

# Immunolocalization of the Yb<sub>1</sub> Subunit of Glutathione S-transferase in the Adult Rat Epididymis Following Orchidectomy and Efferent Duct Ligation

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**ABSTRACT:** In addition to the maturation of sperm, the epididymis also serves to protect sperm from harmful reactive oxygen species. To this end, various antioxidant enzymes are produced by the epididymis, such as glutathione S-transferases (GSTs), a family of dimeric proteins that catalyze the conjugation of glutathione to various electrophilic compounds, thus providing cellular detoxification. In the present study, the regulation of the Yb<sub>1</sub> subunit of GST was examined in Bouin-fixed epididymides of adult control, orchidectomized (O) rats with or without testosterone (T) supplementation and efferent duct-ligated (EDL) rats using light microscope immunocytochemistry with an anti-Yb<sub>1</sub>-GST antibody. The intensely reactive ciliated cells of the efferent ducts and principal cells of the epididymis showing a checkerboard staining pattern were unaltered in their expression of Yb<sub>1</sub>-GST after all experimental procedures, suggesting their regulation by factors other than of testicular origin. On the other hand, the intense reaction of narrow/apical cells and moderate reaction of basal cells of the proximal initial segment of control animals

became negligible in O rats and was not restored with T supplementation. As staining was also absent after EDL, the data suggest that a luminal testicular factor(s), other than androgens, regulates expression of Yb<sub>1</sub>-GST in narrow/apical and basal cells of the proximal initial segment. Although basal cells of the caput and cauda epididymidis were unreactive after all experimental protocols, as also noted in controls, the intensely reactive basal cells of the corpus epididymidis of control animals became unreactive in O animals. However, Yb<sub>1</sub>-GST expression was restored to these cells with T supplementation, and as there was no effect on Yb<sub>1</sub>-GST expression after EDL, the data suggest that circulating testosterone or one of its metabolites regulates expression of Yb<sub>1</sub>-GST in basal cells of the corpus region. Taken together, these data indicate a differential regulation with respect to the expression of Yb<sub>1</sub>-GST in the various cell types and regions of the epididymis.

Key words: Light microscopy, immunocytochemistry.

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The epididymis, in addition to its function in the maturation of sperm, plays an important role in protecting them from harmful substances while they traverse and are stored in this duct (Robaire and Hermo, 1988; Hinton et al, 1995; Aitken, 2002). In many species, the low temperature of the epididymis, especially the cauda region, facilitates sperm storage by enhancing oxygen availability to cells (Djakiew and Cardullo, 1986). However, oxygen implies the increased presence of reactive oxygen species, which are implicated in inducing oxidative damage to DNA, proteins, and lipids. Sperm are particularly susceptible to reactive oxygen species, as they contain high levels of polyunsaturated phospholipids in their membranes. As a result, sperm must be protected as these reactive oxygen species can result in lipid peroxidation of their membranes, membrane fragility, and impaired fertility (Jones and Mann, 1977; Alvarez and Storey, 1989). Thus it has been noted that the epididymis contains various

antioxidant molecules, such as glutathione, that prevent oxygen radical injury, in addition to the presence of numerous antioxidant enzymes such as gamma glutamyl transpeptidase, glutathione peroxidase, superoxide dismutase, and glutathione S-transferase (GST; Hinton et al, 1995; Aitken, 2002; Cornwall et al, 2002; Hermo and Robaire, 2002).

GSTs are a family of soluble isozymes involved in cellular detoxification. They prevent the build up of potentially toxic substances by catalyzing the conjugation of reduced glutathione with various electrophilic substances, thereby protecting cellular components such as DNA, proteins, and lipids. The electrophilic substances can be of cellular origin, such as free radicals formed during normal metabolism or exogenous chemical compounds (Daniel, 1993). In addition to their main function in detoxification, they also play an important role in steroid isomerization (Benson et al, 1977); glutathione peroxidation (Prohaska and Ganther, 1976); leukotriene C biosynthesis (Pemble et al, 1986); and binding noncovalently to a number of nonsubstrate ligands, including steroids (Homma et al, 1986).

Cytosolic, microsomal, and nuclear forms of GSTs have been identified (Hayes and Strange, 2000). They are

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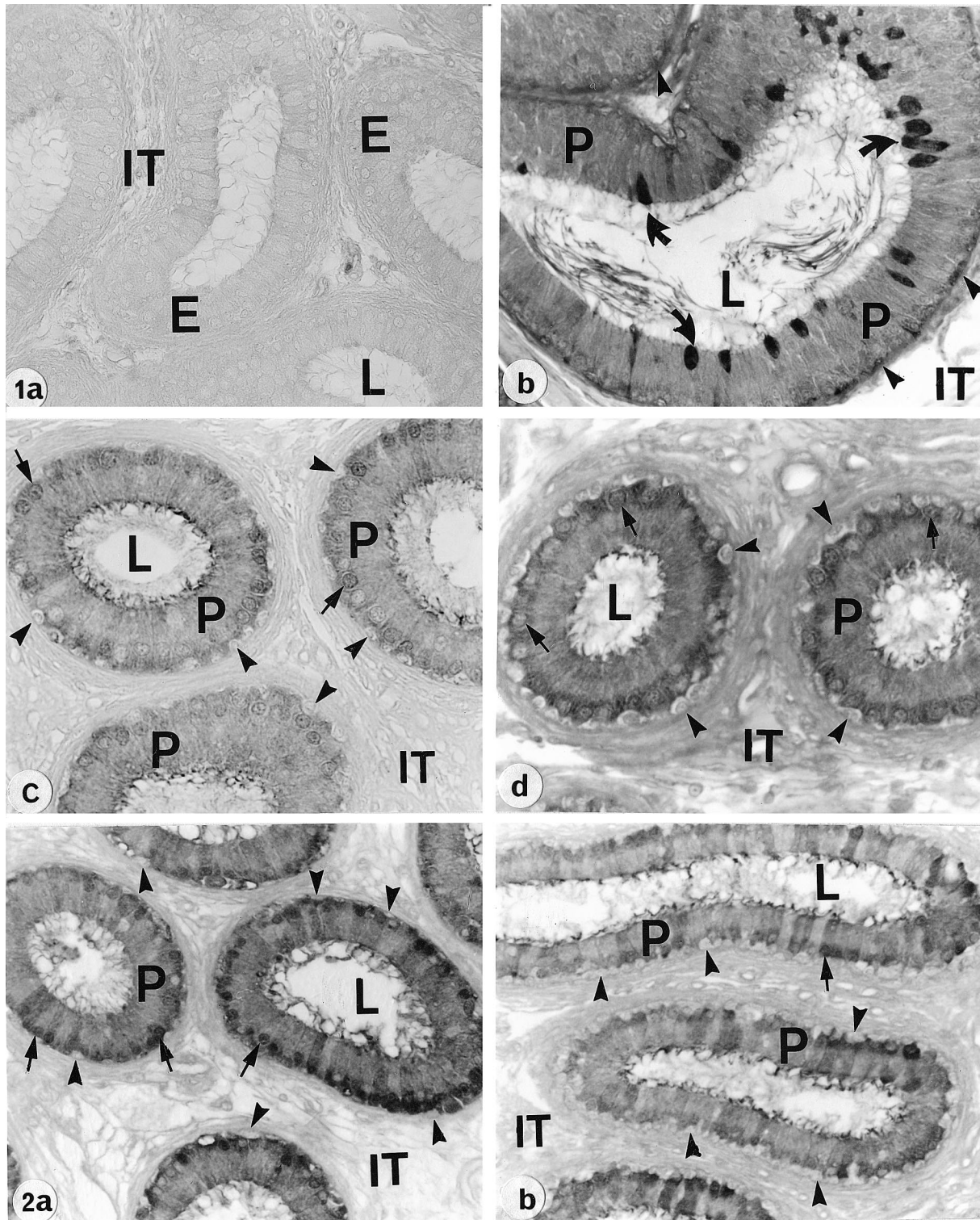


Figure 1. (a) Distal initial segment (DIS) of a 14-day orchidectomized rat treated with normal rabbit serum; (b) proximal initial segment (PIS) of control; (c) 14-day orchidectomized rats; and (d) 14-day efferent duct-ligated rats immunostained with an anti-Yb<sub>1</sub>-GST antibody. In (a), there is an absence of reaction product over the epithelium (E), luminal contents (L), and intertubular space (IT). In (b), the cytoplasm of principal cells (P) of the PIS shows an absence of reactivity, whereas narrow/apical cells (curved arrows) are intensely reactive. Basal cells (arrowheads) show moderate to intense reactivity. In (c), basal cells (arrowheads) and narrow/apical cells become unreactive, whereas principal (P) cells remain unreactive. In (d), principal cells (P) display a comparable cytoplasmic reaction to controls, but basal (arrowheads) and narrow/apical cells, reactive in control animals, continue to be unreactive following efferent duct ligation. Arrows indicate nucleus of principal cells; IT, intertubular space; L, lumen. Magnification 375 $\times$  each.

dimeric proteins from a family of 16 genes grouped into five classes ( $\alpha$ ,  $\mu$ ,  $\pi$ ,  $\theta$ , and  $\sigma$ ) according to the amino acid homology of their subunits (Mannervik and Danielson, 1988; Buetler and Eaton, 1992; Daniel, 1993). GSTs form homo- or heterodimers from subunits that are members of 1 gene family but not between gene families (Mantle et al, 1990). GSTs have similar molecular weights but differ with respect to isoelectric point and substrate specificities (Jakoby et al, 1976). The vast number of different GST subunits implies their importance in the protection of cells against a wide variety of potentially harmful substances that they can encounter.

In previous studies, Hales et al (1980) had shown that the epididymis–vas deferens contains high GST activity, approximately 50% of that found in the liver on a protein weight basis, and that there are a large number of different GSTs in the epididymis–vas deferens, which are differentially localized along the length of this tissue. Recently, we examined with light microscopic immunocytochemistry the distribution of different GST subunits along the epididymis and vas deferens of adult rats. Our studies revealed that the expression of the different GSTs in the epididymis and vas deferens was often cell type– and region-specific (Veri et al, 1993, 1994; Papp et al, 1995; Andonian and Hermo, 1999). The absence of reactivity in 1 cell type of a given region was usually compensated for by the reactivity in another cell type of that region. The varied GST expression in the different cell types was thought to ensure that sperm would be protected from a wide variety of blood-borne electrophiles as they traversed the epididymis and during storage in the cauda region and vas deferens (Papp et al, 1995; Andonian and Hermo, 1999).

Although the activity of GSTs in the epididymis has been shown to be androgen-dependent and at times region-specific (Hales et al, 1980; Robaire and Hales, 1982), the regulation of expression of the different GST subunits has not been examined in a cell-type and region-specific manner, with only one exception. Indeed, in the epididymis, although basal cells were shown to be unaffected in their expression of Yf-GST by the absence of testicular and pituitary factors, the expression of Yf-GST by principal cells was shown to be dependent on testosterone (Herme and Papp, 1996). However, little is known about the regulation of other GST subunits according to the different cell types and regions of the epididymis.

The purpose of the present study was, therefore, to ex-

amine the regulation of the Yb<sub>1</sub>-GST subunit in the epididymis of adult rats following orchidectomy with or without testosterone supplementation and efferent duct ligation using light microscope immunocytochemistry in conjunction with an anti-Yb<sub>1</sub>-GST antibody. The experimental procedures were designed to evaluate the role of androgens on Yb<sub>1</sub>-GST expression, well known regulators of many epididymal functions (Cornwall et al, 2002), as well as luminal testicular factors, other than androgens, which enter the epididymis via the lumen of the seminiferous tubules of the testis and efferent ducts and which have recently been defined as lumicrine factors (Hinton et al, 1998). The data from experimentally treated animals were qualitatively compared with those obtained for control adult rats based on numerous images generated from the 4 animals of each treatment group.

## Materials and Methods

### Animals and Experimental Protocols

Adult male Sprague Dawley rats (350 to 450 g, 3 to 4 months of age) were obtained from Charles River Laboratory Ltd (St. Constant, Quebec, Canada) and were subdivided into 5 groups. The first group consisted of 4 adult control animals. Bilateral ligation of the efferent ducts constituted the second group. After an intraperitoneal injection of sodium pentobarbital (Sommitol, MTC Pharmaceuticals, Hamilton, Ontario, 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 polydimethylsiloxane (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 placed subcutaneously immediately after orchidectomy. The rats (4 per interval) were sacrificed at 3, 7,

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Figure 2. (a) Distal initial segment (DIS) and (b) caput epididymidis of 14-day orchidectomized rats immunostained with anti-Yb<sub>1</sub>-GST antibody. In (a), principal cells (P) show a weak checkerboard-staining pattern of their cytoplasm, whereas their nucleus (arrows) is intensely reactive; basal cells (arrowheads) are unreactive. There is no evidence of staining of narrow/apical cells. The reaction is similar to that seen for control animals. In (b), principal cells show a strong checkerboard-staining pattern of their cytoplasm and nucleus (arrows), whereas basal cells (arrowheads) are unreactive, a situation comparable with that seen for control animals. IT indicates intertubular space; L, lumen. Magnification 375 $\times$  each.

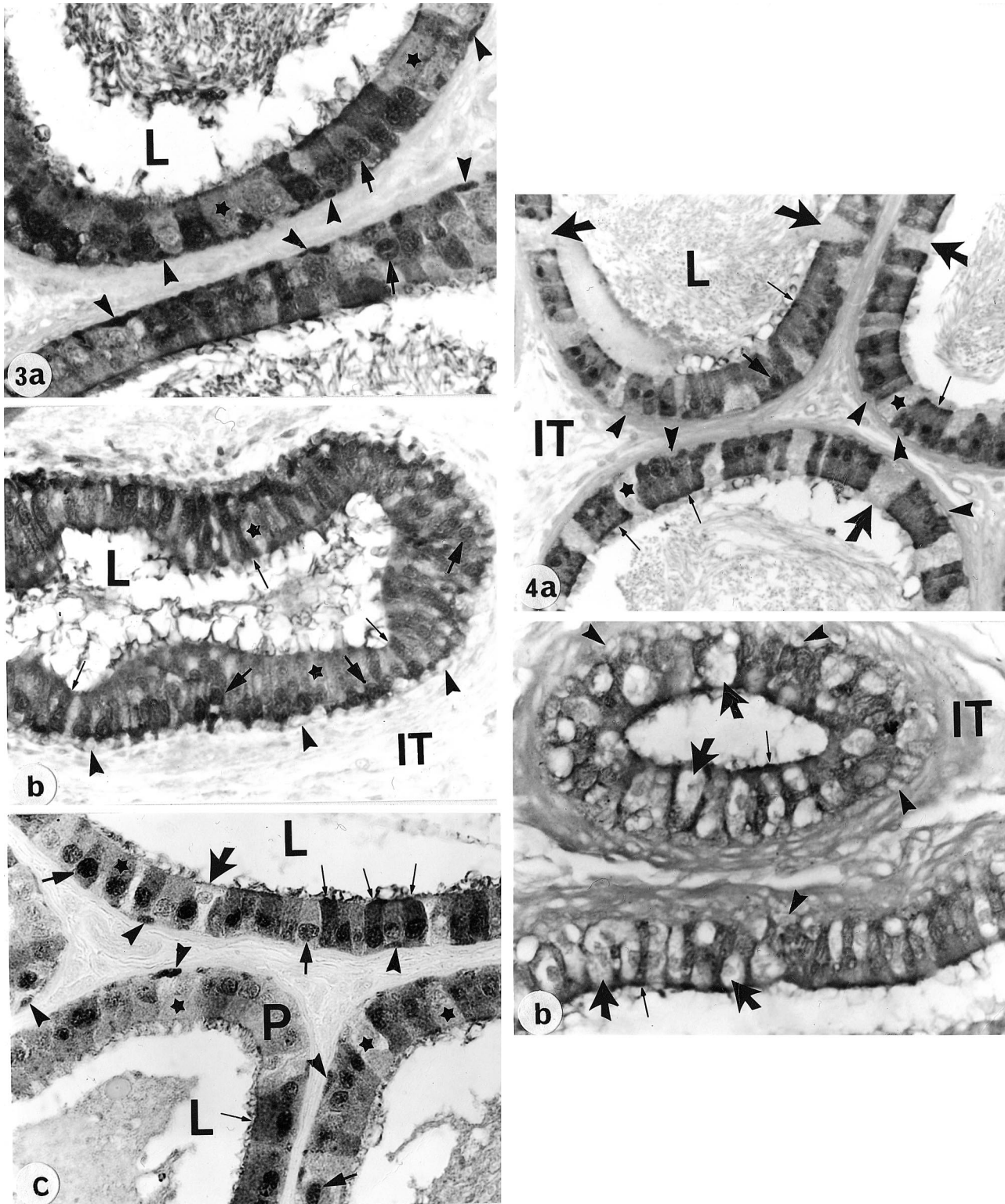


Figure 3. Corpus epididymidis of control (a), 14-day orchidectomized rats (b), and 14-day orchidectomized rats supplemented with testosterone (c) immunostained with an anti-Yb<sub>1</sub>-GST antibody. Principal cells present a checkerboard-staining pattern of their cytoplasm ranging from intense reactivity (thin arrows) to weak or no reactivity (stars); their nuclei (arrows) are at times more reactive than their cytoplasm. No differences in the staining pattern of principal cells are apparent between control (a) and experimentally treated rats (b, c) rats. However, although basal cells (arrowheads) of the corpus region of control animals are intensely reactive (a), they become unreactive in orchidectomized animals (b), but regain their reactivity following testosterone administration (c) comparable with that noted for control animals. This indicates their dependence on testosterone or one of its metabolites for Yb<sub>1</sub>-GST expression. Clear cells (slanted arrows) are consistently unreactive. L indicates lumen; IT, intertubular space. Magnification 375× each.

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

**Light Microscope Immunostaining**—Sections 5  $\mu$ m thick were cut and mounted on glass slides. They were then deparaffinized with xylene and hydrated in graded concentrations of ethanol (from 100% to 50%). During hydration, immersing the tissues in 70% ethanol containing 1% lithium carbonate for 5 minutes neutralized residual picric acid. In order to inactivate any endogenous peroxidase activity, the tissue sections were incubated for 5 minutes in 70% ethanol containing 1% (vol/vol) hydrogen peroxide. Following hydration, the sections were incubated (5 minutes) in a 300 mM glycine solution in order to block free aldehyde groups. The tissue was then blocked with 40  $\mu$ L of 10% goat serum, diluted in TBS (20 mM Tris-HCl saline containing 0.1% bovine serum albumin) at pH 7.4, for 25 minutes at room temperature. The slides were then washed with Tween buffer solution (TBS with 0.1% Tween-20; TWBS). A dilution factor of 1:100 in TBS was used for the affinity-purified polyclonal anti-Yb<sub>1</sub> antibody, which was kindly provided by Dr J. Hayes (University of Edinburgh, Scotland). Its purification and specificity are described in Hayes and Mantle (1986), and the anti-Yb<sub>1</sub>-GST antibody has already been used in one of our previous studies on control animals (Papp et al, 1995).

Each tissue section was incubated in the primary antibody for 1.5 hours. After incubation, the sections were immersed in 4 consecutive wells of TWBS for 2 minutes each. The sections were then blocked with 40  $\mu$ L of 10% goat serum and subsequently incubated with goat anti-rabbit IgG conjugated to peroxidase (Sigma, St. Louis, MO) at a dilution of 1:250 in TBS and incubated for 30 minutes at 35°C in a humidified incubator. After incubation with a secondary antibody, the tissue was washed by immersion in 4 wells of TWBS for 2 minutes each.

The final reaction product was obtained by incubating the slides for 10 minutes in 250 mL of TBS containing 0.03% hydrogen peroxide, 0.1 M imidazole, and 0.05% diaminobenzidine tetrahydrochloride (DAB), pH 7.4. The sections were counterstained with 0.1% methylene blue (2 minutes) and then dehydrated in a graded series of ethanol solutions (30 seconds each) and xylene (3 minutes). Cover slips were mounted onto glass slides using Permount.

Incubation of epididymal tissues with normal rabbit serum at a dilution of 1:100 in TBS and in secondary antibody alone, without primary antibody, served as negative controls.

## Results

### Effects of Orchidectomy and Efferent Duct Ligation on Expression of Yb<sub>1</sub>-GST

In control and all experimental animals, immunostaining with normal rabbit serum or omitting the primary antibody revealed a complete absence of reaction over the entire epididymal epithelium, luminal contents, or intertubular space (Figure 1a). At the different time points after orchidectomy with or without testosterone supplementation or efferent duct ligation, intense staining of ciliated epithelial cells of the efferent ducts was noted when immunostained with an anti-Yb<sub>1</sub>-GST antibody (not shown), a situation comparable with that seen in control animals. In the proximal initial segment of control animals, the most reactive epithelial cell types, immunostained with an anti-Yb<sub>1</sub>-GST antibody, were the narrow/apical cells followed by the basal cells showing a moderate reaction and principal cells without any apparent reaction (Figure 1b). At the different time points after orchidectomy, both the narrow/apical cells and basal cells lost their reactivity, whereas principal cells continued to be unreactive as in control animals (Figure 1c). The administration of testosterone to orchidectomized animals did not restore Yb<sub>1</sub>-GST expression to the narrow/apical or basal cells of the proximal initial segment (not shown), and these cells continued to be unreactive at all time points after efferent duct ligation (Figure 1d). These data suggest that a luminal testicular factor, other than androgens, regulates the expression of Yb<sub>1</sub>-GST in narrow/apical and basal cells of the proximal initial segment.

Principal cells of the distal initial segment in control animals revealed a weak checkerboard staining pattern of their cytoplasm, whereas their nuclei were intensely re-

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Figure 4. Cauda epididymidis of control (a) and 14-day orchidectomized rats (b) immunostained with an anti-Yb<sub>1</sub>-GST antibody. Principal cells present a checkerboard-staining pattern of their cytoplasm ranging from intense reactivity (thin arrows) to weak or no reactivity (stars). In the cauda region, basal cells (arrowheads) are unreactive in (a) and continue to be so in (b). Clear cells (slanted arrows), identified by their larger size and absence of brush border, are especially evident in the cauda region (a, b) and are consistently unreactive in control (a) and experimentally treated (b) animals. L indicates lumen; IT, intertubular space. Magnification 375 $\times$  for each.

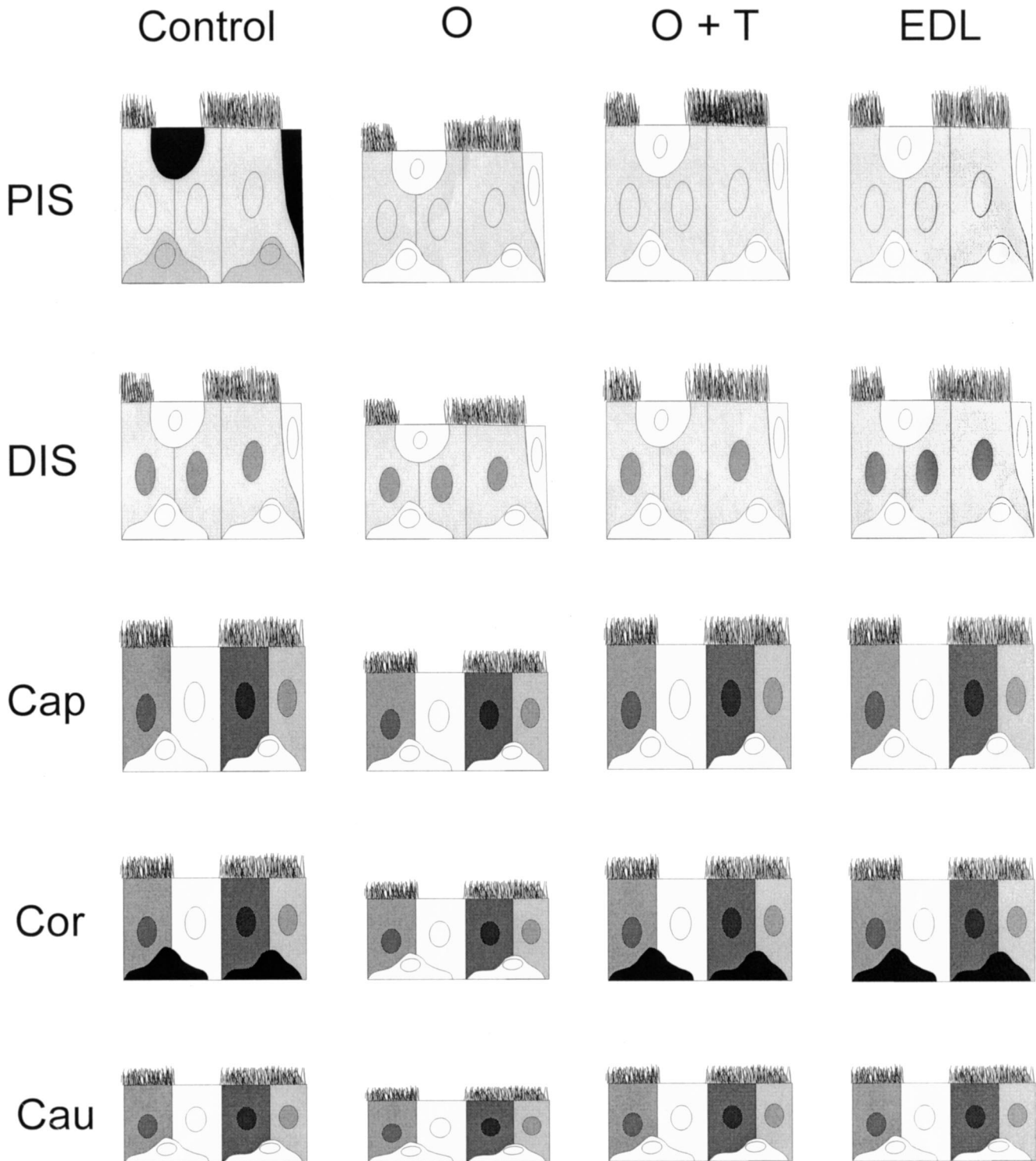


Figure 5. Diagrammatic representation of the staining pattern for  $Yb_1$ -GST in the proximal initial segment (PIS), distal initial segment/intermediate zone (DIS), caput (Cap), corpus (Cor) and cauda (Cau) epididymidis of control animals, orchidectomized animals without (O) or with testosterone (O+T) supplementation, and efferent duct-ligated (EDL) animals. Principal cells are represented as columnar cells with a microvillar brush border and are present in all epididymal regions. Narrow cells, found in the PIS and DIS, are represented as thin attenuated cells at the right margin of each epithelial section, whereas apical cells also present in these regions are shown as cup-shaped cells, which do not reach the basement membrane. Clear cells, present only in the Cap, Cor, and Cau epididymidis, are represented as columnar cells without microvilli. Basal cells, seen as hemispherical cells, do not contact the lumen and are noted in all epididymal regions. After all experimental treatments at the different time points, principal cells maintain their staining pattern comparable with that seen in control animals. Although unreactive in the PIS, their nuclei are intensely reactive in the

active; there was no staining of basal or narrow/apical cells. After each of the different experimental treatments at the various time points examined, no change in the staining pattern of Yb<sub>1</sub>-GST, as compared with control animals, was observed for any epithelial cell type of the distal initial segment (Figure 2a). In the caput epididymidis of control animals immunostained with anti-Yb<sub>1</sub>-GST antibody, principal cells also revealed a checkerboard-staining pattern showing a variable cytoplasmic reaction ranging from intense to moderate to weak reactivity to a complete absence of reaction; their nuclei were at times more reactive than the cytoplasm; basal and clear cells of the caput region were unreactive in control animals. No change was noted in the expression of Yb<sub>1</sub>-GST in any cell type after each experimental treatment at the different time points (Figure 2b).

In the corpus (Figure 3a) and cauda (Figure 4a) epididymidis of control animals immunostained with anti-Yb<sub>1</sub>-GST antibody, principal cells maintained a checkerboard staining pattern, with their nuclei at times being more reactive than their cytoplasm. Although basal cells of the corpus epididymidis were intensely reactive (Figure 3a), these cells were unreactive in the cauda (Figure 4a) region. Clear cells of all regions were consistently unreactive (Figures 3a, 4a).

At all time points after orchidectomy, no change in the staining pattern for Yb<sub>1</sub>-GST expression was noted for principal and clear cells of the caput (Figure 2b), corpus (Figure 3b), and cauda (Figure 4b) regions. However, basal cells displayed a region-specific difference after orchidectomy. Although these cells continued to be unreactive in the caput (Figure 2b) and cauda (Figure 4b) regions, they became unreactive in the corpus epididymidis at all time points after orchidectomy (Figure 3b). In the case of basal cells of the corpus epididymidis, these cells became intensely reactive in orchidectomized rats supplemented with testosterone (Figure 3c), and they were seen to be intensely reactive at all time points after efferent duct ligation (not shown). These data suggest that Yb<sub>1</sub>-GST expression in basal cells of the corpus region is regulated by testosterone or one of its metabolites.

## Discussion

Principal cells are the major epithelial cell type lining the epididymis, which has been shown to be active in secre-

tion and endocytosis of various substances, among other functions (Cooper, 1986; Robaire and Hermo, 1988; Hermo et al, 1994). In the present study, in the case of Yb<sub>1</sub>-GST expression, principal cells of control animals showed no reaction in the proximal initial segment and a checkerboard-staining pattern in all other epididymal regions. The latter is a term reflecting the fact that although some cells showed high levels of Yb<sub>1</sub>-GST immunoreactivity, others showed moderate, little, or no reactivity. This suggests that some principal cells are synthesizing Yb<sub>1</sub>-GST, whereas others are not, and/or that these cells are out of synchrony with respect to their synthesis of Yb<sub>1</sub>-GST at any given moment of time. Such a staining pattern has been already recognized for many other proteins, including secretory and lysosomal (Rankin et al, 1992; Hermo et al, 1994; Igdoura et al, 1995). Nevertheless, no noticeable differences in Yb<sub>1</sub>-GST expression were observed in principal cells of any epididymal region after orchidectomy with or without testosterone administration at the different time points examined (Figure 5, Table). The staining pattern and intensities were comparable in both control and experimentally treated animals. Thus Yb<sub>1</sub>-GST expression in principal cells does not appear to be regulated by androgens.

This data contrasts with data obtained for Yf-GST expression, where principal cells noted to be reactive for Yf GST in control animals became unreactive after orchidectomy. The administration of testosterone to these animals restored Yf-GST expression, indicating that androgens were responsible for regulating Yf-GST expression in principal cells (Herms and Papp, 1996). Clearly differences in the regulation of the various isoforms of GST appear to occur in the case of principal cells. 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, 2002; Ezer and Robaire, 2002), several proteins have been shown to be unaffected by androgen withdrawal in a cell-specific manner, such as SGP-1, cystatin c, SGP-2, and cathepsins A and D (Herms et al, 2000b; Luedtke et al, 2000; Cornwall et al, 2002; Herms and Andonian, in press). This also appears to be the case for Yb<sub>1</sub>-GST expression by principal cells.

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

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DIS. They show a prominent checkerboard-staining pattern of their cytoplasm and nucleus only in the Cap, Cor, and Cau regions. Narrow/apical cells and basal cells, reactive in control animals in the PIS region, become unreactive after all experimental treatments at the different time points, suggesting that they are regulated by testicular lumicrine factors. In the Cap and Cau regions in control animals, basal cells are unreactive and remain so after all experimental treatments. However, in the Cor region, basal cells are intensely reactive in control and EDL animals, become unreactive after O, but regain their intense reactivity after T administration. This suggests that Yb<sub>1</sub>-GST expression in the case of basal cells of the Cor is regulated by testosterone or 1 of its metabolites. Clear cells are consistently unreactive in control and all experimental animals.

*Effects of orchidectomy with or without testosterone supplementation and efferent duct ligation on Yb<sub>1</sub>-GST expression in the adult rat epididymis\**

Groups	Cell Types	PIS†	DIS/IZ	Caput	Corpus	Cauda
Control	Principal	-‡	CB   (N)§	CB (N)	CB (N)	CB (N)
	Narrow/apical¶	+++	-	N/A	N/A	N/A
	Basal	++	-	-	+++ (N)	-
Orchidectomy	Principal	-	CB (N)	CB (N)	CB (N)	CB (N)
	Narrow/apical	-	-	N/A	N/A	N/A
	Basal	-	-	-	-	-
Orchidectomy + testosterone supplementation	Principal	-	CB (N)	CB (N)	CB (N)	CB (N)
	Narrow/apical	-	-	N/A	N/A	N/A
	Basal	-	-	-	+++ (N)	-
Efferent duct ligation	Principal	-	CB (N)	CB (N)	CB (N)	CB (N)
	Narrow/apical	-	-	N/A	N/A	N/A
	Basal	-	-	-	+++ (N)	-

\* The data were similar at all time points after the different experimental treatments.

† PIS indicates proximal initial segment; DIS, distal initial segment; IZ, intermediate zone; Caput, Corpus, and Cauda, regions of the epididymis.

‡ The number of plus signs is directly proportional to the relative intensity of the immunoperoxidase reaction over the cytoplasm, with + representing a weak, ++ a moderate, and +++ an intense reaction, whereas - denotes the absence of a reaction.

§ N indicates a reaction also exists over the nucleus.

|| CB or checkerboard-staining pattern refers to the fact that principal cells of the caput, corpus, and cauda regions display a variable cytoplasmic and nuclear reaction ranging from intense to moderate to weak, and even the absence of reaction. Although qualitative in nature, these data give an overall impression of the status of reactivity based on consistent findings from at least 4 slides for each animal of each experimental group in the case of the different cell types of each epididymal region.

¶ Narrow/apical cells are only present in the PIS, DIS/IZ; N/A, not applicable, indicates that these cells are not found in the caput, corpus, and cauda epididymidis.

lumicrine factors, also play a role in regulating epididymal functions (Hinton et al, 1998; Cornwall et al, 2002). In the present study, efferent duct ligation also had no effect on Yb<sub>1</sub>-GST expression in principal cells, eliminating a role for lumicrine factors on its expression (Figure 5, Table), and this was also observed to be the case for Yf-GST (Herms and Papp, 1996) and several other proteins expressed by the epididymis (Ezer and Robaire, 2002).

In the present study, the anti-Yb<sub>1</sub> antibody often showed a nuclear reaction in principal cells that was more intense than the reaction seen in the cytoplasm (Figure 5, Table), and this was noted throughout most of the epididymis. It has been demonstrated that Yb<sub>1</sub> proteins exist in a soluble form in both the nucleus and cytoplasm; however, they also exist in a bound form in the nucleus, where they bind to DNA (Bennett et al, 1986; Hayes and Mantle, 1986; Ketterer et al, 1990). It has also been shown that Yb<sub>1</sub> in carcinoma cells migrates into the nucleus, providing further evidence for their strong nuclear affinity (Bennett et al, 1986; Bennett and Yeoman, 1987). In the epididymis, the Yb<sub>1</sub> protein may protect DNA and RNA from harmful electrophiles that may be encountered by principal cells. In the present study, at no time was the expression of Yb<sub>1</sub>-GST noted to be different in experimentally treated animals as compared with controls, suggesting that testic-

ular factors did not regulate Yb<sub>1</sub>-GST expression in the nucleus of principal cells.

Narrow/apical cells are found only in the initial segment and intermediate zone of the epididymis (Adamali and Herms, 1996). Aside from a function in endocytosis, these cells also express GSTs in a region-specific manner (Papp et al, 1995; Herms et al, 2000a). Apical cells express Yf-GST in the initial segment and intermediate zone, whereas narrow cells do not. In the proximal initial segment only, both apical and narrow cells express Yo GST (Adamali and Herms, 1996). In the present study, Yb<sub>1</sub>-GST was expressed in narrow/apical cells of the proximal initial segment only. We did not separate these cells from each other, as both the thin narrow cells as well as the cup-shaped apical cells, which did not reach the basement membrane, were reactive (Adamali and Herms, 1996). Although little is known about the regulation of the functions of these cells, the present data indicate that Yb<sub>1</sub>-GST expression is not regulated by androgens. Indeed, expression was lost after orchidectomy and not restored following testosterone supplementation. However, since a lack of expression was also noted after efferent duct ligation (Figure 5, Table), it was concluded that regulation of Yb<sub>1</sub>-GST in narrow/apical cells of the proximal initial segment was via a lumicrine factor. 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 may regulate gene expression in the initial segment include ions, solutes, proteins, steroids, and even germ cells. These factors can up- or down-regulate gene expression after efferent duct ligation (Brooks, 1983). Expression of cystatin-related lipocalin and proenkephalin are among the proteins that have already been shown to be regulated by lumicrine factors (Garrett et al, 1991; Lareyre et al, 2001; Cornwall et al, 2002), and to this list may be now added Yb<sub>1</sub>-GST expression by narrow/apical cells of the proximal initial segment.

Basal cells are small hemispherical cells that reside on the basement membrane and do not reach the lumen of the duct. They also send thin, footlike processes along the basement membrane that collectively encompass a large portion of the circumference of each tubule (Veri et al, 1993). In this way they form a barrier, albeit an incomplete one, between the blood vessels and other contents of the intertubular space and the epididymal lumen. They can, therefore, to a degree effectively eliminate potentially harmful substances emanating from the blood that are trying to access the sperm in the lumen. In this context, basal cells express various isoforms of GSTs, such as the Yf, Ya, Yb<sub>2</sub>, and Yc subunits (Papp et al, 1995), as well as superoxide dismutase (Nonogaki et al, 1992).

Basal cells of control animals express Yb<sub>1</sub>-GST, but only in the proximal initial segment and corpus regions (Figure 5, Table). After orchidectomy and efferent duct ligation, these cells showed a differential response to androgens and lumicrine factors, dependent on their location along the duct. In the proximal initial segment, Yb<sub>1</sub>-GST expression was not restored to orchidectomized animals supplemented with testosterone, and expression was also absent after efferent duct ligation (Figure 5, Table). However, in the corpus region, expression was restored to control levels in orchidectomized animals that received testosterone, and there was no effect on expression in efferent duct-ligated animals (Figure 5, Table). Taken together, the data suggest that in the corpus region, Yb<sub>1</sub>-GST expression in basal cells is regulated by testosterone or one of its metabolites, but in the proximal initial segment, expression is regulated by a lumicrine factor. These data differ dramatically from that obtained for the Yf-GST subunit, where its expression in basal cells was unaltered after orchidectomy and efferent duct ligation, as well as hypophysectomy, indicating that neither testicular nor pituitary factors governed Yf-GST expression in basal cells (Herme and Papp, 1996). Thus basal cells of different regions show differential responses to the absence of androgens or testicular lumicrine factors in their expression of a given GST as well as between different GSTs. Very little is known about what regulates basal cell functions,

as data on the functions of these cells is only slowly being gathered. However, the expression of metallothionein by basal cells, although detectable in all epididymal regions, was shown to be androgen-dependent according to specific regions (Cyr et al, 2001).

Aside from the family of GSTs, the expression of glutathione peroxidase at the message and protein levels has also been noted to be androgen-dependent (Vernet et al, 1997; Schwaab et al, 1998). At the mRNA level, gamma glutamyl-transpeptidases, which show multiple transcripts, are also differentially regulated by androgens and/or lumicrine factors in the different epididymal regions (Palladino and Hinton, 1994), as is their secretion and activity (Agrawal and Vanha-Perttula, 1988). Thus various antioxidant enzymes show a differential regional response in their expression to the presence or absence of testicular factors. However, their regulation has not as yet been determined on a cell-type and region-specific manner. In summary, the present study has revealed that the expression of Yb<sub>1</sub>-GST is regulated along the epididymis in a cell-type and region-specific manner.

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