Preserved tissue structure of efferent ductules in aromatase-deficient mice

Katsumi Toda1, Teruhiko Okada2, Yoshihiro Hayashi3 and Toshiji Saibara4

Departments of 1Biochemistry, 2Anatomy and Cell Biology, 3Pathology and 4Gastroenterology and Hepatology, School of Medicine, Kochi University, Nankoku, Kochi 783-8505, Japan

Abstract

Estrogen receptor α (Esr1) is proposed to play a critical role in the regulation of testicular fluid reabsorption at efferent ductules, and disruption of the Esr1 gene (Esr1−/−) resulted in marked dilation of the lumens of efferent ductules. This study was aimed to clarify whether disruption of the gene for aromatase (Ar), an enzyme responsible for estrogen biosynthesis, results in morphological and transcriptional alterations at efferent ductules as observed in Esr1−/− mice. Histology demonstrated structural preservation of the ducts in aromatase-deficient (Ar−/−) mice. Electron microscopic examinations reveal that endocytic apparatus and tubule-cisternal endoplasmic reticulum are present in non-ciliated cells irrespective of the genotypes. However, electron-dense and acid phosphatase-negative granules and apical tubules, which are components thought to be related to membrane recycling of endosomes, are observed only in wild-type (WT) and Ar−/− mice. By contrast, the Golgi complex is highly developed in Esr1−/− mice when compared with WT and Ar−/− mice. RT-PCR analysis reveals no significant differences in the expression levels of a subset of genes involved in ion transportation. Thus, from the structural and transcriptional points of view, the efferent ductules of Ar−/− mice are indistinguishable from those of WT mice. Moreover, data from electron microscopic examinations indicate the possible involvement of Esr1 in the regulation of vesicle recycling processes.

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Introduction

Testicular development and the maintenance of spermatogenesis are controlled mainly by gonadotropins and androgens (Holdcraft & Braun 2004). However, besides the well-known negative effect of estrogens on the secretion of gonadotropins, they have been shown to directly regulate testicular functions (Simpson et al. 1994, Carreau et al. 2006, Ebling et al. 2006). Estrogens are synthesized by an enzyme complex, aromatase, through the conversion of androgens to estrogens in Sertoli cells in immature animals and in Leydig cells in adults (Rommerts et al. 1982, Simpson et al. 1994, O’Donnell et al. 2001). Furthermore, germ cells were proposed to be one of the major sites for estrogen synthesis in adult mouse testis (Nitta et al. 1993).

Estrogens are thought normally to modulate the transcription of specific genes in estrogen target tissues through binding to estrogen receptors of either α- or β-subtype (Couse & Korach 1999, Nilsson et al. 2001). Both subtypes have been reported to be expressed abundantly in the efferent ductules (Zhou et al. 2002), where a large part of testicular luminal fluid is reabsorbed to concentrate spermatozoa (Clulow et al. 1998, Hess 2000). Esr1 gene knockout (Esr1−/−) mice display dilation of the lumens of the efferent ductules, of which the diameter becomes more than double the size of wild-type (WT) males (Hess et al. 1997), indicating that luminal fluid is not removed causing accumulation of fluids in the lumen of the efferent and seminiferous tubules. As Esr2−/− mice did not display apparent morphological abnormalities in the efferent ductules (Krege et al. 1998, Antal et al. 2008) and the phenotype of Esr1−/−/Esr2−/− mice was similar to that of Esr1−/− mice (Couse et al. 1999), Esr1 rather than Esr2 plays a major role in normal fluid reabsorption at the efferent ductules. Nevertheless, the requirement of aromatase activity for Esr1 to function at the ducts has not been established.

Because estrogens are synthesized by aromatase in the testis, aromatase gene knockout (Ar−/−) mice are a useful animal model to assess the testicular function of estrogens in vivo. Three lines of Ar−/− mice have been generated independently (Fisher et al. 1998, Honda et al. 1998, Toda et al. 2001a). However, the testicular phenotypes are variable among the lines. The Ar−/− mice generated by us were nearly infertile and showed no disruptions in spermatogenesis until 10 months of age, although an apparent reduction in seminiferous epithelial height was observed at that age (Toda et al. 2001b). The Ar−/− males generated by other group are reportedly fertile at 12–14 weeks of age and one of four Ar−/− mice examined displayed grossly dysmorphic seminiferous tubules.
and disrupted spermatogenesis at 4.5 months of age (Robertson et al. 1999, 2001). This phenotypic heterogeneity in spermatogenesis between the $Ar^{-/-}$ lines seems to be attributable to differences in genetic backgrounds rather than in the genomic region used for the gene inactivation, as the neo gene was inserted at the EcoRV site in exon 9 of Cyp19a1 for the disruption of the gene in both $Ar^{-/-}$ lines (Fisher et al. 1998, Toda et al. 2001a). We thus generated fully congenic $Ar^{-/-}$ mice with a C57BL/6J background by repeated backcrossing to C57BL/6J mice. This study was conducted using $Ar^{-/-}$ mice with a C57BL/6J genetic background to examine the effects of aromatase inactivation on spermatogenesis and the efferent ductules with special reference to the changes observed in the tissue sites of $Esr1^{-/-}$ mice.

### Materials and Methods

#### Experimental animals

The animal experiments were carried out according to the Guidelines of our Institutional Animal Regulations. All animals were maintained on a 12 h light:12 h darkness cycle at 22–25 °C and given water ad libitum and a chow, NIH-07PLD, which was developed by the National Institute of Health (USA) in order to lower phytoestrogen contents in the chow (Oriental Yeast Co., Tokyo, Japan; Yamasaki et al. 2002). Cyp19a1 was disrupted by homologous recombination (Toda et al. 2001a) and the genetic background was unified to C57BL/6J by repeated backcrossing. $Esr1^{+/+}$ mice (Lubahn et al. 1993) were purchased from Taconic Farms, Inc. (Hudson, NY, USA) and bred in the animal facility of Kochi University to yield $Esr1^{+/-}$ mice with a C57BL/6J genetic background. $Esr1^{-/-}$ mice were generated by crossing of $Esr1^{+/-}$ mice. Mice at 5 or 10 months of age were used for this study.

#### Histological examination

Testes and efferent ductules were fixed in a solution of 10% (v/v) buffered formalin for 24 h, dehydrated in graded ethanol, and then embedded in paraffin. Samples were cut into 3 μm thick sections and stained with hematoxylin–eosin. The luminal areas of the efferent ductules were quantified using MacScope (ver. 2.5,9) software.

#### Electron microscopic examination

The efferent ductules were fixed in modified Karnovsky's fixative containing 2% paraformaldehyde, 2% glutaraldehyde, and 0.05% CaCl₂ in 0.1 M cacodylate buffer (pH 7.2) for 1 h at 4 °C. The specimens were post-fixed with 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2) containing 0.8% potassium ferrocyanide for 2 h in the dark to preserve the structure of tubule-cisternal endoplasmic reticulum (TCER; Möller et al. 1983). After rinsing, the specimens were exposed to en bloc stain in 1% uranyl acetate in 0.05 M sodium maleate (pH 6.0) for 1 h in the dark. After rinsing in 0.05 M sodium maleate (pH 5.0) for 1 h, the specimens were dehydrated in a graded series of alcohol (Karnovsky 1967), and embedded in Spurr's resin. Thin sections were observed using a Hitachi H-7000H electron microscope. Acid phosphatase activity was detected by means of a cerium-based cytochemical method to identify lysosomes (Robinson et al. 1986).

#### Western blot analysis

The efferent ductules were kept in RNAlater (Ambion, Austin, TX, USA) at −20 °C until use. After removing the fat and connective tissue under a microscope, the tissues were minced using scissors and homogenized in an ice-cold homogenization buffer consisting of 50 mM Tris–HCl buffer (pH 7.4), 0.4 M NaCl, 0.1 mM EDTA, 0.1 mM EGTA, and protease inhibitor cocktail (Roche). The protein concentration of the homogenate was measured with a BCA protein assay reagent (Pierce, Rockford, IL, USA). Each sample (40 μg) was subjected to SDS-PAGE in a 10% gel. The separated polypeptides were then transblotted onto a polyvinylidene difluoride (PVDF) membrane. After treatment with blocking agent, the membrane was incubated with anti-$Esr1$ antibody at a dilution of 1:2000 (MC-20; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

### Table 1 Primer pairs used for RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>5′ Primer primer</th>
<th>3′ Primer primer</th>
<th>Size (bp)</th>
</tr>
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<tbody>
<tr>
<td>Ago1a1</td>
<td>GGGGATTGTGGCTCTGATG</td>
<td>TTTTGCCGTGTGGGGTTTC</td>
<td>328</td>
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<tr>
<td>Ctr</td>
<td>CTTGAGGCGAATGTTGTC</td>
<td>TTGGATGTTATGGGGTCTA</td>
<td>530</td>
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<tr>
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<td>GCCCAACATCCTCCACAAAT</td>
<td>GCTGCTTCATCTCCACCTT</td>
<td>651</td>
</tr>
<tr>
<td>Sclc9a3</td>
<td>GAGTAAAAGCCAAGGAAAGG</td>
<td>AGGGGAGAACACGGGATTAT</td>
<td>343</td>
</tr>
<tr>
<td>Gapdh</td>
<td>CCGATTGTTGTAGTTATGGG</td>
<td>TCCTGGAAGATGTAGTATG</td>
<td>210</td>
</tr>
</tbody>
</table>

*Number of mice showing spermatogenic impairment.

Number of mice examined.

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After washing, the membrane was subsequently incubated with horseradish peroxidase (HRP)–anti-rabbit antibody and the enzyme activity of HRP was detected with an ECL western blotting detection kit. After detaching the anti-Esr1 antibody, the same membrane was reprobed with an HRP–anti-β-actin monoclonal antibody (Sigma–Aldrich, Inc). The results were quantified using Quantity One (ver. 3.0) software (PDI Inc., Huntington Station, NY, USA).

RNA preparation and RT-PCR analysis

Total RNA was extracted from pooled efferent ductules kept in RNA later as described (Zarlenga & Gamble 1987) after removing fat and connective tissue under a microscope. Total RNA (1 μg) was reversed transcribed using oligo-dT primers (Ambion) and Moloney murine leukemia virus reverse transcriptase (Invitrogen) in a total volume of 25 μl per

Figure 1  Histological examination of the testes of Ar−/− and Esr1−/− mice with a C57BL/6J genetic background. (A) Each circle represents an individual mouse, of which both testes were examined histologically. Vertical numerals indicate the sum of the numbers of seminiferous tubules showing impaired spermatogenesis per section in each mouse examined. As no abnormality was found in the testes of WT mice with a C57BL/6J background, the data were not plotted in this figure. (B and C) Representative photographs of the testis of an Ar−/− mouse at 5 months of age displaying various degrees of impairment in spermatogenesis. The seminiferous tubules indicated by arrowhead in (C) are judged to be impaired. (D) The image shown at higher magnification is from the area indicated by a box in (B). Scale bar, 200 μm.
reaction, according to the manufacturer's instructions. The expression levels of genes for ATPase, $\text{Na}^+$/K$^+$ transporting, $\alpha$-1 polypeptide ($Atp1a1$), cystic fibrosis transmembrane conductance regulator homolog ($Cftr$), solute carrier family 9 (sodium/hydrogen exchanger) member 1 ($Slc9a1$), and solute carrier family 9 (sodium/hydrogen exchanger) member 3 ($Slc9a3$) were examined. PCR was carried out with 1.5 μl of the RT samples in a 30 μl reaction volume. Cycling parameters were as follows: an initial melting step of 94 °C for 1 min, amplification by 28 cycles at 94 °C for 30 s, 60 °C for 30 s, and 74 °C for 45 s and then a final 5 min at 74 °C for extension. The amplified DNA products were resolved on 5% polyacrylamide gels. After staining the gels with ethidium bromide, the banding pattern of amplified DNA fragments was recorded using a charged-coupled device camera (Sony Corp., Tokyo, Japan). The results were

Figure 2 Photomicrographs of the testis and efferent ductules in the distal region of two individual $\text{Ar}^{-/-}$ mice at 5 months of age. (A and B) Testis from one $\text{Ar}^{-/-}$ mouse at 5 months of age showed spermatogenic impairment and (D and E) the testis from the other $\text{Ar}^{-/-}$ mouse displayed morphologically normal spermatogenesis. (B and E) The images shown at higher magnification are from the areas indicated by boxes in (A) and (D) respectively. (C and F) Both $\text{Ar}^{-/-}$ mice reveal similar morphology in the distal efferent ductules. Scale bars, 200 μm.
quantified using Quantity One (ver. 3.0) software. The intensity values for each transcript were normalized to the respective expression level of glyceraldehyde 3-phosphate dehydrogenase (Gapdh) and expressed as fold difference over the value of WT mice. The nucleotide sequences of primers and the sizes of amplified fragments are listed in Table 1.

**Statistical analysis**

Data are expressed as the means ± S.E.M. The significance of differences was analyzed using Student’s *t*-test employing InStat software (GraphPad Software, Inc., San Diego, CA, USA, 2003). *P* < 0.05 was considered significant.

**Results**

**Histological examination of the testicular phenotype of Ar<sup>-/-</sup> mice with a C57BL/6J genetic background**

Spermatogenesis was evaluated morphologically at 5 and 10 months of age by measuring the numbers of dysmorphic seminiferous tubules per section. None of the WT males displayed spermatogenic impairment at either age (Table 2). By contrast, variable degrees of aberrant morphological features were observed in the seminiferous tubules of Ar<sup>-/-</sup> mice with a fully congenic C57BL/6J genetic background at 5 months of age (Fig. 1A). Out of 20 Ar<sup>-/-</sup> mice, 2 displayed grossly dysmorphic seminiferous tubules and disrupted spermatogenesis, and 11 mice showed variable degrees of spermatogenic impairment (Fig. 1B–D). As reported previously (Eddy *et al.* 1996), all of the Esr1<sup>-/-</sup> mice examined showed spermatogenic impairment (Fig. 1A; Table 2). When analyzed at 10 months of age, 70% of Ar<sup>-/-</sup> mice (9 out of 13 mice) showed histologically severe impairment of spermatogenesis.

**Morphology of efferent ductule of Ar<sup>-/-</sup> mice**

The morphology of the efferent ductules of an Ar<sup>-/-</sup> mouse showing impairment in spermatogenesis was indistinguishable from that of an Ar<sup>-/-</sup> mouse showing relatively normal spermatogenesis at 5 months of age (Fig. 2). When measuring the luminal areas of the efferent ductules at the distal region, the luminal area of Esr1<sup>-/-</sup> mice at 5 months of age was twofold larger than that of WT mice (Esr1<sup>-/-</sup>, 92.6 ± 6.5 μm<sup>2</sup>; WT, 42.5 ± 2.9 μm<sup>2</sup>; *P* < 0.001; Fig. 3), which is consistent with
the previous study (Hess et al. 1997). By contrast, no significant difference was detected between Ar−/− and WT mice (Ar−/−, 41.0 ± 2.6 μm²; WT, 42.5 ± 2.9 μm²; Fig. 3). In addition, there was no difference in the area at 10 months of age between WT and Ar−/− mice (WT, 59.2 ± 7.8 μm²; Ar−/−, 54.5 ± 6.6 μm²), while the area was significantly enlarged in Esr1−/− mice (93.9 ± 5.6 μm², P < 0.001). Furthermore, the height of epithelial cells was similar between WT and Ar−/− mice at 5 months of age (data not shown), while the height was decreased in Esr1−/− mice (Fig. 4A and B).

**Electron microscopic examinations**

The lumen of the efferent ductule is covered with a single layer of columnar epithelium that is composed of ciliated and non-ciliated cells. Non-ciliated cells had many electron-dense granules (~1000 nm in diameter) at the supranuclear and basal cytoplasm in WT and Ar−/− but not in Esr1−/− mice (Fig. 4A and B). Cytochemical analysis for detecting acid phosphatase activity demonstrated that the granules were totally negative for the activity, indicating that they were not lysosomes (Fig. 4C).

![Figure 4](https://via.placeholder.com/150)

**Figure 4** Transmission electron micrograph analysis of the efferent ductule. Electron micrographs of the epithelium of (A, C, D, and G) WT, (E and H) Ar−/−, and (B, F, and I) Esr1−/− efferent ductules. (A) Non-ciliated epithelial cells of the WT efferent ductule have many electron-dense granules and vacuolar endosomes (asterisk). (B) Non-ciliated epithelial cells of the Esr1−/− efferent ductule have well-developed Golgi complex (G) but do not have electron-dense granules. N and C indicate non-ciliated and ciliated epithelial cells respectively. (C) Acid phosphatase activity was detected cytochemically in epithelial cells of the WT efferent ductules. The reaction products of acid phosphatase are observed at the Golgi complex of the ciliated cells (G) and lysosomes of the non-ciliated cells (large arrow). (D) Electron-dense granules are totally negative for staining. Endosomes are indicated by asterisks. (D) The apical part of a non-ciliated cell of the WT efferent ductules reveals the presence of a well-developed apical tubule (AT) (representatives indicated by arrows), vacuolar endosomes (asterisks), intermicrovillous membrane invaginations (open arrows), and a tubule-cisternal endoplasmic reticulum (TCER) (arrowheads). An insert shows endosomes connecting to the AT. (E) The apical part of a non-ciliated cell of the Ar−/− efferent ductules reveals a similar ultrastructure to that of WT mice. (F) The apical part of a non-ciliated cell of the Esr1−/− efferent ductules reveals vacuolar endosomes (asterisks) and TCER (arrowheads). The Golgi complex (G) is well developed but the AT is not in a non-ciliated cell of the Esr1−/− efferent ductule. (G) The apical part of a ciliated cell of the WT efferent ductule reveals the presence of well-developed TCER (arrowheads). C indicates coated invagination. (H) The apical part of a ciliated cell of the Ar−/− efferent ductule reveals well-developed TCER as seen in that of WT mice. C indicates coated invagination. (I) The apical part of a ciliated cell of the Esr1−/− efferent ductule reveals well-developed TCER. C indicates coated invagination. NC indicates a non-ciliated cell. Scale bars, (A–C) 1 μm and (D–I) 0.5 μm.
Three types of tubule-vesicular components were observed by transmission electron microscopy. One is a component belonging to an endocytic apparatus including intermicrovillous membrane invaginations that are decorated with clathrin coat lining over the cytoplasmic surface of the invaginating region, clathrin-coated vesicles, and vacuolar early sorting endosomes (Hatae et al. 1986a). This component was observed in the WT, Ar−/−, and Esr1−/− efferent ductules (Fig. 4B–I), while the sizes of the invaginations and early sorting endosomes were smaller in non-ciliated cells of Esr1−/− mice relative to those in WT and Ar−/− mice (Fig. 4D–F). The second component was TGER that is well developed in ciliated cells (Fig. 4G–I) but not so prominent in non-ciliated cells (Fig. 4D–F). The third component was referred to as an apical tubule (AT), which had a cylindrical structure with a diameter ranging from 60 to 80 nm and its lumen was filled with moderately electron-dense materials (Fig. 4D and E). Furthermore, it appeared to be covered with a structure with a diameter ranging from 60 to 80 nm and its lumen was filled with moderately electron-dense materials (Fig. 4D and E). Furthermore, it appeared to be covered with smooth membranous materials and occasionally connected to endocytic vesicles with a diameter ranging from 140 to 200 nm (Fig. 4D, insert). The AT was present densely in the apical part of non-ciliated cells of WT and Ar−/− mice, but completely absent in Esr1−/− mice (Fig. 4D–F). Another noticeable feature was the Golgi complex, which was highly developed in the non-ciliated cells of Esr1−/− mice but was not prominent in those of WT and Ar−/− mice (Fig. 4B and F).

**Western blot and RT-PCR analyses**

The expression level of Esr1 in the efferent ductules was examined by western blotting. The antibody against Esr1 recognized a 67 kDa protein as a main band in the extracts of efferent ductules of WT and Ar−/− mice, but not in Esr1−/− mice. In addition to the band, the antibody detected several minor bands, which might represent truncated forms of Esr1, degradation products, or non-specific bands. Nonetheless, the patterns of the bands were essentially the same between the samples of WT and Ar−/− mice (Fig. 5A). Furthermore, the expression level of the main band was not different between WT and Ar−/− efferent ductules (Fig. 5B). These findings demonstrated that Esr1 protein was not overexpressed in Ar−/− efferent ductules. Suppression of gene expressions related to ion transportation including Slc9a3 has been reported to be one of the reasons for impaired testicular fluid reabsorption at the efferent ductules in Esr1−/− mice (Lee et al. 2001, Zhou et al. 2001). Therefore, we examined the expression levels of a subset of genes involved in ion transportation by a semiquantitative RT-PCR procedure. As shown in Fig. 6, no significant differences were found in the expression levels of genes for Atp1a1, Cft, Slc9a1, and Slc9a3 between WT and Ar−/− efferent ductules. By contrast, the expression levels of Cft and Slc9a1 respectively increased 1.8- and 2.4-fold and those of Slc9a3 decreased to 30% in Esr1−/− efferent ductules when compared with those in WT mice (Fig. 6).

**Discussion**

Ar−/− mice with a fully congenic C57BL/6J genetic background were infertile and displayed progressive disruption in spermatogenesis. The Ar−/− mice used in the previous study displayed a more moderate spermatogenic phenotype than that of the present line (Toda et al. 2001b). This difference might reflect an effect of estrogenic compounds in the diet as reported (Robertson et al. 2002), since the current mice were fed a phytoestrogen-lowered diet (NIH-07PLD) instead of a conventional chow diet (NMF; Oriental Yeast Co.) Alternatively, the current strain with a C57BL/6J genetic background might contain subsets of genes that are critical for maintaining normal spermatogenesis and are highly susceptible to aromatase inactivation. The data presented here indicate that aromatase activity is involved in maintaining spermatogenesis in mice; nevertheless, the molecular basis has not been established. It was reported that round spermatids showing apoptosis were detected more frequently in the seminiferous tubules of Ar−/− than WT mice (Robertson et al. 1999, 2001), suggesting that aromatase inactivation results in an increase in apoptosis-inducing molecules. Instead, estrogens might act as a potent inhibitor of apoptosis (Ebling et al. 2000, Pentikainen et al. 2000, Thuillier et al. 2003, Wang et al. 2004, Wählgren et al. 2008), but a number of studies reported that estrogens or estrogenic compounds act as an apoptotic inducer on male germ cells (Koji 2001).

We provided experimental evidence to show that the morphological features of the efferent ductules of Ar−/−
mice are indistinguishable from those of WT mice at 5 and 10 months of age, indicating that morphometric alterations of the efferent ductules are not associated with the abnormalities of spermatogenesis caused by estrogen insufficiency. Electron microscopic examinations demonstrated that TCER is well developed in ciliated cells of the WT, Ar^{-/-} (A), and Esr1^{-/-} (E) mice at 5 months of age, and total RNA was prepared for gene expression analysis. (A) Representative gel patterns of RT-PCR analysis. (B) Bar graphs illustrate the fold difference compared with gene expression analysis. (A) Representative gel patterns of RT-PCR analysis. (B) Bar graphs illustrate the fold difference compared with the signals obtained with specific primers for Gapdh. Data were expressed as mean±s.e.m. *P<0.05 versus WT mice.

Figure 6 Semi-quantitative RT-PCR analysis of Atplα1, Clfr, Slc9α1, and Slc9α3 mRNA expression in the efferent ductules. Whole efferent ductules were collected from WT (W), Ar^{-/-} (A), and Esr1^{-/-} (E) mice at 5 months of age, and total RNA was prepared for gene expression analysis. (A) Representative gel patterns of RT-PCR analysis. (B) Bar graphs illustrate the fold difference compared with the expression level in the WT efferent ductules. Differences in the amount of template cDNA input were normalized by the signals obtained with specific primers for Gapdh. Data were expressed as mean±s.e.m. *P<0.05 versus WT mice.

Membrane invagination and endocytic tubular-vesicle apparatus are present with similar abundance in non-ciliated cells of WT, Ar^{-/-}, and Esr1^{-/-} mice, although the sizes of these cellular structures appeared to be smaller in Esr1^{-/-} than WT and Ar^{-/-} mice. These observations are somewhat inconsistent with the previous studies, in which endocytic apparatus including apical vesicles was markedly reduced in Esr1^{-/-} mice (Hess et al. 2000). The most striking feature is the almost complete absence of the AT in the efferent ductules of Esr1^{-/-} mice. The AT is frequently observed in epithelial cells of tissues such as kidney proximal tubule and visceral yolk sac (Hatae et al. 1986b) where fluids are actively absorbed. Although the roles of the AT have not been established, it was proposed that the components may be related to a membrane recycling process, by which intracellular membrane components are returned to the plasma membrane (Hatae et al. 1986b). Thus, the present findings indicate that membrane recycling activity might be compromised in the Esr1^{-/-} efferent ductules leading to the low activity of fluid reabsorption. The well-developed Golgi complex observed in the Esr1^{-/-} efferent ductules might reflect the aberrant activity of membrane recycling. Involvement of Esr1/ estrogen in maintaining the apical cytoarchitecture of non-ciliated epithelial cells of the efferent ductules was pointed out previously (Zhou et al. 2001).

Several lines of evidence provide clues regarding the less profound effects of aromatase inactivation on the efferent ductule, namely 1) ER ligands other than estrogens might be produced endogenously in an aromatase-independent manner (Weihua et al. 2002, McCarthy et al. 2007), 2) Esr1 is activated estrogen independently by other signaling pathways triggered by growth factors or cAMP (Julie et al. 2001, Aguirre et al. 2007). It is also of interest to speculate that exposure of Ar^{-/-} mice to maternal estrogen during development in the uterus or during weaning through milk might affect the architecture of the efferent ductules or that aromatase inactivation might cause less active fluid secretion. Fluid reabsorption could be mediated by various aquaporins. Expressions of aquaporin 1, 9, and 10 were detected in the efferent ductules leading to the low activity of fluid reabsorption. The well-developed Golgi complex observed in the Esr1^{-/-} efferent ductules might reflect the aberrant activity of membrane recycling. Involvement of Esr1/ estrogen in maintaining the apical cytoarchitecture of non-ciliated epithelial cells of the efferent ductules was pointed out previously (Zhou et al. 2001).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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