Biosynthesis and distribution of Lewis X- and Lewis Y-containing glycoproteins in the murine male reproductive system

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Although Lewis X (LeX) and Lewis Y (LeY) antigens were thought to play important roles in fertility, fucosyltransferase (Fut)-deficient (Fut1, Fut2 and Fut4) mice which lack LeX or LeY antigen are still fertile. In the present study, the Fut-deficient and wild-type mice were used to measure the expression of Fut mRNA along the mouse male reproductive tract and determine the role of each Fut in the biosynthesis of LeX/LeY antigens, which are conjugated to glycoproteins in the male reproductive system. LeX/LeY-containing glycoproteins were detected in the epididymis, vas deferens, seminal vesicle and coagulating gland, but not in the testis. We demonstrate that the synthesis of LeY-containing glycoproteins in the epididymis and vas deferens is catalyzed by Fut1 and Fut4. In the seminal vesicle and the coagulating gland, they are mainly synthesized by Fut2 and an α-(1,3)-Fut, but not Fut4. The synthesis of LeX-containing glycoproteins in the middle caput epididymis is catalyzed by Fut4 and by Fut4 and Fut2 in the seminal vesicle. We provide evidence that LeX is synthesized in the coagulating gland by Fut9. We found that the lack of activity by one Fut does not completely inhibit LeX/LeY antigen expression in the male reproductive tract. This redundancy may help to explain why in vivo studies with Fut-deficient mice do not support the presumption that LeX/LeY antigens play important roles in male fertility. We provide details regarding the phenotypes of established Fut-deficient mice and lay the foundation for elucidating the functions of LeX/LeY antigens in other aspects of the male reproductive system.

Keywords: fucosyltransferase/glycoproteins/Lewis X/Lewis Y/male reproductive system

Introduction

Fucosylation is so important in fertility that both male and female mice that cannot de novo synthesize guanosine diphosphate-fucose are infertile, and fertility is rescued in such mice with dietary fucose supplementation that is employed in the salvage pathway (Smith et al. 2002). Lewis X and Y antigens (LeX and LeY, respectively), characterized by fucose modification, have been reported to be involved in many aspects of mammalian physiology, including tissue development, angiogenesis, fertilization, cell adhesion, inflammation and tumor metastasis (Ma et al. 2006). Also known as stage-specific embryonic antigen-1 (SSEA-1), LeX antigen plays a role in morula compaction (Liu et al. 1999). Meanwhile, LeY, a difucosylated oligosaccharide, plays an essential role at the initial stage of implantation (Zhu et al. 1995; Wang et al. 1998) and is thought to be developmentally regulated in mouse embryo and uterine epithelium (Liu et al. 1999).

The aforementioned in vitro experiments imply that LeX/LeY antigens are important in female fertility. Large quantities of LeX/LeY antigens are found in human semen (HS; Chalabi et al. 2002) and on the surface of sperm (Pang et al. 2007), which implies that they may also play important roles in sperm function; however, the origin and biological functions of LeX and LeY antigens in the mammalian male reproductive system are not well understood.

The general biosynthesis of LeX/LeY is summarized in Figure 1A (Sepp et al. 1997; Becker and Lowe 2003; Ma et al. 2006). LeX is produced by an α-(1,3) subterminal fucosylation on the N-acetylglucosamine residue of Galβ1-4GlcNAc-R. Two α-(1,3)-fucosyltransferases (Fut) are responsible for the synthesis of LeX in mouse: Fut4 is mainly localized in epididymis, stomach and colon; Fut9 is highly expressed in kidney and brain (Kudo et al. 1998). Fut9 is believed to catalyze LeX synthesis in brain (Nishihara et al. 2003; Brito et al. 2007), whereas Fut4 has been implicated in fucosylation of selectin ligands (Homeister et al. 2001). Fut11 is a novel α-(1,3)-Fut studied in human (Mollicone et al. 2009), which needs further...
LeY is formed by α-(1,2)-fucosylation of the galactose residue of Galβ1-4GlcNAc-R following an α-(1,3)-fucosylation on the N-acetylglucosamine residue. Fut1 and Fut2 are the two functional α-(1,2)-Fut in mouse. Fut1 is mainly localized in the pancreas, epididymis, uterus, stomach, thymus and colon (Domino et al. 1997), and Fut2 is expressed in the uterus, stomach and colon (Domino, Zhang, Lowe, 2001).

Several Fut gene knockout mice (Fut1−/−, Fut2−/−, Fut4−/− and Fut9−/−) have been generated to evaluate the functions of α-(1,2)- and α-(1,3)-fucosylation in mammals. Fut4−/− and Fut9−/− mice are characterized by the absence of Fut4- and Fut9-catalyzed LeX antigens in several specific tissues, yet the mice remain fertile and develop normally (Homeister et al. 2001; Kudo et al. 2004). Fut1−/− and Fut2−/− mice are deficient in α-(1,2)-fucosylation, and thus LeY antigen synthesized by Fut1 or Fut2 in the epididymis and uterus, respectively. They are also viable, healthy and fertile (Domino, Zhang, Gillespie et al. 2001). However, the fertile phenotype displayed by these Fut-deficient mice does not necessarily exclude the importance of LeX/LeY antigens in fertility that is implicated by the in vitro data aforementioned. It is unknown whether these modifications were inhibited in the male reproductive tracts of any of the Fut-deficient mice. It is possible that there is a functional compensation between the two α-(1,2)-Fut or between the two α-(1,3)-Fut on LeX/LeY biosynthesis.
LeX and LeY antigens in the male reproductive system

To test the hypothesis of functional compensation among LeX and LeY synthesis, we analyzed the expression profiles of LeX/LeY antigens and Fut in the male reproductive system of Fut-deficient and wild-type mice and elucidated the role of each Fut in the biosynthesis of LeX/LeY antigens in each specific region of the mouse male reproductive system. Our research may help to understand the phenotypes of Fut-deficient mice and reveal the function of LeX/LeY antigens in the male reproductive system.

Results

Comparison of expression profiles of LeX/LeY-containing glycoproteins and Fut in different parts of the male reproductive system to examine Fut-controlled LeX/LeY synthesis

The presence of LeX and LeY antigen-containing proteins in the mouse male reproductive tracts was determined by western blot. Proteins bearing the LeX or LeY antigen were not detected in the testis. However, robust expression of a protein signal among 250 kDa smear area was detected in the epididymis and also at trace levels in the vas deferens (Figures 1B and C and 2A and B). The bands between 72 and 95 kDa resulted from a nonspecific reaction with immunoglobulin (Ig) M; data not shown. The LeX-containing proteins in the seminal vesicle and coagulating gland smeared from 55 to >250 kDa (Figure 1C). LeY antigen-containing proteins in the seminal vesicle and coagulating gland showed a faint, major band at ~148 kDa (Figure 1B). Owing to the difficulty collecting ejaculated mouse semen, we analyzed LeX/LeY-containing proteins in HS. In HS, LeX-containing protein was indicated by a band >250 kDa (Figure 1C), whereas LeY-containing proteins smeared from 36 to >250 kDa (Figure 1B).

Real-time polymerase chain reaction (PCR) analysis of the mRNA levels of α-(1,2)- and α-(1,3)-Fut in the male reproductive system of wild-type mice (Figure 1E and F) yielded results largely consistent with previously reported microarray analysis data (Johnston et al. 2005; Figure 1H). The only exception was a slight difference in Fut9 expression, which may be the result of very low mRNA copy numbers.

The expression profiles of Fut and LeX/LeY-containing glycoproteins were compared to explore which specific Fut controls the synthesis of LeX/LeY-containing glycoproteins in each tissue of the male reproductive system (Figure 1G). Fut1 and Fut4 were highly expressed in the caput epididymis, which may be involved in the synthesis of LeX and LeY antigens in this segment. Fut2 was highly expressed in the seminal vesicle and the coagulating gland, which can synthesize the LeY antigen in these two tissues. Fut9 was highly expressed in the coagulating gland, which can synthesize both LeX and LeY antigens. Lastly, Fut11 was most abundant in the corpus epididymis, seminal vesicle and prostate, but universally expressed throughout the male reproductive system tissues (Figure 1E and F).

In order to verify roles of the Fut in biosynthesis of LeX/LeY along the male reproductive tract, we compared the LeX/LeY-containing glycoproteins in the male reproductive system of wild-type c57BL/6 mice with Fut1−/−, Fut2−/− and Fut4−/− mice.

LeX-containing glycoproteins were detected in the cauda epididymis of Fut4−/− mice at a lower level than in the cauda epididymis of the wild-type mice, and became very weak in the caput epididymis of Fut4−/− mice (Figure 2B). The expression level of LeX in the vas deferens was much lower in Fut1−/−, Fut2−/− and Fut4−/− mice when compared with wild-type mice (Figure 2B). In the seminal vesicle, the LeX-containing glycoproteins were nearly undetectable in both Fut2−/− and Fut4−/− mice (Figure 2B), indicating that both Fut2 and Fut4 could affect the synthesis of LeX antigen in seminal vesicle.

In the coagulating gland, the expression levels of both LeX-containing glycoproteins and Fut9 mRNA were increased in Fut1−/−, Fut2−/− and Fut4−/− mice relative to wild-type c57BL/6 mice, implying that Fut9 may be involved in the synthesis of the LeX antigen in the coagulating gland (Figure 2B and G). Surprisingly, in the coagulating gland, the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was upregulated in Fut1−/−, Fut2−/− and Fut4−/− mice, whereas β-actin expression was downregulated in Fut1−/− mice (Figure 2C and D).

LeY-containing glycoproteins were not detected in the epididymis and vas deferens of Fut1−/− and Fut4−/− mice (Figure 2A), indicating that the synthesis of the LeY antigen in the epididymis and vas deferens is catalyzed by Fut1 and Fut4. The expression of LeY antigen was downregulated in Fut4−/− mice, corresponding to the decrease in the Fut1 mRNA level in Fut2−/− mice (Figure 2A and E), which implied that the Fut2 deficiency downregulated Fut1 mRNA expression, leading to reduction in the level of LeY antigen. In the seminal vesicle and the coagulating gland of Fut2−/− mice, LeY-containing glycoproteins were very weak, indicating that LeY synthesis in these two tissues is mostly catalyzed by Fut2. The expression of LeY decreased in Fut1−/− and Fut4−/− mice, corresponding to reduced Fut2 expression in the seminal vesicle of Fut1−/− and Fut4−/− mice (Figure 2A and F). This implies that a deficiency of Fut1 and Fut4 can downregulate Fut2 mRNA, reducing the level of LeY in the seminal vesicle.

In summary, the synthesis of LeX-containing glycoproteins needs mainly Fut4 in the caput epididymis, Fut2 and Fut4 in the seminal vesicle, and possibly Fut9 in the coagulating gland. LeY-containing glycoprotein synthesis requires Fut1 and Fut4 in the epididymis and vas deferens, and mainly Fut2 in the seminal vesicle and coagulating gland.

Localization of LeX/LeY antigens in the male reproductive system by immunofluorescence analysis

Neither LeX nor LeY antigens were detected by immunofluorescence (IF) in the testis, which is consistent with our western blot analysis. The specificity of the antibodies was also rechecked using FITC (fluorescein isothiocyanate)-conjugated Lotus tetragonolobus lectin, and the result showed that the fluorescence was negative in testis (data not shown). The mouse epididymis is composed of 10 distinct segments (Johnston et al. 2005; Figures 1H and 7). LeX/LeY antigens were expressed at high levels in the epithelium of the middle caput epididymis (triangle Zone 2) as indicated in Figure 3,
Figure 4C and 4A, and at low levels in the remaining areas of the epididymis. LeX/LeY antigen expression was robust in the lumen of epididymis and vas deferens, with the exception of the initial segment (Zone 1; Figures 3 and 4). LeX antigen immunoreactivity was also observed on the luminal surface of specific epithelial cells along the ducts of all segments of the epididymis, magnified in Figure 4B and 4D. Despite the large amount of LeX-containing glycoproteins detected by western
The nuclei of these specific cells were in the intermediate to apical phase (Figure 4B), whereas most of the normal principle cells had nuclei localized in the basal phase (Figure 4C). In the cauda epididymis, the LeX antigen lying on the surface of epithelial cells became flat (Figure 4D). This specific distribution pattern indicates that the specific epithelial cells expressing the LeX antigen may be narrow cells and clear cells (Adamali and Hermo 1996; Da Silva et al. 2007). IF using an anti-ATP6E antibody (Da Silva et al. 2007), a narrow/clear cell marker, indicated that LeX antigen-bearing cells in the initial segment and the caput epididymis were narrow cells, whereas those in the cauda epididymis were clear cells (Figure 6C). Having a typical cap on the luminal face, narrow cell identity was further confirmed by cell morphology (Figure 6C).

Comparing the western blot results in Fut4−/− mice, the synthesis of the LeX antigen was partially catalyzed by Fut4 in the epididymis, which leads to a hypothesis that the nature of LeX distribution might involve an α-(1,3)-Fut other than Fut4 (Figure 2B). In the epididymis of Fut4−/− mice, the expression of secreted LeX was not detected in triangle Zone 2. However, the LeX expressed in narrow/clear cells was elevated in the caput epididymis of Fut4−/− mice compared with wide-type mice (Figure 6A and B).

Fut9 is a functional α-(1,3)-Fut that is capable of synthesizing LeX in the mouse, and Fut11 is a novel α-(1,3)-Fut for which there is no evidence of its catalytic activity in mice. Compared with wild-type mice, neither Fut9 nor Fut11 mRNA levels were increased in the caput epididymis of Fut4−/− mice relative to wild-type controls (Figure 6D), which implies that Fut9 or Fut11 may not synthesize LeX on the luminal phase of narrow/clear cells.

LeX is also localized on the luminal surfaces of narrow cells and clear cells along the epididymis, but is not synthesized by Fut4.

Besides in Zone 2, LeX expression capped the surface of specific epithelial cells in the initial segment of epididymis (Figures 3B and 4B). The coagulating gland, the LeY antigen was detected around the luminal surfaces of epithelial cells (Figure 5D). Fluorescence was detected by IF using an FITC-conjugated secondary antibody. Scale bars represent 200 μm.

Discussion

In this paper, the distribution of LeX/LeY antigens and related Fut were studied to explore the synthesis and regulation of LeX/LeY antigens in the reproductive system of the male mouse. Neither the LeX nor LeY antigen could be detected in the mouse testis, consistent with the data not shown). The LeY antigen was readily detectable in the epithelium of the seminal vesicle, consistent with the IF analysis. This binding of an anti-LeX antibody to the seminal vesicle epithelium by western blot but not by IF. The LeX expressed in narrow/clear cells was elevated in the caput epididymis of Fut4−/− mice compared with wide-type mice (Figure 6A and B).

The nuclei of these specific cells were in the intermediate to apical phase (Figure 5D), whereas most of the normal principle cells had nuclei localized in the basal phase (Figure 5C). In the cauda epididymis, the LeX antigen lying on the surface of epithelial cells became flat (Figure 5D). This specific distribution pattern indicates that the specific epithelial cells expressing the LeX antigen may be narrow cells and clear cells (Adamali and Hermo 1996; Da Silva et al. 2007). IF using an anti-ATP6E antibody (Da Silva et al. 2007), a narrow/clear cell marker, indicated that LeX antigen-bearing cells in the initial segment and the caput epididymis were narrow cells, whereas those in the cauda epididymis were clear cells (Figure 6C). Having a typical cap on the luminal face, narrow cell identity was further confirmed by cell morphology (Figure 6C).

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Fut9 is a functional α-(1,3)-Fut that is capable of synthesizing LeX in the mouse, and Fut11 is a novel α-(1,3)-Fut for which there is no evidence of its catalytic activity in mice. Compared with wild-type mice, neither Fut9 nor Fut11 mRNA levels were increased in the caput epididymis of Fut4−/− mice relative to wild-type controls (Figure 6D), which implies that Fut9 or Fut11 may not synthesize LeX on the luminal phase of narrow/clear cells.
the LeY antigen (Kuo et al. 2009). One possible reason for the incompatibility is that they focused on the fucosylated N-glycan from lumen fluid, while we were concerned about the glycoproteins conjugated with the LeX/LeY antigens in both N- or O-glycan not only in the fluid, but also in the epithelium and sperm. LeX and LeY could also possibly be missed during the sample preparation procedure, or the amount of LeX or LeY released from glycoprotein was too little for the glycome analysis in the epididymis.

Our observations indicate that the synthesis of LeY-containing glycoproteins is catalyzed by Fut1 and Fut4 in the epididymis and vas deferens, and mainly by Fut2 and an α-(1,3)-Fut other than Fut4 in both the seminal vesicle and the coagulating gland. It is unknown whether Fut9 or Fut11 were involved, although they were both detected in coagulating gland, and Fut11 was also detected in the seminal vesicle. Our findings indicate that Fut4 catalyzes the synthesis of LeX-containing glycoproteins in the middle caput epididymis (Zone 2), whereas the synthesis of LeX on the luminal surfaces of narrow cells and clear cells is catalyzed by an α-(1,3)-Fut other than Fut4, Fut9 or Fut11. In the seminal vesicle, the synthesis of LeX-containing glycoproteins appears to be catalyzed by Fut4 and Fut2. LeX antigen synthesis could be catalyzed by Fut9 in the coagulating gland given the simultaneous increases observed in the levels of LeX-containing glycoproteins and Fut9 mRNA in Fut1−/−, Fut2−/− and Fut4−/− mice (Figure 7).

Tissue-specific distribution of LeX/LeY antigens synthesized by specific Fut implies that they may play different roles in different parts of the male reproductive system.
Nevertheless, we did not observe a change in the expression of LeX/LeY antigens among different tissues in normal physiological conditions and should be further studied.

Different expression levels of the LeX antigen in different regions and cell types of the epididymis imply that multiple α-(1,3)-Fut may function in this tissue. One clue is that the LeX antigen was also localized on the cap-like surface of narrow cells and the luminal, flattened surface of clear cells (Figure 6C). Narrow cells and clear cells regulate pH in the lumen (Da Silva et al. 2007). Our data demonstrate that Fut4 is only involved in the synthesis of secreted LeX in Zone 2 of the epididymis, but not the LeX antigen on narrow or clear cells. Our study also indicates that Fut4, Fut9 and Fut11 might not have a role in the synthesis of the LeX antigen on the narrow and clear cells. It is possible that an unknown α-(1,3)-Fut could specifically catalyze the synthesis of the LeX antigen on the surface of narrow cells and clear cells.

Interestingly, LeX-containing proteins were nearly undetectable in the seminal vesicle of Fut2−/− mice. Fut2 is believed to catalyze the transfer of a fucose to the terminal galactose with an α-(1,2) linkage to form the H type-2 antigen (Lin et al. 2001), which is then converted to the LeY antigen by an α-(1,3)-Fut. In our study, lack of Fut2 activity did not decrease the expression level of Fut4 in the seminal vesicle of Fut2−/− mice. This result implies that, like Fut4, Fut2 may also affect the regulation of the synthesis of LeX conjugated to glycoproteins in the seminal vesicle, though it is still unknown how Fut2 is involved. Because LeX-containing glycoproteins were nearly undetectable in the seminal vesicle of Fut4−/− mice and Fut2−/− mice where Fut2 and Fut4 are highly expressed, respectively, it is possible that LeX synthesis in the seminal vesicle needs both the activity of Fut4 and Fut2, rather than Fut4 or Fut2 alone. Another similar affection of an α-(1,2)-Fut on the LeX expression was that the deficiency of Fut1 or Fut2 leads to reduced expression of LeX in the corpus epididymis and the vas deferens. The reduction of Fut4 mRNA in the caput epididymis of Fut1−/− and Fut2−/− (Figure 2E) might be a possible reason. The lack of an α-(1,2)-fucosylation may also affect the activity of α-(1,3)-Fut given that glycosylation can affect the enzyme activity of Fut (de Vries et al. 2001). The localization of α-(1,2)-fucosylated glycoproteins related to the synthesis pathway of LeX might also be affected so that the expression of LeX could be reduced indirectly. However, more solid experimental proofs are necessary to explain the puzzle how the α-(1,2)-Fut affects the expression of LeX antigen in these tissues.

With the exception of the coagulating gland, our work provides suggestive evidence that there is no functional compensation between the two α-(1,2)-Fut and the two α-(1,3)-Fut in Fut1−/−, Fut2−/− or Fut4−/− mouse models. Nevertheless, we did find that LeY- and LeX-containing glycoproteins were always present in the male reproductive tract (epididymis, vas deferens, seminal vesicle or coagulating gland). Their presence is attributable to the differential synthetic regulatory expression of LeX/LeY antigens among different tissues in Fut-deficient mice. The epididymis retained specific fucosylated glycolipids irrespective of targeted deletion of Fut1 or Fut2 (Iwamori and Domino 2004) in the epididymis. The presence of these fucosylated glycolipids might help to explain why fertility in the knockout mouse was not affected by diminished Fut activity. On the other hand, LeX/LeY may also play traditional roles, such as protection from pathogens, in the male reproductive system, which may not be obvious under normal physiological conditions and should be further studied.

Materials and methods

Animals

Adult male wild-type C57BL/6 mice and Fut1−/−, Fut2−/− and Fut4−/− mice (9–11 weeks old) were used in this study. Fut1−/−, Fut2−/− and Fut4−/− mice were kindly donated by Consortium for Functional Glycomics at the Scripps Research Institute which is funded by the NIH (NIGMS). All the mice were housed in the Experimental Animal Center of the Chinese Academy of Science (Shanghai, China).
Protein extraction and western blot analysis

Mouse testis, epididymis (caput, corpus and cauda), vas deferens, seminal vesicle and coagulating gland were homogenized in lysis buffer (10% v/v glycerol, 1% v/v Triton X-100, 50 mmol L\(^{-1}\) Hepes (pH 7.5), 150 mmol L\(^{-1}\) NaCl, 100 mmol L\(^{-1}\) NaF, 1 mmol L\(^{-1}\) phenylmethanesulfonyl fluoride and 10 μg mL\(^{-1}\) aprotinin) on ice, after centrifuged at 12,000 × g for 15 min at 4°C, the supernatants were collected. HS from Shanghai Institute of Planned Parenthood Research (SIPPR) was liquefied, and sperm were separated and removed. Protein concentration of tissue lysate was determined by the Bradford method. Ten micrograms of protein from each tissue sample was separated by SDS–PAGE (10% acrylamide) and electo-transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Buckinghamshire, England). The membranes were incubated with mouse monoclonal antibodies to LeX (1:400 SSEA-1, Santa Cruz Biotechnology, Santa Cruz, CA), LeY (1:200 BG8, Covance Research Products, Inc., Dedham, MA), GAPDH (1:5000, Proteintech Group, Chicago, IL) and mouse anti-β-Actin horseradish

![Fig. 6.](https://example.com/figure6.jpg)

Fig. 6. Lack of expression of *Fut4* only abolishes LeX expression in a specific region of the caput epididymis. LeX antigen was detected by IF. LeX (green) disappeared only in the middle caput (Zone 2) when *Fut4* was deleted, but the LeX antigen level, localized in specific epithelial cells, remained high, or even increased (A and B). Nuclei were stained with propidium iodide (red). Sites 1–5 along epididymis were marked in (A) (×10) and magnified in (B) (×63, scale 50 μm). LeX (green) was localized on the narrow cells and clear cells with ATP6E (red) as a cell marker (×63, scale 10 μm) (C), whereas nuclei were stained by 4,6-diamidino-2-phenylindole (blue). Sites 1–4 (C) are similar to those marked in (A). Narrow cells and clear cells were magnified, and the typical cap of the narrow cell is indicated by a white arrow (C). The mRNA expression of *Fut9* and *Fut11* in the caput epididymis was quantified by real-time PCR and was not increased in *Fut4*–/– mice compared with wide-type mice (D).
peroxidase-conjugated (1:10000 AC-15, Abcam, Cambridge, MA), followed by the addition of horseradish peroxidase-linked anti-mouse IgM for SSEA-1 and BG8, or IgG for GAPDH and ECL visualization of the bands.

RNA preparation and real-time PCR

Total RNA of the testis, epididymis (caput, corpus and cauda) regions of epididymis (cap, cor and cau, respectively); vas, vas deferens; SV, seminal vesicle; co, coagulating gland. Observations are noted as follows: positive (+), negative (−) and very low (+/−). The diagram of epididymis was reproduced from Johnston et al. (2005) with permission of the publisher.

IF analysis

Tissues were fixed for 4–6 h in 4% w/v paraformaldehyde and embedded in paraffin after graded alcohol dehydration. Paraffin sections (5 μm thick) were cut, deparaffinized in xylene and rehydrated in graded ethanol. The slides were blocked with 10% goat serum in 0.1 mol L−1 phosphate-buffered saline overnight at 4°C, incubated in the following primary antibodies for 1 h: SSEA-1 (1:200), BG8 (1:200) and ATP6E (1:300; Genway Biotech Inc., San Diego, CA). Purified mouse myeloma IgM (Zymed Laboratories, South San Francisco CA) was used as a negative control. The secondary antibody for SSEA-1 and BG8 detection was an FITC-conjugated, goat anti-mouse IgM (1:200, Santa Cruz Biotechnology). A rhodamine-conjugated, bovine anti-chicken IgY (1:200, Santa Cruz Biotechnology) was used to detect ATP6E. All cell nuclei were stained with propidium iodide or 4,6-diamidino-2-phenylindole. Fluorescence was detected using an Olympus BX51 fluorescence microscope (Olympus, Tokyo, Japan) and Leica TCS ST2 confocal microscope (Leica, Wetzlar, Germany).

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**Fig. 7.** The biosynthesis of LeX and LeY antigens by *Fut* and their distribution as measured by western blot and IF. TE, testis; cap, corpus and cauda regions of epididymis (cap, cor and cau, respectively); vas, vas deferens; SV, seminal vesicle; co, coagulating gland. Observations are noted as follows: positive (+), negative (−) and very low (+/−). The diagram of epididymis was reproduced from Johnston et al. (2005) with permission of the publisher.

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<tr>
<td>LeY</td>
<td>TCCAAGTGCCTTA TGGCTTCT-3′</td>
<td>LeY</td>
</tr>
</tbody>
</table>

**Table I.** Primer sequences for real-time PCR of *Fut*

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**Conflict of interest**

None declared.

**Abbreviations**

FITC, fluorescein isothiocyanate; Fut, fucosyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HS, human semen; IF, immunofluorescence; Ig, immunoglobulin; LeX, Lewis X; LeY, Lewis Y; PCR, polymerase chain reaction; SSEA-1, stage-specific embryo antigen-1.

**References**


Domino SE, Zhang L, Gillespie PJ, Saunders TL, Lowe JB. 2001. Deficiency of reproductive tract alpha(1,2)fucosylated glycans and normal fertility in mice with targeted deletions of the Fut1 or Fut2 alpha(1,2)fucosyltransferase locus. Mol Cell Biol. 21:8336–8345.


