, these examples illustrate that there are exciting developments in the field regarding the intervention of spermatogenesis that can induce reversible male infertility. However, it must be noted that none of these studies could have been performed without some understanding of the biology of adhesive junctions in the seminiferous epithelium, as this knowledge forms the basis of our understanding of spermatogenesis as depicted in figure 1.

Moreover, other important studies relating to spermatogenesis, such as the biology of spermatogonial stem cells (Kanatsu-Shinohara *et al.* 2008), have significantly impacted other studies in the field, such as the possible use of spermatogonial stem cells for therapeutic applications (i.e. producing non-reproductive tissues for transplantation applications) (Simon *et al.* 2009). Some of these areas of research are discussed in this Special Issue.

3. SPERMATOGENESIS: THE FUTURE

As briefly outlined above, there are many areas that require additional research. For instance, while we have acquired a great deal of knowledge about the transcriptional regulation of spermatogenesis during the past decade, only a few genes (or gene sets) are known to regulate spermatogenesis at the transcriptional level. Moreover, how these genes interact with each other to regulate diverse cellular events during spermatogenesis such as mitosis, meiosis and spermiogenesis remains largely unknown. On the other hand, while the mechanisms that regulate the BTB, which is constituted by coexisting tight junctions, desmosome-gap junctions and basal ectoplasmic specializations between adjacent Sertoli cells, are becoming clearer, there are virtually no functional studies in the literature which attempt to understand the biology of drug transport across this barrier. Without this additional information, it is difficult to develop new contraceptives and/or therapeutic drugs that can target the seminiferous epithelium behind the BTB in order to exert their effects on developing germ cells and/or germ cell tumours, respectively. However, we remain hopeful that significant advances will continue to be made in the next decade.

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