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REVIEW

Hormonal regulation of spermatogenesis

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Summary

Proper functioning of the mammalian testis is dependent upon an array of hormonal messengers acting through endocrine, paracrine, and autocrine pathways. Within the testis, the primary messengers are the gonadotrophins, follicle stimulating hormone and luteinizing hormone, and the androgens. Abundant evidence indicates that the role of the gonadotrophins is to maintain proper functioning of testicular somatic cells. It is the androgens, primarily testosterone, which act through the somatic cells to regulate germ cell differentiation. Despite extensive research in this area, little is known about the cell-specific requirements for androgens and even less is understood about the downstream effectors of androgen signalling. However, recent work using cell-specific ablation of androgen receptor function has demonstrated a clear requirement for androgen signalling at multiple, discrete time points during spermatogenesis. These models also provide useful tools for identifying the targets of androgen receptor activity. The purpose of this review is to provide a brief overview of recent advances in our understanding of hormonal regulation of spermatogenesis, with an emphasis on the role of testosterone within the testis, and to pose important questions for future research in this field.

Keywords: androgens, gonadotrophins, Sertoli, spermatogenesis

Gonadotrophic regulation of spermatogenesis

Spermatogenesis in mammals requires the actions of a complex assortment of peptide and steroid hormones, each of which plays an important role in the normal functioning of the seminiferous epithelium (Fig. 1 and Table 1). These hormonal messengers are critical not only for regulation of male germ cell development, but also for the proliferation and function of the somatic cell types required for proper development of the testis (Sharpe, 1994; McLachlan *et al.*, 2002). These include the interstitial steroidogenic Leydig cells, whose primary function appears to be production of testosterone (Mendis-Handagama, 1997); the myoid cells that surround the seminiferous tubules and provide physical support and contractile motion to these structures (Maekawa *et al.*, 1996); and the Sertoli cells, whose direct contact with proliferating and differentiating germ cells within the seminiferous tubules makes them essential for providing both physical and nutritional support for spermatogenesis (Griswold, 1998). Each of these cell types is a direct target for one or more of the hormones whose actions are essential for unimpaired male fertility.

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are glycoprotein hormones secreted by the anterior pituitary that act directly on the testis to stimulate somatic cell function in support of spermatogenesis. These hormones, part of the transforming growth factor (TGF) β superfamily of secreted growth factors, share a common α-subunit and are distinguished by their hormone-specific

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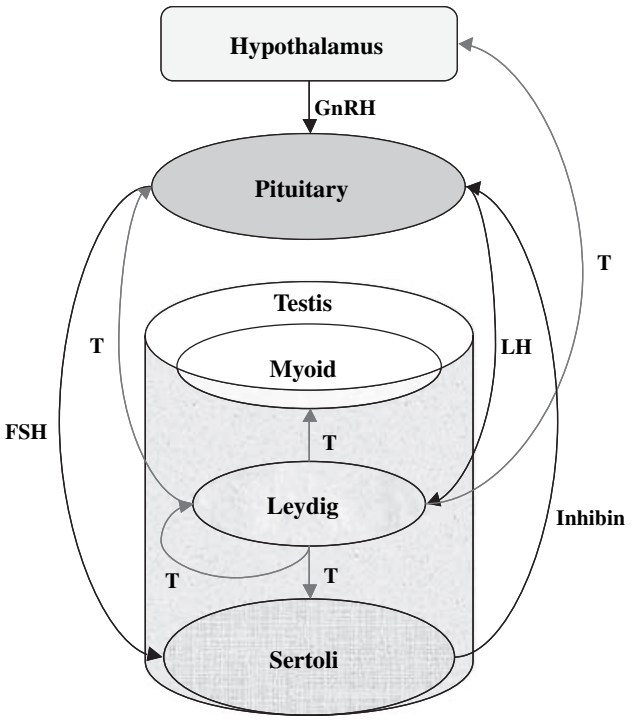

Figure 1. Hormonal regulation of spermatogenesis Most hormones shown can have both positive and negative effects, either at the level of receptor activation/desensitization or through activation and repression of downstream targets. GnRH, gonadotrophin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; T, testosterone.

β-subunit (Pierce & Parsons, 1981). In males, FSH receptor expression (FSH-R) is limited to the testicular Sertoli cells (Rannikki *et al.*, 1995), while LH receptors (LH-R) are found primarily in the Leydig cells, although receptor staining is also observed in spermatogenic cells (Eblen *et al.*, 2001; Lei *et al.*, 2001).

Genetic and pharmacological studies in rodents indicate that the primary role of FSH in spermatogenesis is stimulation of Sertoli cell proliferation during prepubertal development (Heckert & Griswold, 2002). Sertoli cell number largely determines the number of germ cells (Sharpe, 1994). Targeted mutations in the murine FSH-R and FSHβ subunit genes lead to dramatically reduced testis weights and epididymal sperm numbers, although males are fertile in both cases (Kumar *et al.*, 1997; Dierich *et al.*, 1998). These results are consistent with hormone depletion-replacement studies in human males (Matsumoto & Bremner, 1985; Matsumoto *et al.*, 1986), although unlike in rodents, FSH can also rescue spermatogenesis in gonadotrophin-suppressed men independently of T (Matsumoto *et al.*, 1983). However, the authors of this study suggest this effect is because of increased FSH-induced Sertoli cell sensitivity to residual T production within the testis.

Regulation of testosterone (T) synthesis seems to be the only indispensable function of LH within the adult testis. Treatment of LH-R knockout mice with exogenous T is able to fully rescue spermatogenesis in the absence of LH-R function (Lei *et al.*, 2001). Testosterone treatment also confers a qualitative recovery of spermatogenesis in *hpg* mice, which lack both FSH and LH because of an inactivating mutation at the GnRH locus (Singh *et al.*, 1995). In the absence of T replacement, spermatogenesis is arrested during meiosis in *hpg* males (Cattanach *et al.*, 1977). It is also clear that this effect of T replacement is not dependent upon stimulation of other Leydig cell products by T or LH, as T alone completely rescues spermatogenesis in rats treated with the Leydig cell-specific cytotoxin ethane dimethane sulphonate (EDS) (Kerr *et al.*, 1993).

Steroid function in spermatogenesis

Testosterone and its metabolites, dihydrotestosterone (DHT) and estradiol (E2), are collectively referred to as the sex hormones. This is because of their primary role in the regulation of gonadal and germ cell development in both males and females as well as in the sexual differentiation of males. In the male, T assumes the lead role in both morphological development and reproductive function, although E2 and its receptor estrogen receptor (ER)α, but not ERβ, clearly play some role in the maintenance of male fertility. However, these effects appear to be indirect and secondary. Disruption of *Erβ* has no apparent effect in males, as XY animals are morphologically normal and fertile (Krege *et al.*, 1998). Initial observations of *Erα* null male mice suggested a primary role for this gene in regulating spermatogenesis, as animals presented with reduced fertility and dramatically decreased epididymal sperm counts (Lubahn *et al.*, 1993). However, it is now clear that the primary function of ERα in the male reproductive tract is the regulation of luminal fluid reabsorption in the rete testis and efferent ducts linking the testis and epididymis (Hess *et al.*, 2000; Lee *et al.*, 2000). As might be expected, males homozygous for mutations in both *Erα* and β have a similar phenotype to $Er\alpha^{-/-}$ males (Couse *et al.*, 1999).

Targeted disruption of *Cyp19*, whose gene product converts T to E2, results in progressive loss of fertility and disruption of spermatogenesis, although males are initially fertile. It is unclear why loss of E2 synthesis via mutation of *Cyp19* has a less severe reproductive phenotype than disruption of *Erα*. However, it is suggestive of the existence of an as yet unidentified *Cyp19* paralog or an E2 independent function of ER within the $Cyp19^{-/-}$ male. Surprisingly, despite the complete absence of measurable aromatase activity from $Cyp19^{-/-}$ ovaries, there appears to be no difference in serum concentrations of E2 between wild-type and *Cyp19* mutant animals of either sex (Fisher *et al.*, 1998).

In contrast to the role of estrogens in males, androgens and androgen receptor (AR) function are essential for proper sexual differentiation and the maintenance of normal spermatogenesis. AR activity is regulated by T and DHT, whose binding initiates nuclear translocation and the

Table 1. Animal models of hormonal regulation of spermatogenesis

Model	Organism	Phenotype	References
Gnrh$^{hpg/hpg}$	Mouse	Loss of LH, FSH, and T secondary to GnRH deficiency; cryptorchid; spermatogenic arrest at pachytene, rescued by LH and T	Cattanach *et al.* (1977), Singh *et al.* (1995)
Fsh-r$^{-/-}$	Mouse	Loss of FSH-R signalling in Sertoli cells; decreased testis weight and sperm output because of reduced Sertoli cell proliferation; fertile	Dierich *et al.* (1998)
*Fsh*β$^{-/-}$	Mouse	Loss of FSH; decreased testis weight and sperm output as for *Fsh-r*$^{-/-}$; fertile	Kumar *et al.* (1997)
Lh-r$^{-/-}$	Mouse	T deficiency because of loss of LH-R function in Leydig cells; T still detectable in serum; failure of postnatal male sexual development, cryptorchid; spermatogenic arrest at round spermatid stage, rescued by T	Lei *et al.* (2001)
Hypo-physectomy	Rat	Loss of LH, FSH, and T because of removal of pituitary; regression of spermatogenesis to meiotic arrest, round and elongating spermatids rare; phenotype enhanced by EDS, rescued by LH and T	Elkington & Blackshaw (1974), Russell & Clermont (1977), Ghosh *et al.* (1991), Kerr *et al.* (1992), El Shennawy *et al.* (1998), Franca *et al.* (1998)
EDS	Rat	T deficiency because of selective destruction of Leydig cells; phenotype similar to hypophysectomy; rescued by T	Sharpe *et al.* (1990), Kerr *et al.* (1993)
Cyp19$^{-/-}$	Mouse	E2 deficiency because of lack of aromatase enzyme; abnormal male sexual behaviour; progressive disruption of spermatogenesis, although fertile for several months	Fisher *et al.* (1998), Robertson *et al.*, (1999), Robertson *et al.*, (2001)
*Er*α$^{-/-}$	Mouse	Indirect effect on spermatogenesis from loss of ERα signalling in rete testis and efferent ducts; disrupted spermatogenesis because of lack of luminal fluid reabsorption causing increased pressure in seminiferous tubules	Lubahn *et al.* (1993), Hess *et al.* (2000), Lee *et al.* (2000)
*Er*β$^{-/-}$	Mouse	No effect on spermatogenesis because of loss of ERβ	Krege *et al.* (1998)
Ar$^{Tfm/Y}$	Mouse	Null allele of *Ar*; pseudohermaphroditism; cryptorchid; meiotic arrest at pachytene	Lyon & Hawkes (1970)
Ar$^{flox(ex1-neo)/Y}$	Mouse	Hypomorphic *Ar* allele, reduced AR levels in all tissues; significantly elevated LH, FSH, and T levels; spermiogenic arrest during elongating steps	Holdcraft & Braun (2004)
Ar$^{flox(ex1-neo)/Y}$; *Amh-cre*	Mouse	Sertoli cell-specific ablation of AR function, hypomorphic for AR in all other cell types; spermiogenic arrest at the transition from round to elongating spermatids	Holdcraft & Braun (2004)
SCARKO	Mouse	Sertoli cell-specific ablation of AR function; meiosis is arrested during pachytene with corresponding reduction in testis mass; males otherwise indistinguishable from wild-type	De Gendt *et al.* (2004)
S-AR$^{-/y}$	Mouse	Sertoli cell-specific ablation of AR function; decreased testis mass with meiotic arrest at diplotene; hypotesteronemic with elevated serum LH level	Chang *et al.* (2004)

transcriptional regulatory function of AR (Lindzey *et al.*, 1994). In humans, DHT is crucial for the development of the male reproductive tract, although DHT plays little or no role in the development of male mice (Walsh *et al.*, 1974; Mahendroo *et al.*, 2001). As discussed above, T production is regulated by LH, whose release from the pituitary stimulates the steroidogenic Leydig cells in the testis (Mendis-Handagama, 1997). Not surprisingly, AR itself plays an important role in the regulation of T levels through autocrine feedback on the Leydig cells, via endocrine effects on GnRH production, and through inhibition of LH synthesis and secretion by the pituitary (Amory & Bremner, 2001).

In both humans and mice, males carrying a hemizygous null mutation in the X chromosome-linked *Ar* gene exhibit complete Androgen Insensitivity Syndrome (cAIS), characterized by pseudohermaphroditism and sterility (Lyon & Hawkes, 1970; Brown, 1995). This syndrome is recognized by the presentation of XY individuals with a stereotypically

female external appearance, usually diagnosed at puberty in connection with primary amenorrhoea (Brown, 1995). The vagina is incompletely formed and ends abruptly, forming a blunt-ended pouch. Small, abdominally positioned testes are the only internal reproductive organs present with spermatogenesis blocked early in meiosis and some seminiferous tubules having depletion of spermatogonia (Lyon & Hawkes, 1970; Vanha-Perttula *et al.*, 1970).

While the phenotype of cAIS rodents and patients clearly demonstrates the crucial requirement for AR in male development, the suitability of AIS as a model for studying the spermatogenic function of *Ar* is poor. Testicular descent fails in mice with cAIS, and the spermatogenic phenotype mimics that of cryptorchidism in an otherwise normal male, namely early meiotic arrest (Lyon & Hawkes, 1970). Thus it is impossible to discern the contribution to the phenotype of loss of AR function separate from that because of the abdominal positioning of the testes.

Mice homozygous for a mutation in the gonadotrophin releasing hormone gene ($Gnrh^{hpg/hpg}$), which have dramatically lowered serum testosterone levels (Singh *et al.*, 1995), present a testicular phenotype similar to cAIS (Cattanach *et al.*, 1977). Spermatogenesis in these animals can be qualitatively rescued by androgen replacement therapy (Singh *et al.*, 1995). This occurs in the absence of appreciable levels of LH and FSH. Further, FSH alone fails to significantly rescue spermatogenesis beyond the meiotic stages (Singh & Handelsman, 1996; Haywood *et al.*, 2003), providing further support for the contention that T and/or DHT is the major hormonal regulator of spermatogenesis. Interestingly, one study has shown that oestrogen supplementation in $Gnrh^{hpg/hpg}$ males may also rescue spermatogenesis (Ebling *et al.*, 2000). However, the mechanism by which this might occur is unclear.

An absolute requirement for androgens in spermatogenesis is further supported by classic hormone withdrawal experiments in rats. Removal of androgens from adult rats by hypophysectomy (Hx) leads to an acute, stage-specific regression of the seminiferous epithelium (Russell & Clermont, 1977; Ghosh *et al.*, 1991). Elimination of T is manifested initially as loss of mid-stage round spermatids and mature, elongated spermatozoa, indicative of an affect of androgens on spermiation and the transition from round to elongating steps of spermiogenesis. Mid-stage meiotic spermatocytes also undergo an immediate and obvious regression. After long term Hx and elimination of residual testosterone activity by flutamide or EDS treatment, spermatogenesis rarely proceeds beyond meiosis, with very few round spermatids observed and elongated spermatids nearly non-existent (Kerr *et al.*, 1992; Franca *et al.*, 1998). As with *Gnrh* null mice, androgen or LH replacement leads to qualitative recovery of spermatogenesis while FSH has little direct stimulatory effect (Elkington & Blackshaw, 1974; Russell & Clermont, 1977; El Shennawy *et al.*, 1998). Similar results are seen in response to suppression of *Gnrh* activity (Szende *et al.*, 1990), and destruction of Leydig cells with the Leydig-specific cytotoxin EDS (Sharpe *et al.*, 1990; Kerr *et al.*, 1993).

While androgens have a positive regulatory influence on differentiating germ cells, there is a well-established negative effect of androgens on the differentiation of spermatogonial stem cells. This observation stems from work aimed at understanding the prolonged suppression of spermatogenesis in men following radiation or chemotherapy treatment for cancer (Meistrich, 1998). Following treatment, spermatogonia are present, but fail to proliferate or differentiate. It was found that in irradiated rats, stimulation of spermatogenesis occurred following treatment with GnRH agonist or T, which both act to suppress intratesticular T concentration. This work was later extended to show that a GnRH antagonist stimulates spermatogonial proliferation and inhibits apoptosis following irradiation (Shuttlesworth *et al.*, 2000). In addition, recent studies have shown that this effect is because of inhibition of androgen function, as the androgen antagonist flutamide stimulates, while testosterone inhibits, spermatogonial proliferation and differentiation (Shetty *et al.*, 2000, 2002). A similar effect has been demonstrated in juvenile spermatogonial depletion (*jsd*) mice, whose germ cells regress to a spermatogonia-only phenotype following the first wave of spermatogenesis. Stimulation of spermatogonial proliferation and differentiation, along with completion of spermatogenesis, is observed in the testes of these animals following treatment with GnRH antagonist or flutamide. The stimulatory action of GnRH antagonist is prevented by co-administration of T, while flutamide reverses the repressive effect of testosterone (Shetty *et al.*, 2001).

In the testis, AR protein is expressed in the somatic Leydig, myoid, and Sertoli cells (Bremner *et al.*, 1994; Vornberger *et al.*, 1994; Suarez-Quian *et al.*, 1999; Zhou *et al.*, 2002). Some groups have reported the presence of AR within mouse fetal and postnatal germ, human spermatogonia, and rat spermatids (Kimura *et al.*, 1993; Vornberger *et al.*, 1994; Zhou *et al.*, 2002). However, it is clear from work with AR-null chimeric mice and from germ cell transplantation experiments that AR is not required in male germ cells for normal fertility (Lyon *et al.*, 1975; Johnston *et al.*, 2001). While expression in Leydig and myoid cells is continuous, Sertoli cell expression of AR occurs in a stage dependent fashion. Although it is apparent that other factors must be involved to confer stage-specific expression in the complex environment of the testis, hormone withdrawal-replacement experiments have shown that testosterone can support stage-specific Sertoli cell AR expression (Bremner *et al.*, 1994; Zhu *et al.*, 2000). In addition, a DHT responsive promoter has been defined upstream of the *Ar* gene itself, providing further support for androgen/AR auto-regulation of testicular AR expression (Grossmann *et al.*, 1994). Interestingly, stages VII–VIII, during which AR expression in Sertoli cells is highest, correspond directly with those most acutely affected by androgen withdrawal (Russell &

Clermont, 1977; Ghosh *et al.*, 1991; Kerr *et al.*, 1993). It is also during these stages where androgens have been shown to stimulate a stage-specific increase in overall protein secretion by the seminiferous tubule, including the induction of several specific androgen-regulated proteins (McKinnell & Sharpe, 1992).

Sertoli cells are also the only somatic cell type in direct contact with differentiating germ cells. They provide both physical and nutritional support for spermatogenesis, which occurs in the intercellular spaces between Sertoli cells (Griswold, 1998). Adhesion between germ cells and Sertoli cells may be androgen dependent, as testosterone withdrawal leads not only to retention and phagocytosis of mature, elongated spermatids, but also to the premature release of round spermatids (Russell & Clermont, 1977; Kerr *et al.*, 1993; O'Donnell *et al.*, 1996). Taken together, these observations have led to the general belief that Sertoli cells are the primary mediators of AR regulation of spermatogenesis.

To examine the consequences of loss AR function specifically in Sertoli cells, several groups have recently created conditional alleles of the *Ar* gene, and used them to generate Sertoli cell-specific ablation of *Ar* (Chang *et al.*, 2004; De Gendt *et al.*, 2004; Holdcraft & Braun, 2004). The results of theses studies suggest there are multiple AR-dependent steps during spermatogenesis. In the first study, a conditional allele of *Ar* was created by flanking exon 2 with *loxP* sites (De Gendt *et al.*, 2004). In the absence of Cre, the allele has full wild-type AR function. However, when crossed to mice expressing the Cre recombinase driven by the Sertoli cell-specific anti-Müllerian hormone (*Amh*) promoter, the germ cells undergo meiotic arrest, predominantly during the pachytene stage, although some diplotene spermatocytes, secondary spermatocytes, and round spermatids are produced. Other than sterility, the males appear to be normal with fully descended testes, and a grossly normal appearance.

In a second study, a hypomorphic conditional allele of *Ar* was created by introducing a neomycin-resistance gene cassette in the first intron of *Ar*, and flanking the first exon of *Ar* with inverted *loxP* sites (Holdcraft & Braun, 2004). The hypomorphic allele, which results in reduced levels the wild-type *Ar* mRNA in all AR-expressing cells, causes severe oligospermia, despite fully descended testes, indicating maximal sensitivity to loss of AR function during spermatid elongation. The males also exhibit severe alterations in serum hormone levels, with significant increases in LH, FSH, and T concentrations. Crossing the allele to an *Amh-Cre* line causes a more severe spermatogenic phenotype than hypomorphism alone. Although the males have no apparent defect in meiosis, the germ cells are defective in making the transition from round to elongating spermatids, as indicated by a significant reduction in elongated spermatids, and the continued presence of round spermatids in stage IX tubules. The elevated hormone levels in mice carrying the hypomorphic allele may explain the apparently contradictory results between the two studies, as elevated T and/or intratesticular E2 levels may be suppressing meiotic arrest. While serum E2 levels are normal in the males, it is possible that elevated intratesticular E2 levels, because of increased conversion of supraphysiological T levels, suppress the meiotic arrest phenotype. In support of this possibility, one previous study of *Gnrh*-null males has shown that E2 can support qualitatively normal spermatogenesis in the absence of testosterone (Ebling *et al.*, 2000). Alternatively, lack of meiotic arrest may be the result of residual AR activity in a subset of Sertoli cells because of incomplete inversion of the first exon. This latter hypothesis is consistent with the phenotype of LHβ mice (R. Kumar, personnel communication), and LH receptor-null mice (Lei *et al.*, 2001), in which a round spermatid arrest is observed as a consequence of basal levels of androgen action in the absence of LH signalling (Lei *et al.*, 2001).

A third conditional *Ar* allele has recently been reported that also has the second exon flanked by *loxP* sites (Chang *et al.*, 2004). Recombination-induced deletion of the allele in Sertoli cells, again by crossing to *Amh-Cre* mice, also causes azoospermia. In this model spermatogenesis is primarily blocked at the diplotene spermatocytes stage, instead of during pachytene (De Gendt *et al.*, 2004). It is unclear why the two alleles have different meiotic arrest phenotypes, although it could be because of the genetic backgrounds of the mice, the efficiency of recombination, or changes in steroid levels. The mice also exhibit hypotestosteronemia, while at the same time displaying significantly elevated serum LH levels. The authors suggest that the decreased concentration of serum T is because of upregulation of anti-Müllerian hormone expression by Sertoli cells, a conclusion supported by their own observation of elevated *Amh* mRNA in these animals, as well as by studies showing lowered serum T, Leydig cell hypoplasia, and inhibition of steroidogenesis, all induced by AMH (Behringer *et al.*, 1990; Lyet *et al.*, 1995; Fynn-Thompson *et al.*, 2003).

Taken together, the observations from these studies reveal a differential requirement for AR activity in Sertoli cells for at least three steps of spermatogenesis. AR is first required for progression through meiosis I, again during the transition from the round to elongating steps of spermatogenesis, and finally during the terminal stages of spermiogenesis. Surprisingly, it is the meiotic requirement for AR function that appears to be least sensitive to absolute levels of AR activity, as evidenced by the differential effects on meiosis observed between these models of Sertoli cell AR ablation. Whether AR function in Sertoli cells also affects steroidogenesis remains an open question.

Future studies

Many important questions remain to be answered regarding the androgenic regulation of male reproduction.

As discussed above, little is known about the phenomenon of stage-specific regulation of Sertoli cell AR expression beyond a presumptive role for androgens and for AR itself. Identification of potential co-activators or repressors that act in conjunction with AR at these stages, or the discovery of other transcription factors that regulate stage-specific AR expression, would provide insight into the important functional differences between Sertoli cells during the different stages of spermatogenesis. It would also be of interest to determine the role that the specific germ cell complement of tubules at different stages plays in the regulation of Sertoli cell function. Additionally, the discovery of novel factors that regulate AR expression or unique targets of AR transcriptional regulation within Sertoli cells would provide intriguing candidates for future male contraceptive design.

While androgens, acting through AR, are clearly essential for normal spermatogenesis, the crucial downstream targets of AR activation in the testis remain elusive. Androgens may also mediate effects in Sertoli cells via a non-classical receptor pathway, as androgens have been shown to activate signalling cascades in multiple cell types independently of a direct androgen-AR-DNA interaction (Walker, 2003). Few AR responsive genes have been defined in the testis, and none of these have been demonstrated to play a role in spermatogenesis. The homeobox transcription factor PEM has been shown to be positively regulated by androgens in Sertoli cells specifically during the androgen-sensitive stages of spermatogenesis (Lindsey & Wilkinson, 1996). However, targeted disruption of the *Pem* gene in the mouse produces no discernible phenotype (Pitman *et al.*, 1998). Overexpression of PEM in Sertoli cells was shown to increase the frequency of DNA strand breaks in adjacent germ cells, but again no effect was observed on the reproductive fitness of these males (Wayne *et al.*, 2002). The advent of microarray technology now allows for the rapid identification of androgen responsive genes by comparing RNA levels from the testes of appropriate mutant and control mice. One recent study has validated this approach using androgen-replacement in *Gnrh*-null males (Sadate-Ngatchou *et al.*, 2004), suggesting that AR is predominantly a suppressor rather than activator of transcription. The generation of conditional *Ar* alleles provides useful tools to further investigate this question. These models will also be useful for investigating the potential role of AR in Leydig and peritubular myoid cells. The proximity of myoid cells to the spermatogonial stem cells, presents the possibility that AR may regulate some aspect of stem cell proliferation or differentiation via this cell type. This is a particularly intriguing possibility in the context of androgenic suppression of stem cell differentiation and spermatogenic recovery in *jsd* males and following chemotherapeutic insult. Similarly, conditional removal of AR function if Leydig cells will allow dissection of the role of AR in the maturation of Leydig cells during puberty (O'Shaughnessy *et al.*, 2002), and in feedback regulation of steroidogenesis in the adult.

Finally, a long-term goal in the field should be the delineation of AR function as having either an instructive, supportive, or dual role in the differentiation and development of male germ cells. To date, it is unclear whether the ultimate outcome of AR function is to produce a healthy environment within which germ cells can thrive, or whether the downstream effect of AR activity is the generation of a signal(s) that impinges directly upon germ cell migration, differentiation and survival. The development of these new genetic models of *Ar* regulation of spermatogenesis might provide the tools necessary to answer this question and many others in the fields of androgen and *Ar* biology.

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