Expression of estrogen receptor-α and -β mRNAs in the male reproductive system of the rat as revealed by *in situ* hybridization

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ABSTRACT

We mapped the cellular expression of estrogen receptor (ER) α and ERβ mRNAs in the male reproductive system of the rat during development and adulthood by *in situ* hybridization. The expression patterns of ERα mRNA in the gonad, efferent duct and initial segment of the epididymis during the perinatal period were essentially similar to those of the adult: ERα mRNA signals were expressed most intensely in the epithelia of the efferent ducts and initial segment of the epididymis, and in the interstitial cells of the testis from the prenatal period to adulthood. However, ERα mRNA signals in the primordial epididymis and vas deferens during the prenatal period were confined to the outermost cellular layer of the ducts, whereas thereafter they were only expressed weakly in the epithelium and stroma of the epididymis and moderately in the muscle layer of the vas deferens. ERβ signals were expressed intensely (1) in primordial germ and Sertoli cells only during the prenatal period, (2) in arterial walls in the adult testis, and (3) in the epithelium of the sex accessory glands from the perinatal period to adulthood. This report is the first to describe the cellular distribution of ER mRNA in the male reproductive organs during the perinatal period, and complements and confirms earlier data on its distribution in the adult. The broad expression of ERs in male reproductive organs suggests roles for estrogen in regulating tissue development and reproductive events.
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INTRODUCTION

One of the primary roles of estrogen is to induce the growth and function of reproductive and neuronal cells that are related to sexual development and behavior (Galand *et al*. 1971). Produced mainly in the ovary and adrenal gland in the female, estrogen exerts a strong influence over reproductive events (Freeman 1988). Although the presence of estrogen in the male gonad has been well documented for more than 50 years (see review by Carreau *et al*. 1999), it is only recently that studies on the exact role of estrogen in male reproduction and fertility have been undertaken (Meistrich *et al*. 1975, Couse & Korach 1999, Hess 2000, Lee *et al*. 2000). Aromatase, a microsomal enzymatic complex which irreversibly converts androgens into estrogen, has been demonstrated in testicular cells including Sertoli and Leydig cells and, more recently, in germ cells (Janulis *et al*. 1998, Carreau *et al*. 1999). Even though the overall production of estrogen by the testis, which is the major source of estrogen in the male, is much less than that produced by the ovaries, the concentration of estrogen in the rete testis and caput epididymis fluids has been reported to be higher than serum estradiol in the female (Hess *et al*. 1997*b*). Consequently, the influence of estrogen on male sexuality is expected to be profound (vom Saal 1980, Byskov & Hoyer 1988).

Estrogenic effects on target tissues are principally mediated by estrogen receptors α (ERα) and β (ERβ), members of the steroid/thyroid hormone receptor superfamily, which regulate gene transcription through estrogen-responsive elements (Koike *et al*. 1987, Kuiper *et al*. 1996). Investigators have demonstrated the presence of ER using

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different techniques including steroid autoradiography and immunohistochemistry in the testis and reproductive tract of rodents during development and adulthood (Cooke *et al*. 1991, Greco *et al*. 1992, Hess *et al*. 1997*a*,*b*, Fisher *et al*. 1998, Saunders *et al*. 1998, Jefferson *et al*. 2000, Pelletier *et al*. 2000). *In situ* hybridization, immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR) using tissues and cultured cells of adult rat testes have demonstrated predominant expression of ERα in the Leydig cells, while ERβ expression has been localized to the Sertoli and germ cells (Zhai *et al*. 1996, Fisher *et al*. 1997, Saunders 1998, Saunders *et al*. 1998, Pelletier *et al*. 2000). In tissues other than the testis, the most intense signals of ERα mRNA in the adult rodents were found in the epithelia of the efferent duct and initial segment of the epidydimis, sites responsible for semen concentration (Hess *et al*. 1997*b*, Fisher *et al*. 1998). ERα mRNA expression is also intense in the muscle layer of the vas deferens in the rat, while ERβ mRNA is weakly expressed in the efferent duct, epidydimis, and vas deferens (Hess *et al*. 1997*a*). Although significant data on the cellular distribution of ER mRNA in the male reproductive system have been generated, our current knowledge of its cellular expression during development and adulthood is still far from complete and, in some tissues, the data are inconsistent. The most obvious is the absence of data on the cellular expression of ERα and ERβ mRNAs in the male reproductive system during the prenatal period.

A thorough knowledge of the cellular distribution of ER in tissues is essential to identify which of the many heterogenous cell types respond to estrogen; subsequently, this knowledge provides a foundation for understanding the role of estrogen in male reproduction. Thus, this study was designed to map, systematically and in detail, the cellular distribution of ERα and ERβ mRNAs in the whole reproductive system of the male rat, from the prenatal period through adulthood, using *in situ* hybridization. The data on ER mRNA expression of the penis have been submitted for publication elsewhere. Our findings confirm and extend the data of earlier reports on ER mRNA distribution in the male reproductive system.

MATERIALS AND METHODS

Animals

Normal immature and adult Wistar male rats, from fetal day 14 to 90 days after birth, were killed for histological procedures as described below. The older rats were anesthetized by intraperitoneal injection with pentobarbital sodium solution (Nembutal, Abbot, Chicago, IL, USA), 0·5 ml/kg body weight, whereas the neonates were anaesthetized with ether vapor. The animals were treated in accordance with the Guidelines on the Handling and Training of Laboratory Animals published by the Universities Federation for Animal Welfare, UK.

Probes

Proven ERα and ERβ probes, complementary to 301–346 base pairs of ERα cDNA (Koike *et al*. 1987) and 45–90 base pairs of ERβ cDNA (Kuiper *et al*. 1996) respectively, were utilized in this study (Mowa & Iwanaga 2000). The oligonucleotides were labeled with ^{35}S-dATP, using terminal deoxyribonucleotidyl transferase (Promega, Madison, WI, USA) at a specific activity of $0{\cdot}5 \times 10^9$ d.p.m./μg DNA.

In situ *hybridization*

The *in situ* hybridization procedure was performed as previously described (Mowa & Iwanaga 2000). Briefly, 20 μm cryostat sections were mounted on glass slides, fixed in 4% paraformaldehyde and then acetylated with 0·25% acetic anhydride in 0·1 M triethanolamine-HCl (pH 8·0).

The prepared sections were prehybridized for 2 h in a buffer containing 50% formamide, 0·1 M Tris–HCl (7·5), 4 × SSC (1 × SSC = 150 mM NaCl and 15 mM sodium citrate), 0·02% Ficoll, 0·02% polyvinylpyrrolidone, 0·02% bovine serum albumin, 0·6 M NaCl, 0·25% sodium dodecyl sulfate (SDS), 200 μg/ml tRNA, 1 mM EDTA and 10% dextran sodium sulfate. Hybridization was performed at 42 °C for 10 h. The slides were washed in 2 × SSC containing 0·1% sarkosyl (Nacalai Tesque, Kyoto, Japan) and twice at 55 °C in 0·1 × SSC containing 0·1% sarkosyl. The sections were exposed to Hyperfilm-β max (Amersham International, Amersham, Bucks, UK), dipped in Kodak NTB2 nuclear track emulsion and exposed.

The specificity of the *in situ* hybridization was confirmed by the disappearance of signals when an excess dose of corresponding cold oligonucleotides was added to the hybridization fluid. Consistent ER mRNA signals above background levels were considered positive and scored as strong, moderate or weak.

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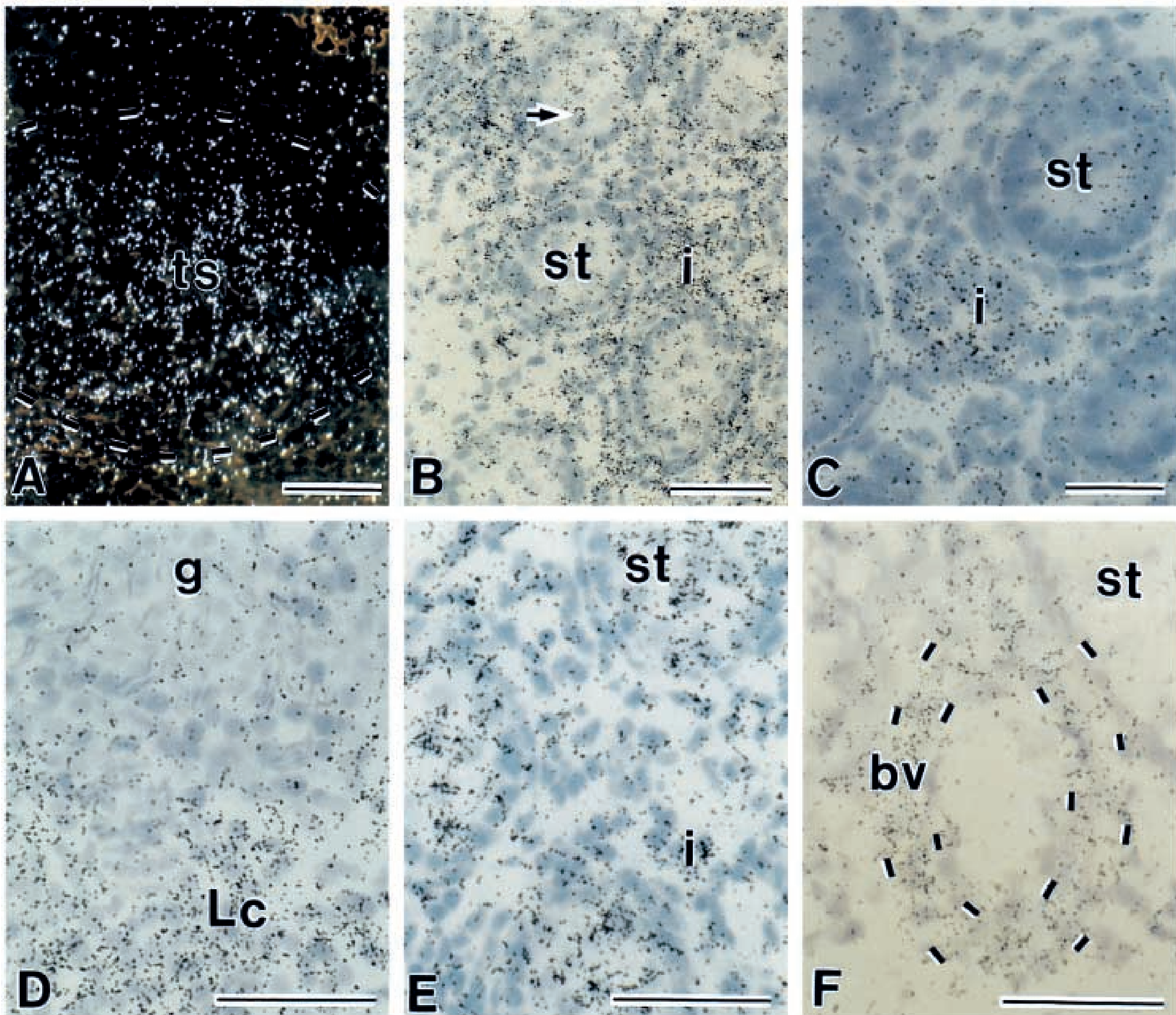

FIGURE 1. Distribution of ERα and ERβ mRNAs in the rat testis. (A) Initial signals for ERα mRNA in the testis (ts) are found at fetal day 14, as shown by the dark-field image. (B–D) At fetal day 17 (B) and postnatal day 14 (C), ERα mRNA signals are essentially confined to the interstitial cells (i), with weak and scattered signals in the germ cells (arrow) (st, seminiferous tubule). The signals are also restricted to the interstitial cells in the adult (D) (g, germ cells; Lc, Leydig cells). (E) Signals of ERβ mRNA are found largely within the seminiferous tubule (st) at fetal day 17 (i, interstitial cells). (F) In adults, ERβ mRNA signals are localized to the testicular blood vessels (bv), with very weak signals in the seminiferous tubules (st). Bar=100 μm (A–C), 200 μm (D–F).

RESULTS

Testis

Initial signals of ERα mRNA in the primordial gonad were diffusely expressed in the mesenchyme by fetal day 14 (Fig. 1A). By fetal day 17 its signals aggregated largely to the interstitial cells, identified as precursor Leydig cells (Fig. 1B). Scattered round cells within the developing seminiferous tubules, presumed to be gonocytes, expressed detectable signals for ERα mRNA from the late prenatal to the late neonatal periods (Fig. 1B,C). By adulthood, testicular ERα mRNA signals were exclusively confined to the Leydig cells (Fig. 1D).

Like ERα mRNA expression, initial signals of ERβ mRNA in the primordial gonad appeared by fetal day 14 and were diffusely distributed. By fetal day 17, intense signals were localized to the cells found within and along the basal lining of the seminiferous tubules, identified as gonocytes and precursor Sertoli cells respectively (Fig. 1E). The interstitial cells during the prenatal period

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possessed only weak signals of ERβ mRNA, in contrast to the intense signals located within the seminiferous tubules (Fig. 1E). The signal intensity of ERβ mRNA in the germ and Sertoli cells gradually decreased with development, and the signals were barely detectable in adulthood. Furthermore, the signals in Leydig cells progressively weakened with age, becoming undetectable after birth. However, in adulthood, distinct signals of ERβ mRNA were localized to the walls of arteries located between seminiferous tubules (Fig. 1F).

Reproductive tract

By fetal day 17, distinct and intense signals of ERα mRNA appeared in the epithelium of the mesonephric tubules, which gives rise to the efferent ducts and, presumably, the initial segment of the epididymis (Fig. 2A). In contrast, ERα mRNA expression in the mesonephric ducts, from which the epididymis and vas deferens arise, were limited to the outermost cell layer of the ducts at the onset of their appearance (fetal day 14) (Fig. 2D). ERα mRNA expression in the primordial epididymis and vas deferens gradually spread inwards towards the centrally located epithelium during the perinatal period (Fig. 2E–F). The epithelia in the mesonephric ducts lacked signals of ERα mRNA (Fig. 2D,F). In the adult rat, the expression pattern and intensity of ERα mRNA in the efferent duct and initial segment of the epididymis remained unchanged (Fig. 2B,C), whereas the stroma and epithelia in the rest of the epididymis (Fig. 2G) and smooth muscle layer in the vas deferens (Fig. 2H) expressed weak to moderate signals for ERα mRNA.

ERβ mRNA signals in the mesonephric tubules ducts were weak during the perinatal period and were localized to the stroma but were not in the epithelia. This expression pattern remained unchanged after the differentiation of these structures into the efferent duct, initial segment of the epididymis, epididymis and vas deferens. In the adult rat, ERβ mRNA expression was diffusely localized to the stroma and muscle layer of the efferent ducts, epididymis (Fig. 3B,C) and vas deferens.

Sex accessory glands and urethra

By fetal day 17, distinct and intense signals of ERα mRNA were detectable in the subepithelial stroma of the glandular structures adjacent to the developing bladder (Fig. 2I) and of the urethra (Fig. 2K) respectively. The glandular structures were identified as the primordia of the prostate and seminal vesicular glands, which develop precociously. Following parturition and differentiation, ERα mRNA signals persisted in the stroma and, in some glands, they also extended to the smooth muscle layers of the excretory ducts, but were never localized to the glandular and ductal epithelium. This expression pattern remained unchanged in adulthood. In contrast, the initial signals of ERβ mRNA, recognizable by postnatal day 8, in the sex accessory glands were localized diffusely in the stroma and epithelia of the glands. Similar to ERα mRNA, ERβ mRNA signals in the urethra were localized to the muscle layer and became detectable by fetal day 17 (Fig. 3A). Thereafter, they were observed mainly in the epithelial cells of the seminal vesicles (Fig. 3D), prostate (Fig. 3E), and urethra, bulbo-urethral (Fig. 3F) and urethral glands (Fig. 3G).

A summary of the differential expression patterns of ERα and ERβ mRNAs in the cells of the male reproductive organ of the rat as revealed by *in situ* hybridization is shown in Table 1 and Fig. 4.

DISCUSSION

ER mRNA expression in the testis

In rodents, spermatogenesis commences soon after the appearance of the seminiferous tubules, around fetal day 15 (Rugh 1988). The undifferentiated gonocytes proliferate and increase in cell size up to fetal days 16–17, and thereafter remain in a quiescent state until after birth (Rugh 1988). The intense prenatal expression of ERβ mRNA in the undifferentiated gonocytes and Sertoli cells, which coincides with the onset of their proliferation, suggests that estrogen may play a role in regulating early germ cell production via ERβ. In support of this idea is the finding that estrogen stimulates proliferation of gonocytes isolated from the testes of postnatal day 3 rats (Li *et al*. 1997). Similarly, the onset of ERα mRNA expression in interstitial cells of the testis correlates with the beginning of androgen production and the presence of aromatase in these cells (Ojeda & Urbanski 1988). Thus, estrogen may also be involved in regulating steroidogenesis during perinatal development via ERα. Furthermore, the demonstration of aromatase activity and ER mRNAs in both the spermatozoa and the Leydig cells (Levallet *et al*. 1998) indicates the existence of local estrogen autocrine systems in the testis. Finally, the findings that intratesticular administration of estrogen inhibits testosterone production by Leydig cells and that the initial response of Leydig cells to luteal hormone treatment in the adult rat results in increased secretion of testosterone, followed by a significant

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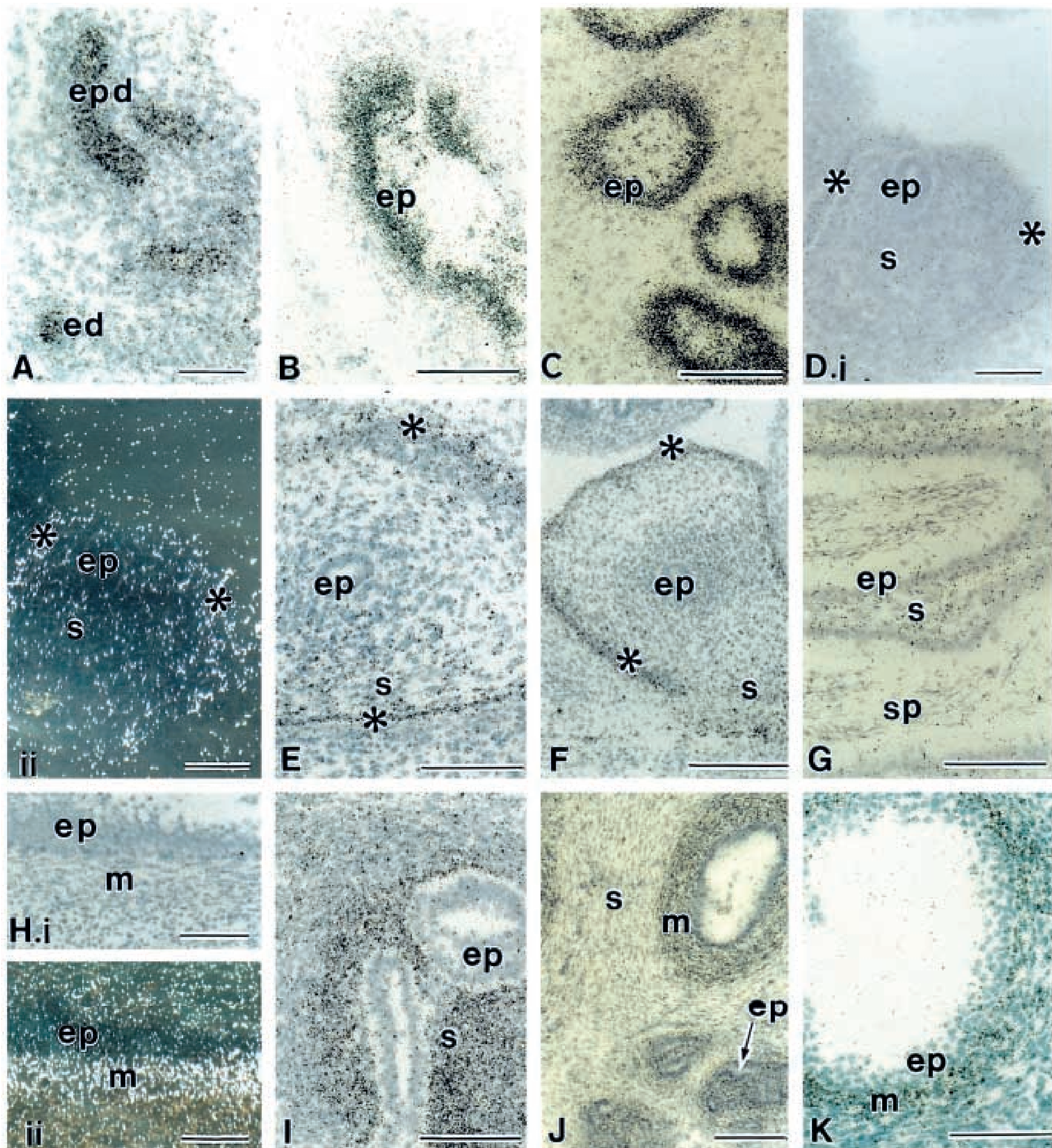

FIGURE 2. Cellular localization of ERα mRNA in the reproductive tract and sex accessory glands of the rat during development and adulthood. (A) Intense ERα mRNA signals are localized to the epithelia in primordial efferent duct (ed) and initial segment of the epididymis (epd) at fetal day 17. (B–C) In the adult, signals of ERα mRNA are confined to the epithelia (ep) of the efferent duct (B, short straight part of the efferent duct) and initial segment of the epididymis, located at the borders with the efferent duct (C, epithelium type – columnar). (D) Detectable signals of ERα mRNA are first seen at fetal day 14 in the outermost cellular layer (*) of the primordial epididymis and vas deferens, as revealed by the bright (i) and dark-field (ii) images (ep, epithelia; s, stroma). (E, F) At fetal day 17, ERα mRNA signal intensity increases significantly in the serosa (*) of the primordial epididymis (E) and vas deferens (F) and significant signals spread to the stroma (s) (ep, epithelia). (G, H) In the adult, ERα mRNA signals are localized to the epithelia (ep) and stroma (s) of the epididymis (G), and to the smooth muscle cells (m) of the vas deferens (sp, spermatozoa) (H.i,ii). (I) In the sex accessory glands, the first signals of ERα mRNA are observed at fetal day 17 in the stroma (s), not in the epithelium (ep). (J) At postnatal day 24, strong signals of ERα mRNA are detected in the lamina propria (lp) and muscle layer (m) of the sex accessory glands. (K) Signals of ERα mRNA in the primordial urethra are limited to the smooth muscle (m) (fetal day 17). Bar=100 μm (A, C–E, H, J, K), 200 μm (B, F, G, I).

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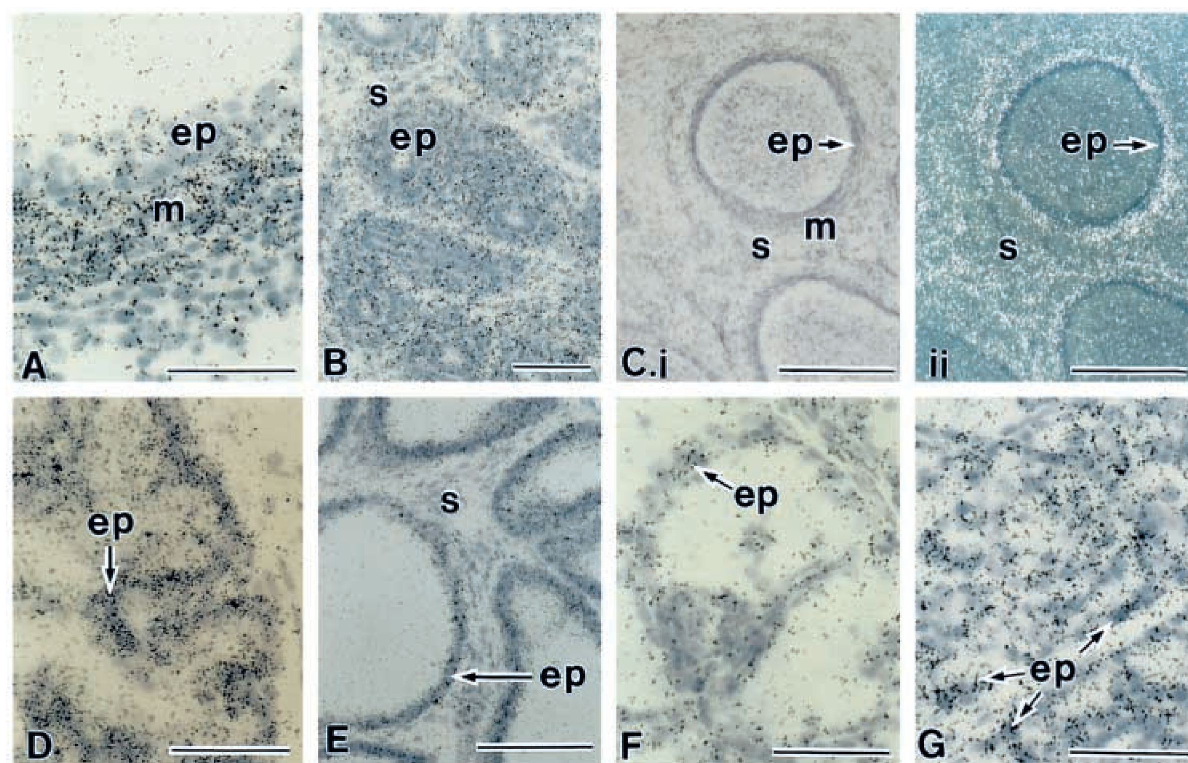

FIGURE 3. Cellular localization of ERβ mRNA in the reproductive tract and sex accessory glands of the rat during development and adulthood. (A) ERβ mRNA signals in the reproductive tract are limited to the smooth muscle cells (m) of the urethra at fetal day 17 (ep, epithelium). (B,C) In the adult, ERβ mRNA signals in the cauda epididymis (B) are diffusely expressed, whereas in the caput epididymis (C.i,ii) fairly distinct signals aggregate in the muscle layer (m) and stroma (s), but not in the epithelium (ep). (D,E) The epithelia (ep) of the seminal vesicles (D) and prostate glands (E) show intense signals of ERβ mRNA in the adult. (F) Weak to moderate signals of ERβ mRNA are expressed in the epithelia (ep) of bulbo-urethral glands of the adult. (G) The most intense signals of ERβ mRNA in the reproductive tract and glands of the adult male rat are localized to the epithelia (ep) of the urethral glands. Bar=100 μm (B), 200 μm (C–G), 210 μm (A).

increase in estrogen production, confirms the above conclusion (Saez 1994). It is noteworthy that the differential expression pattern of ER subtypes in the testis is analogous to that seen in the ovary: ERβ is localized to the granulosa cells (oocyte-nurturing cells, i.e. Sertoli cells), whereas ERα is expressed in the theca/interstitial complex (steroid-producing cells, i.e. Leydig cells) (Mowa & Iwanaga 2000).

The differential cellular expression of ERα and ERβ mRNA in the testis, as revealed in this study, is generally consistent with previous immunohistochemical and *in situ* hybridization studies using rodents, with minor differences (Saunders *et al*. 1997, 1998, Fisher *et al*. 1997). In accordance with the results of the present study, the expression of ERβ mRNA was demonstrated in the total RNA of a 14-day postconception mouse testis by ribonuclease protection assay (RPA) (Jefferson *et al*. 2000). The signal intensities of ERβ mRNA and protein, and ERα mRNA, significantly decreased after postnatal days 5 and 19 respectively (Jefferson *et al*. 2000). In their earlier study, Saunders and colleagues (1997) failed to detect any significant immunoreactivity of ERβ in the adult testis, while a different research group (Fisher *et al*. 1998) localized ERα largely in the interstitial cells from the fetal period through to adulthood in rats, in agreement with our observation. Furthermore, similar to the present data, no ERβ immunoreactivity was found in the germ cells of adult testis by Pelletier *et al*. (2000). On the contrary, some immunohistochemical and *in situ* hybridization studies have reported expression of ERβ protein and mRNA in the differentiated germ cells of the adult testis (Saunders *et al*. 1998, van Pelt *et al*. 1999) and ERα protein in the Sertoli cells, round spermatocytes and developing spermatids of adult rats (Pelletier *et al*. 2000). Although the cause of this

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TABLE 1. Chronological order of appearance and changing patterns of ERα and ERβ mRNA expression in the reproductive organs of the male rat

		ER expression*	
	Age (days)	ERα	ERβ
Tissue			
Testis	14f		
Germ cells	19f, 7p, adult	+/−, +, −	+++, ++, +/−
Sertoli cells	19f, adult	−, ∼	+, +/−
Leydig cells	17f, 7p, adult	+++, +, ++	+, +/−, ∼
Efferent duct			
Epithelium	17f, ∼	+++, ∼	−, ∼
Stroma	17f, adult	+, +/−	+/−, −
Epididymis			
Epithelium	14f, adult	−, +	−
Stroma	14f, 19f, adult	++, +++, +	+/−, +/−, +
Vas deferens			
Epithelium	14f, ∼	−	−
Stroma	14f, 19f, adult	++, +++, ++	+/−, +/−, +
Sex accessory glands			
Epithelium	17f, 8p, adult	−, ∼	−, +, +++
Stroma	17f, 8p, adult	+++, 11, ∼	−, +/−, −

f=fetal; p=postnatal.
*ER signal strength: +, weak; ++, moderate; +++, strong; +/−, inconsistent signals; ∼, signal unchanged thereafter.
The column under Age indicates when significant ER mRNA signals become detectable; the middle and last figures (where applicable) indicate the age at which ER mRNA signal strength sharply increases and plateaus respectively.

discrepancy is unclear, the idea that ERβ plays no role in gametogenesis during adulthood is supported by analyses of ERβ-knockout mice, which are fertile and show no apparent morphological and functional abnormalities in the testis (Couse & Korach 1999).

ER mRNA expression in the efferent duct, epididymis and vas deferens

The role of estrogen and the expression of its receptors in the efferent duct have been elaborately investigated in the past largely by immunohistochemistry, steroid autoradiography and RT-PCR, notably by the laboratories of Hess (Cooke *et al.* 1991, Hess *et al.* 1997*a*,*b*, 2000, Hess 2000, Lee *et al.* 2000). However, data on the cellular distribution of ER mRNA in the rat from efferent duct to vas deferens, particularly in development, are lacking. This study is the first to describe the cellular expression of ER mRNA in the efferent duct, epididymis and vas deferens during development, and extends our knowledge of ER mRNA expression in the adult rat.

Clulow *et al.* (1994) have shown that the efferent duct concentrates spermatozoa by re-absorbing more than 90% of the fluid released from the testis, a process essential for sperm maturation and storage and, subsequently, crucial for optimal male fertility. Among the factors involved in this process are solutes such as Na^+ and Cl^- in luminal fluids, and the water channel protein, aquaporin-1 (AQP-1), expressed in the epithelium (Clulow *et al.* 1994, Fisher *et al.* 1998). In the efferent ducts of the adult, estrogen is thought to regulate fluid re-absorption, in part, by modulating the expression of AQP-1 (Fisher *et al.* 1998). Thus, loss of ERα function in the ERα-knockout mice leads to low sperm count and marked structural alterations such as decrease in epithelia height, number of cilia, height of microvillus brush border and density of lysosome and endocytotic organelles (Hess *et al.* 2000). Consequently, these changes interfere with the resorptive functions of the efferent duct (Couse & Korach 1999, Hess 2000, Lee *et al.* 2000). It is interesting to note that AQP-1 signals, like those of ERα reported here, are intensely expressed in the efferent duct from the prenatal period through adulthood (Fisher *et al.* 1998). Furthermore, in perinatal rats treated with diethylstilbesterol, AQP-1 immunoreactivity was abolished by postnatal day 18, accompanied by severe dilatation of efferent ducts, suggesting impaired function of fluid re-absorption (Fisher *et al.* 1999). These

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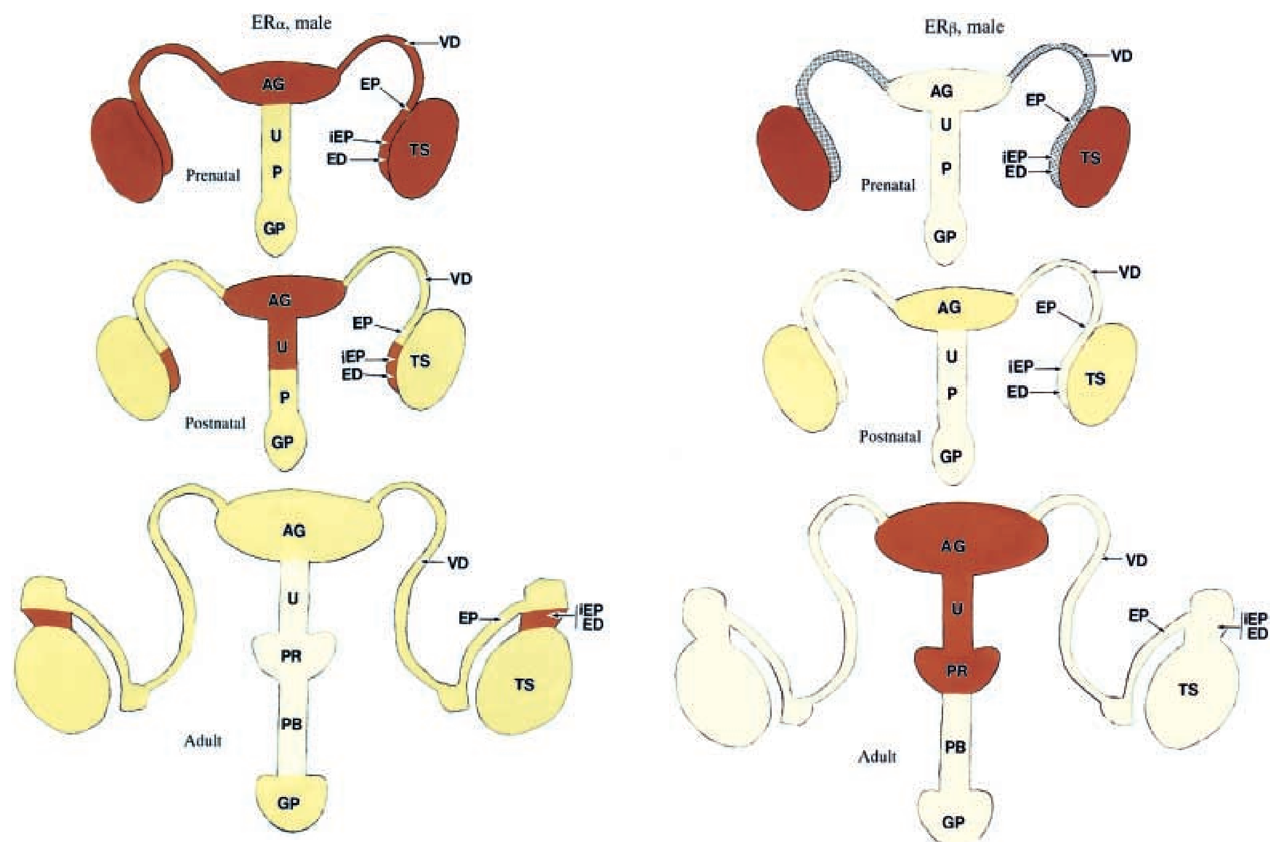

FIGURE 4. Schematic representation of the overall distribution and signal intensity of ERα and ERβ mRNAs in the reproductive organs of the male rat during the prenatal and postnatal periods and in adulthood. Signal intensity is represented by: red, intense signals; bright yellow, moderate signals; light yellow, weak signals; stipple, insignificant signals. TS, testis; ED, efferent duct; iEP, initial segment of epididymis; EP, main epididymis; VD, vas deferens, AG, accessory gland; U, urethral gland; P, penis; PR, penis root; PB, penis body; GP, glans penis.

observations, taken together with the present findings, indicate that provision for fluid reabsorption is established long before the onset of sexual maturation. Thus, estrogen may play a role in both the development and function of the efferent duct during the perinatal period. ERα and ERβ have been localized in the developing and mature epididymis and ductus deferens of rodents by previous studies using immunohistochemistry, RT-PCR, RPA and steroid autoradiography, but not by *in situ* hybridization (Cooke *et al*. 1991, Greco *et al*. 1992, Hess *et al*. 1997*a*, Jefferson *et al*. 2000). The data of these studies are largely consistent with the present data. Studies by steroid autoradiography, which detects cells expressing both ER subtypes, demonstrated that fetal mesenchyme accumulates labeled estrogen (Cooke *et al*. 1991). Following their differentiation into stroma, fibroblasts and smooth muscle cells, the signal pattern changed, with stroma and fibroblasts retaining the signals until adulthood, whereas the smooth muscle cells had weak or no signals (Cooke *et al*. 1991). The signal pattern of fetal mesenchyme described above may correspond to the pronounced expression of ERα mRNA in the primordial epididymis and vas deferens, as observed in the present study, whereas the stromal signals seen in the adult may correspond to the expression of both ER mRNA subtypes in the stroma of the same structures.

ERα mRNA expression in the epididymis, originally detected in the outermost cell layer of the duct and later in the epithelia and stroma, suggests roles for estrogen in its development and function. The epididymis is a negligible site of sperm concentration in comparison to the efferent duct (Clulow *et al*. 1994). It is, instead, a site of sperm storage and maturity characterized by accumulation of high concentrations of organic compounds (Clulow *et al*. 1994). Hence, the presence of ERα mRNA signals in the epididymis implies that

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estrogen modulates secretion of factors that promote the maturity and viability of spermatozoa. This conclusion is consistent with data for ERα-knockout mice, whose spermatozoa lack motility and the ability to fertilize oocytes *in vitro* (Couse & Korach 1999). Like the epididymis, the initial signals of ERα expression in the vas deferens were localized to the outermost cell layer of the duct. The similarity in the expression patterns between the two ducts may reflect their common ontogenic origin (Rugh 1988). In the adult, the presence of ERα mRNA in the muscle layer of the vas deferens, in accordance with previous immunohistochemical studies, confirms an earlier proposal that estrogen may regulate contraction of the vas deferens, essential for transporting the spermatozoa. Interestingly, our previous study revealed significant expression of ERα mRNA in the muscle coat of the rat oviduct (Mowa & Iwanaga 2000), and physiological studies have demonstrated that estrogen administration accelerates transportation of the oocyte in the oviduct (Harper 1988), possibly via ER expressed in the oviductal muscle coat.

ER mRNA expression in the sex accessory glands

Prins *et al*. (1997, 1998) have eloquently described in detail the pattern of ERα and ERβ mRNAs/proteins expression in the prostate gland of the rat from birth to adulthood using immunohistochemistry, *in situ* hybridization and RT-PCR. They, with others, have demonstrated that ERβ mRNA signals are intense in the epithelium of the prostate gland and that this intensity increases with development and age, in contrast to the situation in the stroma where intensity decreases with age (Prins *et al*. 1998, Pelletier *et al*. 2000). The present study confirms these earlier findings and provides new data on the expression of ERα and ERβ mRNAs in the primordia of the sex accessory glands, in general, during the prenatal period. However, our data are inconsistent with the findings of Jefferson *et al*. (2000) who, using RPA, were only able to detect low and varying levels of ERβ mRNA in the seminal vesicle and prostate glands of CD-1 mice between postnatal days 1–26. The cause of the discrepancy is unclear. It is, however, interesting to note that a different study group, which also used mice, found signals in the prostate and bulbourethral glands during development (Cooke *et al*. 1991).

The seminal vesicles and prostate glands in rodents develop into large structures by fetal day 18 and increase significantly in size after birth (Rugh 1988). The detection of intense ERα signals in the developing accessory glands suggests the involvement of ERα in the growth of these glands. The presence of ERβ in the epithelia of the glands after the neonatal period suggests that the regulatory role of estrogen in their growth and/or secretion is mediated by ERβ. Indeed, ERβ-knockout mice show growth impairment in the secretory portion of the accessory glands, manifested as hyperproliferation which is absent in ERα-deficient mice (Gustafsson 1999). On the other hand, ERα expression in the stroma and later in the excretory ducts of these glands indicates that estrogen may modulate the development of these structures and, following maturation, transportation of their secretory products via ERα.

Pheromones, which are vital for social and sexual intercourse, are released via the urine in adult rats (Sasaki *et al*. 1999). Other than the preputial glands, the source of pheromones in the reproductive organs is unclear. Since pheromone production is partly influenced by estrogen as well as by testosterone, the intense expression of ERβ in the secretory portion of the urethral gland indicates that this gland may be another source of pheromones.

Our findings of ERα and ERβ mRNA expression in the male reproductive tract confirm observations of earlier steroid autoradiographic, *in situ* hybridization, RT-PCR and immunohistochemical studies, which demonstrated expression of ER in the efferent ducts, epididymis, sex accessory glands and urethra (Fisher *et al*. 1997, Hess *et al*. 1997*a*, Prins & Birch 1997, Saunders *et al*. 1997, Prins *et al*. 1998, Pelletier *et al*. 2000). Furthermore, this study provides the first report on the onset and expression pattern of ER mRNA subtypes in the developing efferent duct, epididymis, some accessory glands and urethra.

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