

# The androgen receptor in spermatogenesis

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**Abstract.** Androgens are steroid hormones that are necessary for normal male phenotype expression, including the outward development of secondary sex characteristics as well as the initiation and maintenance of spermatogenesis. Many physiological actions of androgens are mediated by the androgen receptor (AR), a member of the nuclear receptor superfamily. AR functions as a ligand-dependent transcription factor,

regulating expression of an array of target genes that are important in male pubertal development and fertility. In this review, the expression and necessity of AR in specific testicular cell types that are important in spermatogenesis will be discussed, and recent information obtained through the study of complete and cell type-specific AR null mouse models will be presented.

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Spermatogenesis is a process, occurring in the testis, by which germ cell precursors develop and mature into functional spermatids. Androgens, principally testosterone and 5-dihydrotestosterone (DHT), by signaling through the androgen receptor (AR), mediate a wide range of physiological responses and developmental processes, involving both reproductive and non-reproductive systems in the male (reviewed in Chang et al., 2002; Collins and Chang, 2002; Shumazaki, 2002). Proper regulation of androgen production via the hypothalamic-pituitary-gonadal axis is necessary for development of the male phenotype, as well as for initiation and maintenance of spermatogenesis (reviewed in Collins and Chang, 2002; Quigley, 1998) (Fig. 1). Many of the biological actions of androgens are me-

diated by AR, a member of the nuclear receptor superfamily (Chang et al., 1988a; Chang et al., 1988b; Lubahn et al., 1988; Tilley et al., 1989), and androgen signaling is also modulated by specific receptor coregulators (reviewed in Heinlein and Chang 2002a). Additionally, more recent evidence suggests that androgens can act independently of AR, without DNA-binding, and therefore produce non-genomic effects (reviewed in Heinlein and Chang, 2002b; Rommerts, 1998).

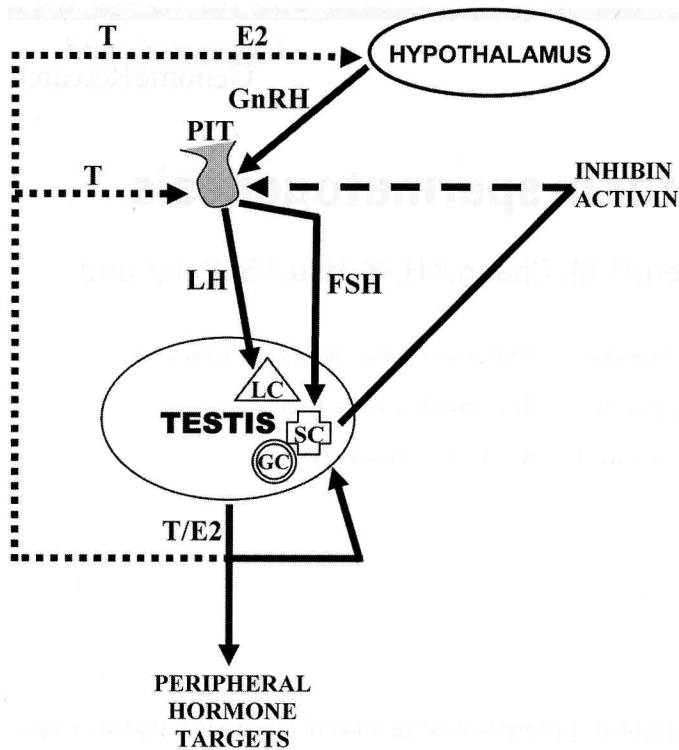
The testis is structured in such a way that cellular factors necessary for germ cell development and maturation are available, yet an immunologically secure intratubular space, in which germ cell development occurs, is provided via the blood-testis barrier (Schlatt et al., 1997). The testosterone-producing Leydig cells are located interstitially (Saez, 1994), or between the seminiferous tubules, along with blood vessels and macrophages. At the basal aspect of the seminiferous tubule, peritubular myoid cells, Sertoli cells, and spermatogonia are found. Peritubular myoid cells display contractility and function to induce peristalsis-like waves which push mature spermatids through the tubules and into the epididymis. Sertoli cells, often referred to as “nurse cells,” provide an essential link between the interstitial and basal aspects of the testis and the developing germ cells within the tubule adluminal space (Griswold, 1995). In addition to providing physical and nutritional support for germ cells, Sertoli cells provide structural support for the semi-

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**Fig. 1.** Endocrine signaling via the hypothalamic-pituitary-gonadal axis. Solid lines represent stimulatory effects and dashed lines represent inhibition. Gonadotropin-releasing hormone (GnRH) produced in the hypothalamus stimulates leuteinizing hormone (LH) and follicle-stimulating hormone (FSH) production by the pituitary (PIT). Once secreted, LH signals the production of testosterone (T) in the Leydig cells (LC) that reside between the seminiferous tubules of the testis, while FSH acts within the tubules (where the Sertoli cells, SC, and germ cells, GC, are located) to stimulate spermatogenesis. T secreted from Leydig cells inhibits hormone production in the hypothalamus and pituitary, and estradiol (E2) produced in the testis also suppresses GnRH production in the hypothalamus. In addition, the peptides activin (stimulatory) and inhibin (inhibitory), which are produced in Sertoli cells, alternately stimulate or inhibit production of FSH by the pituitary.

niferous epithelium (de Krestler and Kerr, 1994). Spermatogonia, or germ cell precursors, are also located basally in the seminiferous tubule. Testosterone produced in the Leydig cells is necessary for spermatogenesis to proceed (Sharpe et al., 1988), demonstrating that paracrine communication between interstitial, basal intratubular and adluminal cell types is essential, and suggesting a role for AR in spermatogenesis.

Indeed, expression of AR has been demonstrated in Sertoli, Leydig, peritubular myoid, and vascular smooth muscle cells of the testis (Anthony et al., 1989; Sar et al., 1990; Ruizeveld de Winter et al., 1991; Kimura et al., 1993; Bremner et al., 1994; Vornberger et al., 1994; Zhou et al., 1996; Pelletier et al., 2000; Zhou et al., 2002). It was initially thought that AR was not expressed in germ cells of the testis (Grootegoed et al., 1977; Anthony et al., 1989; Sar et al., 1990), but more recent evidence indicates expression of AR in spermatogonia (Kimura et al., 1993; Zhou et al., 1996), spermatocytes (Kimura et al., 1993), and elongated spermatids at spermatogenic stage XI (Vornberger et al., 1994). Such germ cell type and spermatogenic

stage specificity suggest precisely targeted roles and potential AR-mediated feedback regulatory mechanisms based on dynamic hormone and growth factor expression patterns throughout spermatogenic cycles.

The recent generation and characterization of male androgen receptor knockout (ARKO) mice confirmed the necessity of AR signaling for both external and internal male phenotype development (Yeh et al., 2002; Matsumoto et al., 2003). Male ARKO mice displayed feminized external genitalia and reduced (female-like) body weight. Internally, the accessory sex organs of the ARKO were agenic, and the testis were cryptorchid and appeared to contain fewer cells and thinner seminiferous tubules than the wildtype AR testis. In contrast to the normal compliment of germ cells in various stages of maturation, including late stage elongated spermatids, observed in the wtAR testis, some tubules in the ARKO sections lacked germ cells altogether, and others contained few germ cells (Yeh et al., 2002). The complete absence of round spermatids, elongated spermatids, and mature spermatozoa throughout the ARKO testis was striking, and suggests that spermatogenic arrest occurs at the pachytene spermatocyte stage in these mice (Yeh et al., 2002). In Tfm mice, a strain lacking functional AR in all cell types, Leydig cell development has been investigated. Leydig cells develop through an initial fetal stage, and are later replaced by an adult population in the adult mouse (Vergouwen et al., 1991). In Tfm mice, it was shown that fetal Leydig cells developed and functioned normally, whereas adult Leydig cells failed to mature properly (O'Shaughnessy et al., 2002).

Evidence for roles of AR relevant to male gonadal development and spermatogenesis has been generated through the study of AR-deficient mice (O'Shaughnessy et al., 2002; Yeh et al., 2002), but due to the developmental roles of AR in establishing the male phenotype, the disruption of AR throughout an experimental animal does not allow the study of AR function specifically in cell types intimately involved in the spermatogenic process. To determine whether it is the early developmental consequences of loss of AR signaling that causes the spermatogenic defects in AR-deficient mice, or a combination of these developmental abnormalities and the dysregulation of AR function in specific testis cell types, disruption of AR function exclusive to particular cell types has recently been studied. In male mice lacking AR only in the Sertoli cells (S-AR-*y*), the testes were acellular and atrophied, with spermatogenesis arrested at the diplotene stage prior to the first meiotic division (Chang C., pers. commun.). Evidence of significantly reduced testosterone, and increased leuteinizing hormone levels in the serum of S-AR-*y* mice suggest that AR function in Sertoli cells is essential for the maintenance of the appropriate hormone levels that support spermatogenesis (Chang C., pers. commun.). Interestingly, loss of AR specifically in the germ cell lineage of the male mouse resulted in no defects in spermatogenesis or fertility, suggesting that AR function is not essential in this cell type and that paracrine interactions among testicular cell types is sufficient to communicate any signals originating from androgen-AR pathways that may be necessary for normal germ cell development and maturation (Tsai, M.Y., pers. commun.).

## Conclusion

Androgens and AR are required for normal spermatogenesis, yet AR expression is not essential in all cell types in the testis. AR-deficient mice, both Tfm and ARKO, are infertile and display feminized internal and external phenotypes. In the ARKO mouse model, the lack of post-meiotic germ cell types and mature spermatids highlight the necessity of AR for proper spermatogenesis. Although not yet explored in a cell-specific knockout model, the steroid-producing Leydig cells have been shown to require functional AR for maturation to their adult stage. In male mice lacking AR only in the Sertoli cells of the

testis, spermatogenesis arrested at the diplotene stage prior to the first meiotic division, and loss of AR specifically in the germ cell lineage of the male mouse caused no defects in spermatogenesis or fertility. Androgen-AR signaling is necessary for spermatogenesis to occur, yet the cell type-specific requirement of AR in the testis highlights the complex paracrine signaling mechanisms present in the male reproductive system. Further dissection of the mechanisms of AR action using cell type-specific models may lead the development of new, more effective strategies for the prevention and treatment of male reproductive disorders.

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