

# Immunolocalization and Regulation of Cystic Fibrosis Transmembrane Conductance Regulator in the Adult Rat Epididymis

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**ABSTRACT:** Cystic fibrosis is the most common serious autosomal recessive condition in whites, and more than 95% of men with cystic fibrosis are infertile. The cystic fibrosis transmembrane conductance regulator (CFTR), a cyclic adenosine monophosphate (cAMP)-regulated chloride channel, has been localized in the efferent ducts; however, to our knowledge, its expression and regulation in the epididymis by testicular factors have not been examined. In the present study, these parameters were examined immunocytochemically by the light microscope with an anti-CFTR antibody in Bouin-fixed, paraffin-embedded control adult rat epididymides and both orchidectomized adult rats with or without testosterone supplementation and efferent duct-ligated rats sacrificed at different time points. In control animals, a thick dense band of immunoperoxidase reaction product was visualized over the apical plasma membrane of the principal cells but not their microvilli. The apical band was prominent only in the corpus and cauda regions. While there was no CFTR expression

in basal cells, clear cells of the corpus and cauda regions showed a weak-to-moderate band of apical plasma membrane staining. An examination of orchidectomized, orchidectomized and testosterone, and efferent duct-ligated rats revealed that CFTR was no longer expressed as an intense band on the apical plasma membrane of the principal cells of the corpus and cauda regions. However, under these conditions, an intense apical/supranuclear reaction was noted in the form of small vesicular structures. Clear cells were unaffected by the different experimental treatments. Together, these data indicate that CFTR is expressed in a cell- and region-specific manner and that, while its synthesis in principal cells is not under the control of testicular factors, targeting to the apical plasma membrane is regulated by a testicular luminal factor.

Key words: Principal and clear cells, orchidectomy, efferent duct ligation, lumicrine testicular factor.

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Sperm passage through the epididymis is important for their maturation, and the formation of a suitable luminal environment is the major role of the epithelial cells lining the entire epididymal duct (Bedford, 1967; Orgebin-Crist, 1967). To this end, the epithelial cells secrete and reabsorb various electrolytes and fluid to create the optimal medium for sperm maturation (Cornwall et al, 2002; Wong et al, 2002). Although the reabsorption of fluid (from the lumen to the blood) is an element of epithelial dynamics, fluid secretion (from the blood to the lumen) also plays an important role in the epididymis. Reabsorption is driven by active sodium import, while secretion is driven by active anion export, both of which influence the movement of water inward and outward, respectively, across the epithelium (Wong, 1986, 1998; Wong and Huang, 1989). Secretion may act as a counterbalance to reabsorption, thereby exerting a fine control over the net movement of water across the epididymal

epithelium (Wong et al, 2002). Indeed, the epithelium of the epididymis is highly permeable to water because of the expression of various aquaporin water membrane channels (AQP-9) in their membranes (Pastor-Soler et al, 2001; Badran and Hermo, 2002; Hermo and Robaire, 2002).

Several anion channels with different characteristics have been described in the epididymis (Chan et al, 1993, 1994). However, the cystic fibrosis transmembrane conductance regulator (CFTR), a small conductance cyclic adenosine monophosphate (cAMP)-activated chloride channel, has been demonstrated to have the greatest relevance to transepithelial secretion of electrolytes and water (Pollard et al, 1991; Tizzano et al, 1994; Wong et al, 2002). CFTR, a 168-kd membrane transport protein (Riordan et al, 1989; Fiedler et al, 1992), is composed of 5 domains: 2 membrane-spanning domains, 2 nucleotide-binding domains, and 1 regulatory domain (Sheppard and Welsh, 1999). CFTR is located primarily in the apical membrane of polarized epithelial cells (Crawford et al, 1991; Denning et al, 1992). The importance of CFTR has been borne out by the genetic disease cystic fibrosis, caused by mutation of CFTR. From the most severe forms of mutations to the least, it is apparent that the male reproductive system is highly dependent on CFTR for nor-

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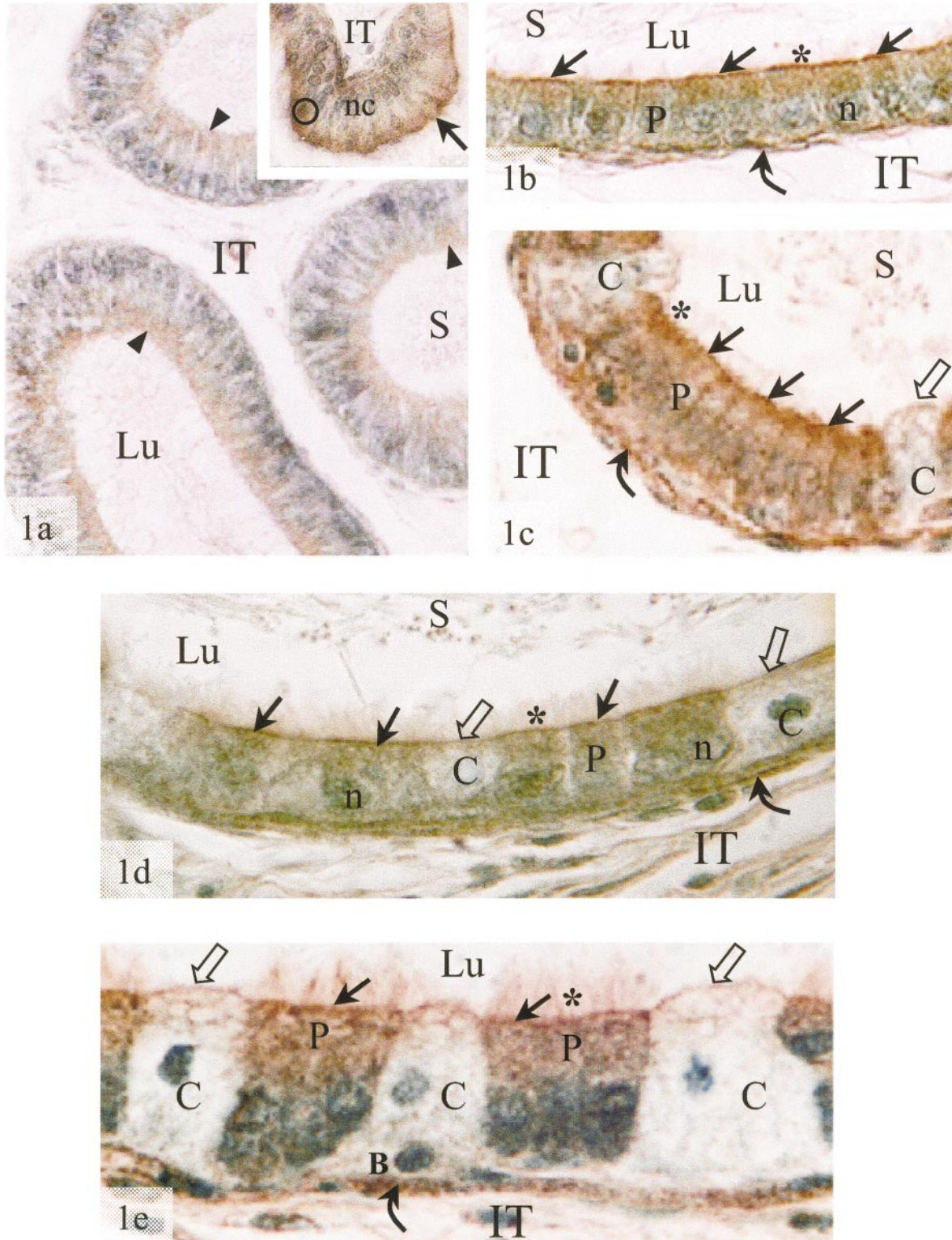


Figure 1. Efferent ducts (a) (inset), caput (a), corpus (b, c), and cauda (d, e) epididymidis at low (a, b) and high (c–e) magnification of 90-day-old control adult rats immunostained with an anti-cystic fibrosis transmembrane conductance regulator (CFTR) antibody. In the inset of (a), a band of intense reaction is visible over small apical vesicles (circle) and the apical plasma membrane of nonciliated cells (nc) but not over their microvilli (arrow). In (a), a weak diffuse reaction product is evident over the apical cytoplasm of the principal cells (arrowheads). In (b–e), the most conspicuous reaction appears as an intense band of reaction product over the apical plasma membrane of these cells (arrows), which is not evident in those of the caput epididymidis (a). In addition, a weak-to-moderate vesicular reaction is evident over the apical and supranuclear regions of the principal cells. Clear cells (C) show a weak-to-moderate apical plasma membrane staining (open arrows) (c–e). No reaction is visible over the microvilli of the principal cells (stars) or sperm (S) in the lumen (Lu), other than background levels of staining. Note that a reaction is also prominent over the peritubular myoid

mal function. While there is still debate as to the consequences of CFTR mutation, congenital unilateral or bilateral absence of the vas deferens has been suggested along with poor sperm quality (Anquiano et al, 1992; Patrizio et al, 1993; Oates and Amos, 1994; Chillon et al, 1995; Van der Ven et al, 1996; Rave-Harel et al, 1997).

Previous studies have shown that CFTR is expressed on the apical domains of the nonciliated epithelial cells of the rat efferent ducts (Leung et al, 2001) and that it is functional in the epididymal epithelium (Wong et al, 2002). In the normal human epididymis, CFTR messenger RNA (mRNA) was identified by *in situ* hybridization in the epithelium mainly of the caput epididymidis as well as the vas deferens (Patrizio and Salameh, 1998). Secretion of Cl<sup>-</sup> by CFTR across a membrane provides a driving force for sodium and water secretion. By effecting fluid secretion, CFTR acts counter to fluid reabsorption and serves to fine-tune the fluidity of the epididymal microenvironment (Wong et al, 2002). Indeed, it has been demonstrated that the luminal milieu of the epididymis is actively maintained and that this plays a crucial role in the maturation process of spermatozoa (Hamilton, 1975; Robaire and Hermo, 1988; Cornwall et al, 2002). While CFTR has been documented to be present and functional in the epididymis, studies on its immunolocalization have not been performed in detail, nor have studies examining its regulation by testicular factors, whether gaining access to the epididymis via the circulation or lumen of the duct.

In the present study, the expression of CFTR in the epididymis was examined on Bouin-fixed adult rat epididymides using an anti-CFTR antibody. In addition, the regulation of CFTR was examined in orchidectomized and efferent duct-ligated adult rats to determine the role of testicular factors on its expression.

## Materials and Methods

### *Animals and Experimental Protocols*

Adult male Sprague-Dawley rats (350–450 g, aged 3–4 months) were obtained from Charles River Laboratory Ltd (St Constant, Quebec) and were divided into 5 groups. The first group consisted of 4 normal adult control animals. Bilateral ligation of the efferent ducts constituted the second group. After an intraperitoneal injection of sodium pentobarbital (Somnitol; MTC Pharmaceuticals, Hamilton, Canada), the testes and epididymides of adult rats were exposed through an incision of the anterior abdominal wall. Using a dissecting microscope, a ligature was placed around both right and left efferent ducts at a site close to and further removed from the rete testis, with care being taken

to avoid interference of the blood vessels entering the testis. The interval between the 2 ligatures was then excised to ensure that no sperm or fluids would enter the epididymis from the testis. The animals (4 per interval) were sacrificed at 3, 7, 14, and 21 days following surgery. Bilateral orchidectomy constituted the third group. After anesthesia, both testes of each rat were removed after a ligature was placed around the efferent ducts and testicular blood vessels. The animals (4 per interval) were sacrificed at 3, 7, 14, and 21 days after surgery. Bilaterally orchidectomized rats that received three 6.2-cm testosterone-filled implants constituted the fourth group. Testosterone-filled polydimethyl-siloxane (silastic) implants were prepared according to the method of Stratton et al (1973) and have well-characterized steroid release rates (Brawer et al, 1983). Subsequent to anesthesia, both testes were removed from each rat, and the implants were placed subcutaneously immediately after orchidectomy. The rats (4 per interval) were sacrificed at 3, 7, 14, and 21 days after surgery. The fifth group consisted of 4 sham-operated animals, 2 of which received 3 empty 6.2-cm-long implants, with all rats being sacrificed 14 days after the initiation of the experiment. All experimentation was carried out with minimal stress and discomfort being placed on the animals both during and after surgery as set up by the guidelines and approval of the McGill University Animal Care Committee.

### *Tissue Preparation for Light Microscope Immunocytochemistry*

At the end of each experiment, the epididymides of each rat were fixed by perfusion with Bouin fixative via the abdominal aorta for 10 minutes. Following perfusion, the epididymides were removed and cut so that given sections would include all the major regions of the epididymis (ie, the initial segment, intermediate zone, caput, corpus, and cauda) (Herme et al, 1991). The tissue was then immersed in Bouin fixative for 72 hours, after which it was dehydrated and embedded in paraffin.

Immunoperoxidase staining of sections was carried out according to the procedure of Oko and Clermont (1989). Monoclonal, affinity-purified anti-CFTR antibody was tried at different dilutions (1:50–1:200) in Tris-buffered saline, pH 7.4, with the 1:100 dilution showing the optimal reaction for the type of fixation and immunostaining method used. The anti-CFTR antibody was obtained from NeoMarkers (Fremont, Calif). The antibody, well-characterized and specific for its respective peptide, was purified from ascites fluid by Protein G chromatography and raised against a 10-amino acid synthetic peptide within the carboxy terminus of the protein (Kartner et al, 1992). It was supplied as a 200- $\mu$ g/mL solution in phosphate-buffered saline (PBS), pH 7.4, with 0.2% bovine serum albumin as a stabilizer. The antibody solution also contained 15 mM sodium azide as a preservative.

Paraffin sections, 5  $\mu$ m thick, were deparaffinized in Histo-clear (Diamed Lab Supplies Inc, Mississauga, Canada) and hydrated in a series of graded ethanol solutions. During hydration,

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cell layers enveloping each epididymal tubule (curved arrows). IT indicates intertubular space; P, principal cells; B, basal cells; and n, nuclei of the principal cells. (a, b): 320 $\times$ ; inset of (a, c, d): 512 $\times$ ; (e): 1280 $\times$ .

residual picric acid was neutralized in 70% ethanol containing 1% lithium carbonate, and endogenous peroxidase activity was abolished in 70% ethanol containing 1% (vol/vol) H<sub>2</sub>O<sub>2</sub>. Once hydrated, the tissue sections were washed in distilled water containing glycine to block free aldehyde groups.

Antigen was retrieved by treatment in 100  $\mu$ L of 0.025% trypsin solution for 9 minutes. Sections were then rinsed 3 times in PBS. After rinsing with tap water and PBS, sections were incubated in normal blocking serum (Vectastain Elite ABC kit, Vector K-6101; Vector Laboratories, Burlingame, Calif) for 30 minutes and then with the monoclonal anti-CFTR antibody, diluted to 1:100 with PBS. Sections were then washed 3 times with PBS and incubated with biotinylated secondary antibody (ABC kit) for 30 minutes. After washing 3 times with PBS, sections were incubated with the ABC reagent for 30 minutes and finally washed again 3 times with PBS. Visualization of the stain was achieved by incubating sections with 0.05% diaminobenzidine tetrahydrochloride (Bio Fx Laboratories, Owings-Mills, Md) until the desired staining intensity was achieved. Slides were rinsed with tap water for 5 minutes and then counterstained with 0.1% methylene blue. Passing them through a graded ethanol series dehydrated the tissues. Thereafter, the tissue sections were mounted on glass slides with Permount for observation. Negative controls were obtained by omission of the primary antibody.

## Results

### *Expression of CFTR in the Efferent Ducts and Epididymis of Control Adult Rats*

In the efferent ducts of control adult rats immunostained with an anti-CFTR antibody, an intense band of immunoperoxidase reaction product was noted over the apical plasma membrane of the nonciliated epithelial cells, in addition to a moderate vesicular reaction immediately subjacent to it (Figure 1a, inset). No reaction was evident in the supranuclear region of these cells, nor was any reaction visible on their microvilli. Ciliated cells were unreactive.

In the epididymis, a cell- and region-specific expression of CFTR was noted. In the initial segment and caput epididymidis, the principal cells showed a weak diffuse reaction product over their apical cytoplasm but no discrete reaction product over their apical plasma membrane or microvilli (Figure 1a). Narrow and apical cells of the initial segment and intermediate zone were consistently unreactive. In contrast to these 2 epididymal regions, the principal cells of the corpus and cauda epididymidis showed an intense band of immunoperoxidase reaction product over their apical plasma membrane, while a mod-

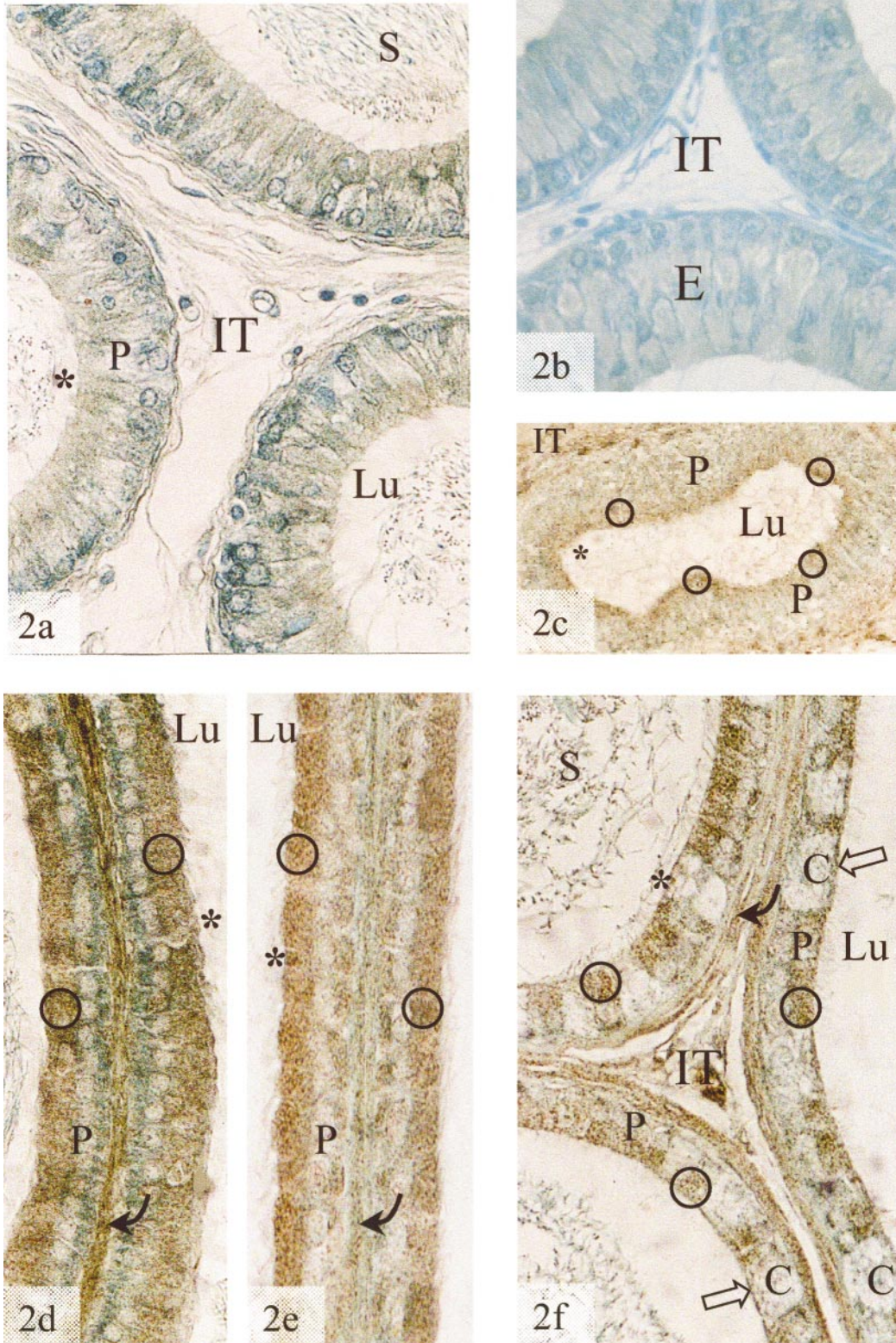
erate diffuse reaction, at times vesicular in appearance, was noted apically and supranuclearly; there was no staining of their microvilli (Figure 1b through e). While clear cells of the caput region were unreactive, those of the corpus and cauda epididymidis showed a weak-to-moderate reaction (Figure 1c through e). There was no reaction over the basal cells of any epididymal region. The peritubular myoid cell layers enveloping the tubules of the corpus and cauda epididymidis often displayed moderate-to-intense levels of reaction product (Figure 1b through e). Throughout the epididymis, sperm in the lumen were consistently unreactive (Figure 1a through e). The elimination of the primary antibody to the epididymal sections showed the complete absence of a reaction product over the epithelium or lumen of all epididymal tubules, as well as the underlying connective tissue areas of the entire epididymis, whether sections were examined of normal or experimental animals. In fact, in all cases when the different experimental conditions were examined, control sections were included when the primary antibody was not added and, in such cases, no reaction was observed (eg, Figure 2b).

### *CFTR Immunolocalization in Adult Rats After Various Experimental Treatments*

After orchidectomy at the 3-, 7-, 14-, and 21-day intervals, there was a dramatic redistribution in the expression of CFTR in the principal cells of the epididymis. This was evident as early as the 3-day interval and persisted at all later time points. In the initial segment and caput regions, only a weak diffuse reaction was evident over the apical cytoplasm of the principal cells (Figure 2a), not unlike that seen in control animals. However, in the corpus and cauda epididymidis, there was an absence of the intense band of reaction product over the apical plasma membrane of these cells (Figure 2c and e). Noteworthy, however, was the presence of an apical and supranuclear cytoplasmic reaction in the principal cells of these 2 regions, which was visualized as numerous small intensely reactive vesicular profiles (Figure 2c and e). The same staining pattern in the various epididymal regions was observed for orchidectomized rats supplemented with testosterone at the different time points examined, with Figure 2f being representative of the type of reaction seen in testosterone-supplemented animals. While the illustrations were selected at apparent random time points, each serves to demonstrate the type of staining seen at all time points after orchidectomy or orchidectomy with testosterone supplementation.

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Figure 2. Initial segment (a, b), proximal cauda (c), corpus (d, e), and middle cauda (f) epididymidis of a 3-day efferent duct-ligated rat (a), the negative control of a normal adult animal (b), a 21-day orchidectomized rat (c), a 7-day efferent duct-ligated rat (d), a 14-day orchidectomized rat (e), and a 14-day orchidectomized rat supplemented with testosterone (f). (a, c-f) Tissues were immunostained with an anti-cystic fibrosis transmembrane



conductance regulator (CFTR) antibody and (b) tissue treated without a primary antibody. In (b), no reaction appears over the epithelium (E) or intertubular space (IT). While a weak diffuse reaction is noted over the apical cytoplasm of the principal cells in (a), as also noted for control animals, only an intense vesicular reaction product (circles) is prominent and uniformly distributed over the apical and supranuclear regions of the principal cells of the corpus and cauda epididymidis (c–e), suggesting the presence of numerous small CFTR-reactive vesicular structures after the different experimental treatments. The clear cells (C) maintain weak-to-moderate staining of their apical plasma membrane (open arrows) (f), as do the peritubular myoid cells (curved arrows). No reaction is evident over sperm (S) in the lumen (Lu) or microvilli of the principal cells (c–f). n indicates nuclei of the principal cells. (a, b): 320×; (c–e): 512×.

After efferent duct ligation at the 3-, 7-, 14-, and 21-day intervals, the same type of staining pattern was noted in the principal cells as was seen for orchidectomized animals with or without testosterone treatment, and this was noted as early as at the 3-day interval. Illustrations once again were selected randomly, but each serves to illustrate the type of staining noted at the different time points. CFTR expression in the principal cells of the initial segment and caput epididymidis continued to be diffuse and restricted to the apical cytoplasm; however, the prominent thick band of reaction product noted over the apical plasma membrane of the principal cells of the corpus and cauda regions of control animals was conspicuously absent. In its place was the presence of numerous small reactive vesicular profiles distributed throughout the apical and supranuclear cytoplasm of these cells (Figure 2d).

After the different experimental treatments and at all time points, clear cells of the caput epididymidis remained unreactive, while those of the corpus and cauda regions maintained their apical plasma membrane staining. The peritubular myoid cell layers of the corpus and cauda regions also remained intensely reactive at the various time points after each experimental condition (Figure 2d through f).

## Discussion

### *Expression of CFTR in the Epididymis of Control Adult Rats*

CFTR, a cAMP-dependent protein kinase-activated chloride channel, has been located primarily in the apical plasma membrane of polarized epithelial cells of a variety of different tissues (Crawford et al, 1991; Riordan, 1993). In the rat testis, the CFTR message has been associated with developing germ cells, Sertoli cells during early postnatal development, and cultured Sertoli cells (Boockfor et al, 1989; Trezise and Buchwald, 1991). In the epididymis, the presence of CFTR is not novel, as a functional role for CFTR has been well described for several years (Wong et al, 2002). However, in the present study, it is revealed that CFTR shows cell- and region-specific expression, with principal, clear, and peritubular cells of the corpus and cauda regions presenting the most prominent reaction. There was no evidence of CFTR expression in narrow, apical, or basal cells of the entire epididymis. Thus, CFTR appears to play an important role in the distal regions of the epididymis.

The presence of weak CFTR expression in the initial segment would support the finding that water in this region is driven mainly from the lumen to the intertubular space. This has been suggested to be of importance for concentrating sperm in the small luminal diameter of the

initial segment so as to better facilitate interactions of the sperm surface with the secretion products of the epithelial cells lining this region of the duct (Robaire and Hermo, 1988; Hess et al, 1997, 2002; Hess, 2002). In fact, in the initial segment, intense expression of AQP-9 has been localized to the microvilli of the principal cells, which may aid the passage of water from the lumen to the underlying intertubular space (Badran and Hermo, 2002).

In the corpus epididymidis, where there is a progressive accumulation of sperm, and in the cauda region, where they are stored, a maximal expression of CFTR was observed in the principal cells, and a weak-to-moderate reaction was observed in the clear cells. It has been noted that under basal conditions, various transporters located in the basolateral plasma membranes of the epididymal epithelial cells take up  $\text{Cl}^-$  into their cytoplasm so that intracellular chloride activity is held above its electrochemical equilibrium. When cells are stimulated, there is an increase in intracellular cAMP, which activates CFTR, allowing chloride efflux into the lumen (Wong, 1988; Chan et al, 1995). Under such stimulated conditions, the net secretory flux is increased to a level that exceeds the reabsorptive flux, resulting in a net secretion of water into the lumen. In the cauda epididymidis where most of the testicular fluid would have been reabsorbed, the secretion of electrolytes and fluid would be imperative to prevent luminal dehydration (Wong et al, 2002). Thus, sperm in the corpus and cauda regions, while encountering immobilin, a protein that gradually fills the lumen of the cauda epididymidis to eventually immobilize them (Hermo et al, 1992), would nevertheless be maintained in a fluid environment created by the input and output of water, the fine-tuning of which is exerted by various transporter proteins (Wong et al, 2002), including the presence of CFTR in the principal and clear cells as demonstrated in the present study.

In addition to being a chloride channel, CFTR acts as a regulator of other membrane transport proteins, such as the epithelial  $\text{Na}^+$  channels and the outwardly rectifying  $\text{Cl}^-$  channels (Schwiebert et al, 1995; Schreiber et al, 1999). CFTR has also been shown to activate AQP-3 in *Xenopus* oocytes, and the water permeability of respiratory cells was enhanced with CFTR activation (Schreiber et al, 1997, 1999). The present localization of CFTR on the apical surface of the principal and clear cells of the corpus and cauda regions, along with previous findings of AQP-9 expression in these 2 cell types (Badran and Hermo, 2002), suggests that these 2 proteins functionally interact with one another in the epididymis. Recently, the interaction between rat epididymal CFTR and AQP-9 has been visualized by expressing both in *Xenopus* oocytes. The results demonstrated that AQP-9 alone resulted in an increase in oocyte water permeability, which was further augmented by CFTR, and these results were also con-

firmed in the rat epididymis by *in vivo* studies (Cheung et al, 2003). Thus, there appears to be a synergistic effect of the 2 proteins in conferring water permeability. In this way, the fluidity of the epididymal lumen appears to be fine-tuned by the presence of both AQPs and CFTR, resulting in a microenvironment that is conducive not only for sperm maturation but also for their passage down the duct and storage in the cauda epididymidis. The finding of CFTR expression in peritubular myoid cells suggests that they also aid in the movement of water from the circulation to the lumen of the tubules.

#### *Regulation of CFTR in Orchidectomized and Efferent Duct-Ligated Adult Rats*

In the present study, there was a dramatic alteration in the staining pattern of CFTR in the epididymis following orchidectomy at early and late time points, and this was also observed with testosterone treatment. The alteration was seen as the complete absence of the thick intense band of reaction product over the apical plasma membrane of the principal cells of the corpus and cauda epididymidis. However, under such conditions, numerous small CFTR-positive vesicles appeared in the apical and supranuclear cytoplasm of the principal cells, which were not prominent in control animals. It is suggested, therefore, that the synthesis of CFTR-positive secretory vesicles from the Golgi apparatus is ongoing and unaffected following orchidectomy but that these vesicles in the absence of testicular factors do not bind with the apical plasma membrane. The administration of testosterone to orchidectomized animals also failed to restore staining to the apical plasma membrane, excluding the possibility that androgens are responsible for the targeting and binding of CFTR-Golgi-derived vesicles with the apical plasma membrane. As efferent duct ligation demonstrated similar results at early and late time points, it is suggested that testicular factors are not essential for regulating the synthesis of CFTR and its presence in Golgi-derived secretory vesicles. However, it would appear that luminal factors emanating from the testis are essential for the targeting and binding of Golgi-derived CFTR-secretory vesicles with the apical cell surface of the principal cells.

In contrast to the principal cells, the clear cells of the corpus and cauda epididymidis did not exhibit changes to CFTR expression after any experimental treatment or at early or late time points. In addition, the layers of the peritubular myoid cells, which envelop each epididymal tubule (Robaire and Hermo, 1988), maintained their intense reactivity. Thus, regulation of CFTR in the epididymis appears to be cell-specific.

Although it is well established that many epididymal functions are under the control of androgens (Robaire and Hermo, 1988; Orgebin-Crist, 1996; Cornwall et al, 2001; Ezer and Robaire, 2002), several proteins have been

shown to be unaffected in their protein or mRNA expression by androgen withdrawal (Hermo et al, 2000; Luedtke et al, 2000; Cornwall et al, 2002; Hermo and Andonian, 2003). However, in addition to the regulation mediated by androgens, factors emanating from the testis that enter the epididymis via the lumen of the duct, defined as lumicrine factors, play a role in regulating epididymal functions (Hinton et al, 1998; Cornwall et al, 2002; Ezer and Robaire, 2002). Lumicrine factors derived from the testis have been shown to regulate several proteins synthesized by the epididymis. Indeed, ligation of the efferent ducts induces changes in epididymal gene and protein expression (Cornwall et al, 2002). The luminal testicular factors that regulate gene expression in the initial segment include ions, solutes, proteins, steroids, and even the spermatozoa themselves. These factors can up-regulate or down-regulate gene expression after efferent duct ligation (Brooks, 1983). In fact, spermatozoa have been suggested to regulate expression of proencephalin in the rat initial segment (Garrett et al, 1991). While orchidectomy and efferent duct ligation prevent the entry of spermatozoa into the epididymis, they continue to be present in the corpus and cauda epididymidis even at the later experimental time points. This suggests that spermatozoa are not the lumicrine factor involved in regulating CFTR expression; however, further experimentation would be needed to confirm this point. In addition, while the same cell type along the epididymal duct may express a given protein, different factors appear to regulate its expression in different epididymal regions (Cyr et al, 1992; Andonian and Hermo, 2003). In the present study, regulation of CFTR adds an additional twist. While its synthesis by principal cells is not regulated by testicular factors, it appears that its targeting and binding with the apical plasma membrane, where it is normally found, are regulated by a lumicrine testicular factor, the identity of which remains to be determined.

An alternative possibility to the targeting and binding of *de novo* Golgi-derived CFTR secretory vesicles with the apical membrane may be the inhibition of CFTR vesicles recycling from endosomes to the apical plasma membrane. However, the recycling of apical membrane receptors normally occurs via tubular structures emanating from endosomes, referred to as the CURL, located at the apex of the cell (Geuze et al, 1983). In the present study, numerous CFTR-positive vesicles, not tubules, were evident, and these were also prominent in the supranuclear region of the principal cells where recycling does not normally occur.

It is of interest to compare the expression and regulation of CFTR with AQP-9. Principal cells express AQP-9 in the initial segment and cauda regions, while CFTR is expressed in the corpus and cauda regions. Clear cells express AQP-9 in the cauda region, while CFTR is ex-

pressed in the corpus and cauda regions. Thus, region-specific differences exist for these 2 proteins. AQP-9 expression in principal cells of the initial segment and clear cells of the cauda region was substantially reduced after efferent duct ligation and orchidectomy. As its expression was not restored to control levels by testosterone replacement, it was concluded that AQP-9 expression was regulated by a lumicrine testicular factor (Badran and Hermo, 2002). However, in the case of AQP-9 regulation, no accumulation of AQP-9-reactive vesicles was observed in the cytoplasm. Furthermore, no changes to the expression of AQP-9 were noted in the case of the principal cells of the caput, corpus, or cauda regions after either treatment (Badran and Hermo, 2002). Thus, differences clearly exist in the regulation of CFTR and AQP-9 expression in the principal and clear cells of the epididymis.

It has been documented that multiple regulatory pathways control the secretion of electrolytes and water transport across the epididymal epithelium, such as neurotransmitters, prostaglandins, bradykinins, and other peptide hormones (Wong et al, 1999). Prostaglandins (PGE<sub>2</sub>) synthesized in basal cells diffuse out and act on prostaglandin receptors on the basolateral membrane of the principal cells. This causes an increase in intracellular cAMP through receptor-G protein-coupled adenylate cyclase, which then activates CFTR, resulting in the secretion of anions and water (Wong et al, 2002). The present study further suggests that in vivo, a lumicrine factor derived from the testis regulates the targeting and binding of CFTR-Golgi-derived vesicles with the apical plasma membrane and that, in its absence, such vesicles are maintained and accumulate in the cytoplasm. The nature of this regulatory factor (or factors) needs to be examined in future studies.

In summary, the present data show that, in the epididymis, CFTR is distributed in a cell- and region-specific manner and that, although its synthesis is not under the control of testicular factors, its targeting to the apical plasma membrane of the principal cells is regulated by a testicular luminal factor.

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