Journal of Andrology, Vol. 32, No. 3, May/June 2011

Postnatal Changes in the Expression of Claudin-11 in the Testes and Excurrent Ducts of the Domestic Rabbit (*Oryctolagus cuniculus domesticus*)

CHAN JIN PARK,*‡ JAE EUN LEE,*‡ YOUNG SEOK OH,* SARA SHIM,* DONG MIN KIM,* NAM CHEOL PARK,† HYUN JUN PARK,† AND MYUNG CHAN GYE*

*From the *Department of Life Science, College of Natural Sciences, Hanyang University, Seoul, Korea; and the †Department of Urology, College of Medicine, Pusan National University, Pusan, Korea.*

ABSTRACT: We examined the expression of claudin-11 (CLDN11) in the testes and male reproductive tracts of rabbits. The rabbit CLDN11 cDNA sequences were nearly identical with human, mouse, and bovine CLDN11. The levels of CLDN11 mRNA and protein (22 kDa) were markedly increased in the testis during adult development. On postnatal day (PND) 10, CLDN11 was colocalized with ZO-1 at the lateral contacts between adjacent Sertoli cells and was perpendicular to the basal lamina. In adult testis on PND 180, CLDN11 was codistributed with ZO1, and the pattern of immunoreactivity consisted of wavy linear tracts parallel to the basal lamina, which was different according to the spermatogenic stage. These results suggest that CLDN11 participates in inter–Sertoli cell tight junctions (TJs) at the blood-testis barrier in adult rabbits. CLDN11 was also found in the basal regions of Sertoli cells adjacent to the basal lamina in adult testis, suggesting that CLDN11 also participates in the adhesion between Sertoli cells and the basal lamina. CLDN11 mRNA and protein expressions were decreased in the adult epididymis compared with those in immature animals. In adults, CLDN11 mRNA levels were relatively high in the efferent duct, followed by those in the vas deferens, proximal corpus, and distal cauda, although low levels were observed in the initial segment and caput. On PND 10, CLDN11 immunoreactivity was identified at the apicolateral contacts between adjacent epithelial cells in the epididymis and vas deferens. In adults, CLDN11 was found in the nonciliated cells in the efferent duct and at the lateral contacts in the epithelial cells in the epididymal segments. In the caput, CLDN11 was found at the apicolateral contacts between adjacent epithelial cells, but expression was weak to negligible in the corpus of the vas deferens. CLDN11 may play an important role in TJs and cell adhesion in immature rabbit excurrent duct epithelia. In adult rabbits, CLDN11 in efferent duct epithelium and epididymal epithelium may contribute to the specific environment for sperm maturation.

Key words: Testis, epididymis.

J Androl 2011;32:295–306

Epithelial tight junctions (TJ) separate adjacent epithelial cells and restrict the movements of small molecules from the basolateral to the adluminal surface and vice versa; they are also essential in establishing and maintaining cell polarity (''fence function''; Tsukita et al, 1999). Furthermore, by regulating the epithelial microenvironment, TJs are critical for various biologic functions, including control of cell proliferation (Mitic et al, 2000; Tsukita et al, 2008). To date, multiple integral membrane proteins that compromise TJs have been found, including claudins (CLDNs), occludin, junctional adhesion molecules, tricellulin, coxsackievirus and adenovirus receptors, and several associated peripheral proteins, such as zonula occludens 1 (ZO-1), 2, and 3, cingulin, and AF-6 (Ikenouchi et al, 2005; Tsukita et al, 2008).

In the testis, TJs between Sertoli cells form the blood-testis barrier (BTB), an important entity in the maintenance of a special physiologic milieu for spermatogenesis in the seminiferous tubules and in the protection of germ cells from the immune system (Russell and Peterson, 1985; Russell et al, 1989). In humans, pathologic conditions of the BTB have been found to be related to changes in male fertility (Landon and Pryor, 1981; Cavicchia et al, 1996). In the epididymis, where the final events of sperm maturation occur (Orgebin-Crist, 1969), TJs between adjacent epithelial cells create the paracellular diffusion barrier and permit the formation of a specific luminal microenvironment. This barrier maintains the high osmotic state of the luminal environment, preventing dilution of its contents via paracellular routes and thus allowing

Supported by grant A080921 from the Korean Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea, and by Pusan National University (2006).

Correspondence to: Dr Hyun Jun Park, Department of Urology, College of Medicine, Pusan National University, Pusan, Korea (e-mail: hjpark@pnu.ac.kr); and Dr Myung Chan Gye, Department of Life Science, Hanyang University, Seoul, 133-791 Republic of Korea (e-mail: mcgye@hanyang.ac.kr).

Received for publication April 13, 2010; accepted for publication October 20, 2010.

‡ These authors contributed equally to this article.

DOI: 10.2164/jandrol.110.010611

sperm maturation and survival in the epididymis (Cyr et al, 1999). Indeed, functional alterations of the blood-epididymal barrier (BEB) have been shown to lead to altered sperm motility in mice (Hermo et al, 2007).

To date, several TJ proteins have been identified in the mammalian testis and epididymis (Moroi et al, 1998; Gow et al, 1999; Morita et al, 1999; Hellani et al, 2000; Gye, 2003a,b, 2004; Guan et al, 2005; Gregory and Cyr, 2006; Dubé et al, 2007; Yoon et al, 2009). Of these genes, the CLDN family of transmembrane proteins accounts for at least 24 members, the genes of which are expressed in a tissue-specific manner (Van Itallie and Anderson, 2006). CLDNs interact with multiple proteins and are intimately involved in TJ-related signal transduction (Tsukita et al, 1999; Mitic et al, 2000). CLDNs 1, 3, 4, 5, 7, 8, and 11 have been shown to be expressed in the mammalian testis (Gow et al, 1999; Morita et al, 1999; Hellani et al, 2000; Gye, 2003a,b; Meng et al, 2005; Wang et al, 2006; Morrow et al, 2009), suggesting that multiple CLDNs contribute to the BTB. Among the CLDNs expressed in the testis, CLDN11 is crucial for the development of the BTB and spermatogenesis because male mice lacking CLDN11 were found to be sterile and demonstrated testicular atrophy and an absence of inter–Sertoli cell TJs (Gow et al, 1999). In the epididymis, mRNA for CLDNs 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 16 and proteins for CLDNs 1, 3, 4, 5, 6, 8, and 10 have been identified (Gregory et al, 2001; Guan et al, 2005; Gregory and Cyr, 2006; Dubé et al, 2007, 2010; Inai et al, 2007), suggesting the complex nature of CLDN-TJs in the BEB. Although the ultrastructures of the BTB and BEB have been described in some mammalian species (Connell, 1978; Pelletier, 1988; Bielli et al, 1995; Lopez et al, 1997; Cambrosio Mann et al, 2003), details of the molecular architectures of the BTB and BEB are still not well understood. Although the expressions of several TJ genes change during testis and epididymis development under the control of endocrine and paracrine factors (Cyr et al, 1999; Gye et al, 2000; Hellani et al, 2000; Gregory et al, 2001; Gye, 2003a,b; Meng et al, 2005; Gregory and Cyr, 2006; Wang et al, 2006; Tarulli et al, 2008), developmental changes in the expressions of most TJ genes in the testis and epididymis have not been described. In addition to the advantage in anatomic size of the rabbit excurrent duct system for the analysis of segmental differences in gene expression in immature as well as adult animals, rabbit CLDN11 expression in developing testes and the excurrent duct system may provide comparative information regarding the molecular structures and developments of the BTB and BEB in mammals. In the present study, we cloned rabbit CLDN11 cDNA and examined the developmental changes in the expressions of CLDN11 mRNA and protein in the rabbit testis and excurrent duct system.

Materials and Methods

Tissue Sampling

New Zealand white male rabbits (*Oryctolagus cuniculus*) at 10 and 180 postnatal days (PND) of age, representing the neonatal and adult stages of testis development, respectively, were obtained from a local farm (Kapyung, Korea) during the breeding season (April–May). The testes and epididymides were dissected from the animals after CO_2 asphyxiation. All animal experiments were performed in accordance with the Guide of Hanyang University for Care and Use of Laboratory Animals. The immature epididymis was divided into the caput, corpus, and cauda regions. The adult epididymis was divided into the efferent duct, initial segment, proximal caput, distal caput, proximal corpus, distal corpus, proximal cauda, distal cauda, and vas deferens (Figure 1). For the analysis of CLDN11 cDNA sequences and mRNA expressions in testes and epididymides, decapsulated tissue samples were frozen in LN_2 and stored at $-70^{\circ}C$ until analysis. For immunohistochemical analysis, tissue samples were fixed in a Bouin fixative overnight, after which they were processed for paraffin embedding. Nonfixed testes were directly embedded in OCT compound (TissueTek; Sakura, Torrance, California) and processed for cryosectioning and a double-immunofluorescence experiment.

Cloning of Rabbit CLDN11 Partial cDNA

Total RNA was isolated from adult rabbit testes and epididymides using a TRI reagent (Molecular Research Center Inc, Cincinnati, Ohio), as directed by the manufacturers. One microgram of RNA sample was reverse transcribed for 60 minutes at 42°C in a 20-μL reaction with 50 units of MuLV reverse transcriptase and 2.5 μM oligo$(dT)_{16}$ primer, following the standard protocol from the supplier (Applied Biosystems, Foster City, California). To clone the rabbit CLDN11 cDNA sequences, a pair of polymerase chain reaction (PCR) primers of rabbit CLDN11 cDNA was designated within conserved nucleotide sequences of the open reading frame (ORF) of human (GenBank accession number NM_005602.5), bovine (GenBank accession number NM_001035055.1), and mouse (GenBank accession number NM_008770.2) CLDN11 cDNA; the forward primer was 5′-TTCGTCACGAGCTTCGTGG G-3′, and the reverse primer was 5′-TAGCCAAAGCTCAC-GATGGT G-3′. PCR of testis cDNA using these primers resulted in the generation of a 436-bp amplicon, which was ligated into pGEM-T Easy vector (Promega, Madison, Wisconsin) and transformed into DH-5α–competent cells. Positive clones were selected based on blue/white screening. Sequencing was performed using M13 primers, and the nucleotide sequences of the rabbit CLDN11 cDNA fragments were compared to those of humans, bovines, and mice using BLAST.

Reverse Transcription–PCR Analysis of CLDN11 mRNA Expressions in Rabbit Testes and Epididymides

For semiquantitative reverse transcription–PCR (RT-PCR) analysis of CLDN11 mRNA expression, we used a pair of secondary primers, 5′-GACCTGC GGCTACACCATC-3′ (forward) and 5′-CTAGGATGAGCAGAACGCC A-3′ (reverse),

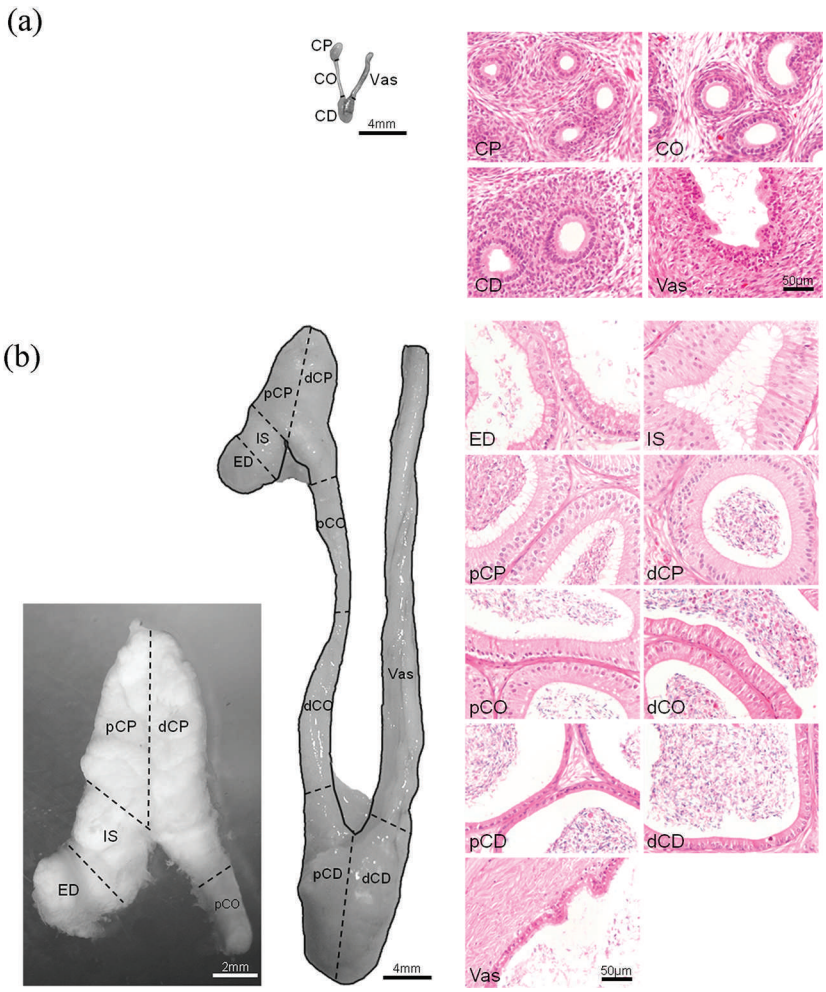

Figure 1. Rabbit excurrent ducts. **(a)** On postnatal day (PND) 10. **(b)** On PND 180. Left: external morphology. Right: hematoxylin-eosin staining. Analysis of claudin-11 (CLDN11) mRNA expression was analyzed in each segment, as depicted in the left panel. CP indicates caput; CO, corpus; CD, cauda; Vas, vas deferens; ED, efferent duct; IS, initial segment; pCP, proximal caput; dCP, distal caput; pCO, proximal corpus; dCO, distal corpus; pCD, proximal cauda; dCD, distal cauda. Color figure available online at www.andrologyjournal.org.

within the cloned CLDN11 cDNA sequences (Figure 2). GAPDH mRNA was used as an internal control, and the primers for rabbit GAPDH were 5′-AGAACATCATCC-CTGCCTCC-3′ (forward) and 5′-CCACCACCCTGTTGC-TGTAG-3′ (reverse; GenBank accession number NM_001082253.1). The optimal annealing temperatures for PCR of CLDN11 and GAPDH cDNA were determined by performing 30 cycles of amplification using the iCycler (Bio-Rad, Hercules, California) temperature gradient mode. The optimal annealing temperature was found to be 65°C for both the CLDN11 and GAPDH primer sets. Each cycle consisted of the following: 95°C for 30 seconds; 65°C for 30 seconds; and 72°C for 45 seconds. The PCR products (20 μL) were then analyzed on 2% agarose gels containing 0.5 μg/μL ethidium bromide and were photographed under ultraviolet light. To quantify CLDN11 (293 bp) and GAPDH (366 bp) mRNA levels in the testes and epididymal segments, real-time PCR was carried out using the same primers and iQ SYBR Green Supermix reagent (Bio-Rad), according to the manufacturer's instructions. PCR was performed in a MyiQ i Cycler (Bio-Rad), with 1 cycle of 3 minutes at 95°C, followed by 40 cycles at 95°C for 30 seconds, annealing temperature for 30 seconds, and 72°C for 30 seconds. PCR amplification was performed in 3 independent samples.

The CLDN11 mRNA level was expressed in arbitrary units relative to those of the GAPDH mRNA internal control level, and the relative quantification was performed using the comparative C_T method according to the manufacturer's protocols (Bio-Rad). The statistical significance of the CLDN11 mRNA level among the samples was analyzed using 1-way analysis of variance (ANOVA) followed by Tukey's post hoc test using the SPSS 17.0 program (SPSS Inc, Chicago, Illinois). Statistical significance was accepted when the *P* value was smaller than .05.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Western Blot Analysis

Tissues were homogenized in 10 volumes of cold extraction buffer (20 mM Tris-HCl, pH 7.5, and 1% Triton X-100)

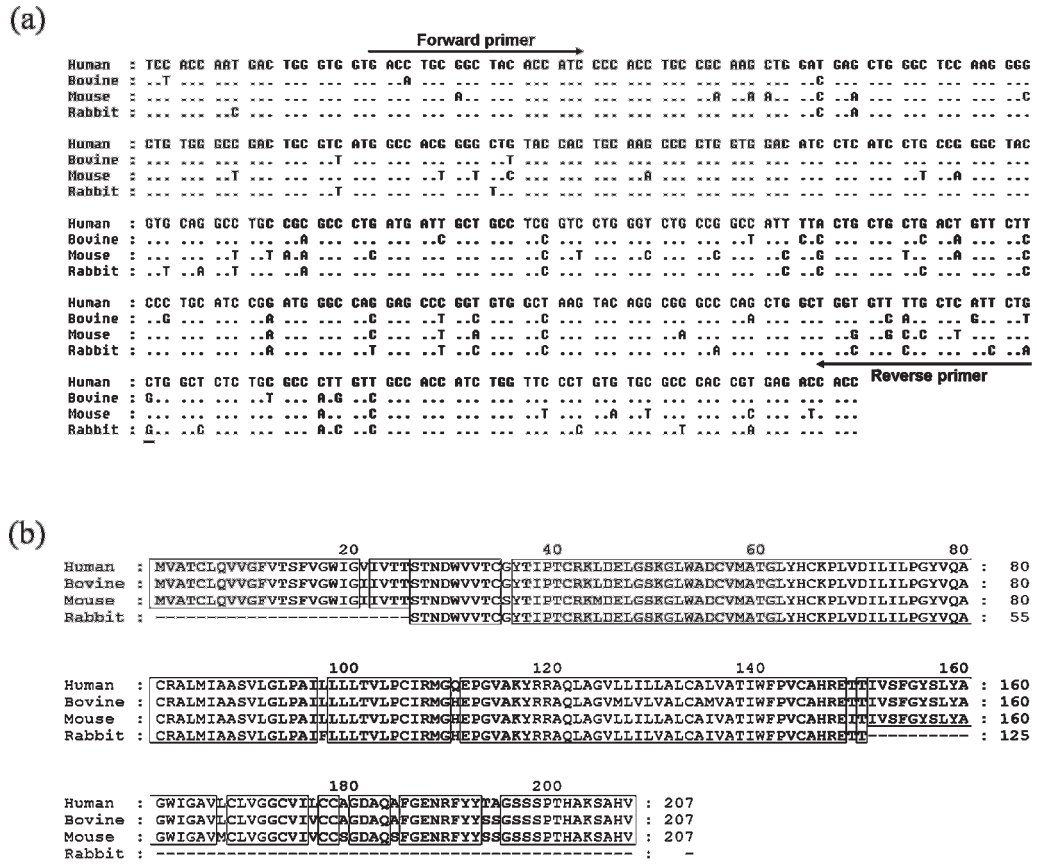

Figure 2. Nucleotide sequence and deduced amino acid sequence of the rabbit claudin-11 (CLDN11) cDNA fragment. **(a)** Partial nucleotide sequences from the open reading frame region of rabbit CLDN11 cDNA compared with those of the human (GenBank accession number NM_005602.5), bovine (GenBank accession number NM_001035055.1), and mouse (GenBank accession number NM_008770.2) CLDN11 cDNA. **(b)** Deduced amino acid sequence of rabbit CLDN11 cDNA compared to human, bovine, and mouse sequences. Open boxes indicate conserved amino acid sequences; dashed lines, undetermined sequences; F, forward primer; R, reverse primer used for reverse transcription–polymerase chain reaction (RT-PCR) of CLDN11 mRNA in rabbit testes and epididymides. Please see Figure 1 caption for further explanation of abbreviations.

containing a complete protease inhibitor (Roche, Mannheim, Germany). Homogenates were then centrifuged at 12 000 × *g* for 1 hour at 4°C. The supernatant was collected, and the protein concentration was determined using a commercial protein assay kit (Bio-Rad). To remove the contaminating immunoglobulin G (IgG) fraction in the tissue lysate, samples (12.5 μg/μL) were mixed with an equal volume of Protein A/G PLUS-agarose–conjugated beads (sc-2003; Santa Cruz Biotechnology, Santa Cruz, California) and were rocked overnight at 4°C. After centrifugation, the supernatants were mixed with 6× sample buffer (Laemmli, 1970) containing 0.1 M dithiothreitol, boiled for 5 minutes, and cooled to room temperature. After centrifugation, the clear supernatants (20 μg of protein per lane) were resolved on 12% sodium dodecyl sulfate–polyacrylamide gels. After electrophoretic transfer to a nitrocellulose membrane (Amersham Biosciences, Little Chalfont, United Kingdom), the membrane was blocked in Tris-buffered saline (TBS) containing 1% bovine serum albumin (BSA) overnight at 4°C. After rinsing 3 times with TBS/0.1% Tween 20 (TBST) for 10 minutes each, the membrane was incubated with rabbit CLDN11 antibody (ab53041; Abcam, Cambridge, United Kingdom) diluted in TBST (1:1000) for 2 hours at room temperature. After rinsing with TBST 3 times for 10 minutes each, the membrane was incubated with a Clean-Blot IP Detection kit (peroxidase conjugate; 1:2000 dilution in TBST; Pierce Biotechnology, Rockford, Illinois) for 1.5 hours. The membrane was washed with TBST for 10 minutes and then with TBS for 10 minutes. Signal was detected using an ECL Plus (for testis CLDN11) or an ECL Advanced (for epididymis CLDN11) kit (Amersham Biosciences). The same blot was deprobed through incubation for 15 minutes in stripping buffer (2 M glycine, pH 2.5), washed in TBST, and then reprobed with rabbit normal IgG (sc2027; Santa Cruz Biotechnology) diluted 1:1000 in TBST as a negative control. Rabbit anti-GAPDH antibody (LF-PA0018; LabFrontier, Seoul, Korea) was used as an internal control.

Immunohistochemistry

For immunohistochemical analysis, paraffin sections (5 μm) of Bouin solution–fixed testes and epididymides were prepared. After deparaffinization, endogenous peroxidases were blocked with 3% H_2O_2 for 15 minutes. For antibody labeling, sections

were incubated for 1 hour with a blocking solution (3% BSA in phosphate-buffered saline [PBS]). The slide was incubated with the CLDN11 antibody (ab53041; Abcam) diluted 1:500 (actual protein concentration, 2 μg/mL) in blocking solution for 2 hours at room temperature. Rabbit normal IgG (sc-2027; Santa Cruz Biotechnology) diluted 1:200 (actual protein concentration, 2 μg/mL) in blocking solution was used in place of the primary antibodies to act as a negative control. After washing twice in PBS, the slide was incubated with a peroxidase-labeled goat anti-rabbit IgG (C81-6120; Zymed, San Francisco, California) in a 1:200 dilution in blocking solution. After a coloring reaction using oxidized 3,3-diaminobenzidine as the chromogen, the slide was counterstained with Harris hematoxylin (Sigma, St Louis, Missouri). The permanently mounted slide was observed and photographed using a microscope equipped with a digital imaging system (DFC320; Lieca, Heerbrugg, Switzerland). Otherwise, CLDN11 immunofluorescence was conducted with ZO-1 as a TJ marker. Briefly, cryocuts (5 μm thick) were placed on a poly-L-lysine–coated slide, air dried for 30 minutes, permeabilized in ice-cold acetone for 5 minutes, and air dried. After hydration in PBS for 5 minutes, the slide was blocked through incubation with 2% normal chicken serum (sc-2479; Santa Cruz Biotechnology) in PBS and incubated with rabbit anti-CLDN11 antibody (36-4500; Zymed) and goat anti–ZO-1 antibody (sc-8146; Santa Cruz Biotechnology) diluted 1:100 (actual protein concentration, 2 ~ 2.5 μg/mL) in 1% normal chicken serum in PBS in a humidified chamber overnight at 4°C. For the negative control, normal rabbit IgG diluted 1:100 (actual protein concentration, 4 μg/mL) in 1% normal chicken serum in PBS was used in place of the primary antibodies. After washing twice in PBS, Texas Red–conjugated chicken anti-goat IgG (sc-3923; Santa Cruz Biotechnology) and fluorescein isothiocyanate–conjugated chicken anti-rabbit IgG (sc-2990; Santa Cruz Biotechnology) diluted 1:200 in 1% normal chicken serum in PBS were applied and incubated for 1 hour at room temperature. After washing in PBS, nuclear staining was conducted with ProLong Gold (Invitrogen, Eugene, Oregon) containing 4′,6-diamidino-2-phenylindole (DAPI). Slides were observed under an epifluorescence microscope (IX71; Olympus, Tokyo, Japan) equipped with a digital imaging system (DP71; Olympus). Fluorescein isothiocyanate, Texas Red, and DAPI images were captured and merged using DP-BSW software (version 3.03; Olympus).

Results

Nucleotide Sequences of Rabbit CLDN11 cDNA

Partial nucleotide sequences spanning the ORF region of rabbit CLDN11 cDNA showed 91%, 87%, and 92% homology with human, mouse, and bovine CLDN11, respectively. The amino acid sequence identities of the corresponding ORF regions of rabbit CLDN11 were 96% homologous with those of human, mouse, and bovine CLDN11 (Figure 2).

Expressions of CLDN11 mRNA and Protein in the Rabbit Testes and Epididymides

Real-time PCR analysis revealed that the CLDN11 mRNA level was significantly increased in adult testes on PND 180 compared with immature testes on PND 10. In the immature epididymis, the CLDN11 mRNA level was the highest in the caput (including the efferent duct), but was moderate in the corpus, cauda epididymis, and vas deferens. Compared with immature epididymides, adult epididymides showed markedly reduced CLDN11 mRNA levels, with the level being the highest in the efferent duct, followed by those of the distal cauda, vas deferens, and proximal corpus. Low levels of CLDN11 mRNA were expressed in the initial segment, caput, distal corpus, and proximal cauda (Figure 3). There was no specific amplification product in the negative control experiment, which did not contain RT-reactive RNA.

On the Western blot of rabbit testis and epididymis, a 22-kDa CLDN11 protein was detected. A 22-kDa CLDN11-positive control protein was also detected in mouse brain. When the Western blot was probed with nonimmune rabbit normal IgG, the 22-kDa protein band was absent, suggesting a specific antibody reaction. CLDN11 protein levels were markedly increased in the testis but decreased in the epididymis during adult development (Figure 4).

Localization of CLDN11 in the Rabbit Testis

On PND 10, CLDN11 immunoreactivity was found at the contacts between adjacent Sertoli cells. In the negative control where rabbit normal IgG was used in place of primary antibodies, the perinuclear structure in the gonocytes showed nonspecific staining. On PND 180, CLDN11 immunoreactivity was markedly increased in the basal part of the Sertoli cells in which CLDN11 immunoreactivity was parallel or perpendicular to the basal lamina; this varied among the seminiferous tubules. The basal region of the Sertoli cells adjacent to the basal lamina was also positive for CLDN11 immunoreactivity. In the negative control with rabbit normal IgG instead of primary antibodies, the nonspecific reaction was not observed (Figure 5). In a double-immunofluorescence labeling experiment, CLDN11 immunoreactivity was largely perpendicular to the basal lamina and colocalized with ZO-1 at lateral contacts between adjacent Sertoli cells on PND 10. On PND 180, strong CLDN11 immunoreactivity was found together with ZO-1 at the periphery of the seminiferous epithelium parallel to the basal lamina. In most of the seminiferous tubules, CLDN11 immunoreactivity delineated pachytene spermatocytes from early-stage germ cells in the basal part of the seminiferous

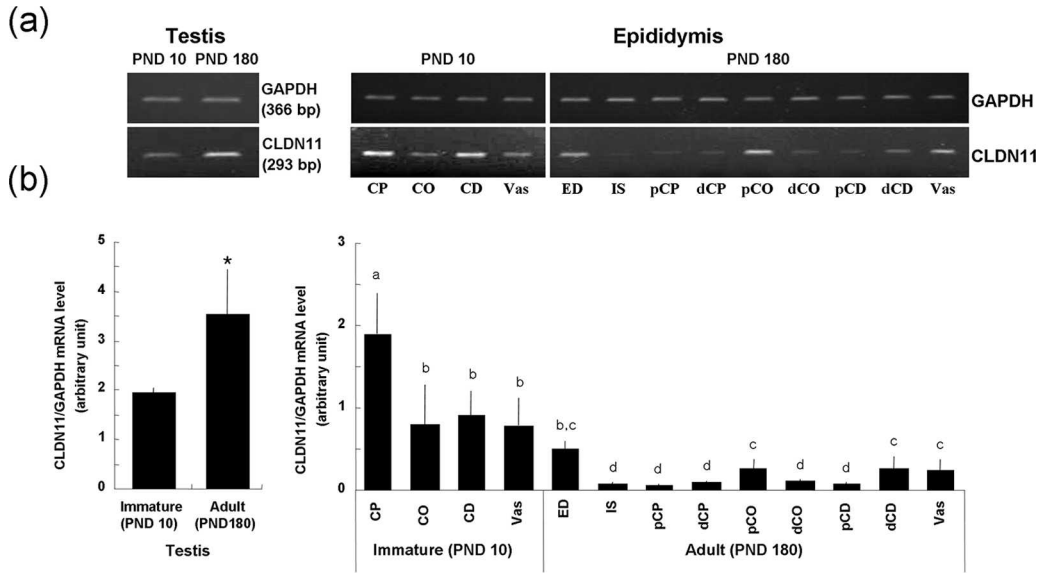

Figure 3. Reverse transcription–polymerase chain reaction (RT-PCR) analysis of claudin-11 (CLDN11) mRNA expression in rabbit testes and epididymides. **(a)** RT-PCR of CLDN11 and GAPDH mRNA in the testes and epididymal segments on postnatal days (PND) 10 and 180. **(b)** RT-PCR analysis of CLDN11 mRNA levels in the testis and epididymis. GAPDH mRNA was used as an internal control. *Significantly different from those on PND 10. For superscript letters (a, b, c, and d), the same letter represents a lack of significant difference. Error bar = SD (n = 3). Please see Figure 1 caption for further explanation of abbreviations.

epithelium. Moderate CLDN11 immunoreactivity perpendicular to the basal lamina was also found in the Sertoli cells. In part of the seminiferous tubule, the basal region of the Sertoli cells adjacent to the basal lamina was also positive for CLDN11 immunoreactivity. No specific signal was found in the negative control, in which rabbit and goat normal IgG were used instead of primary antibodies (Figure 6). The subcellular localization of CLDN11 in testes is summarized in Table 1.

Localizations of CLDN11 in the Rabbit Efferent Duct, Epididymis, and Vas Deferens

On PND 10, CLDN11 was found in the apicolateral contacts between epithelial cells and in the apical cytoplasm of epithelial cells in all epididymal segments and in the vas deferens. Although the epididymal lumen is already developed on PND 10, there is no marked difference in the subcellular localizations of CLDN11 in the epithelium among the epididymal segments. On

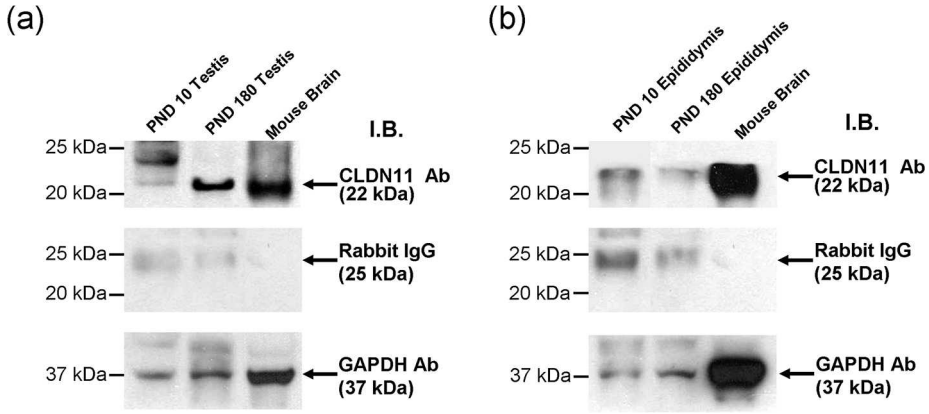

Figure 4. Western blot analysis of claudin-11 (CLDN11) in the adult rabbit testis and epididymis. **(a)** Testis. **(b)** Epididymis. Tissue extracts from adult rabbit testes and epididymides were resolved on a 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel, transferred to a nitrocellulose membrane, and probed with CLDN11 antibody. A major CLDN11 band (22 kDa) was detected. When probed with rabbit normal immunoglobulin G (IgG), no specific band was detected. As a positive control, adult mouse brain was examined. PND indicates postnatal days; I.B., immuno blot.

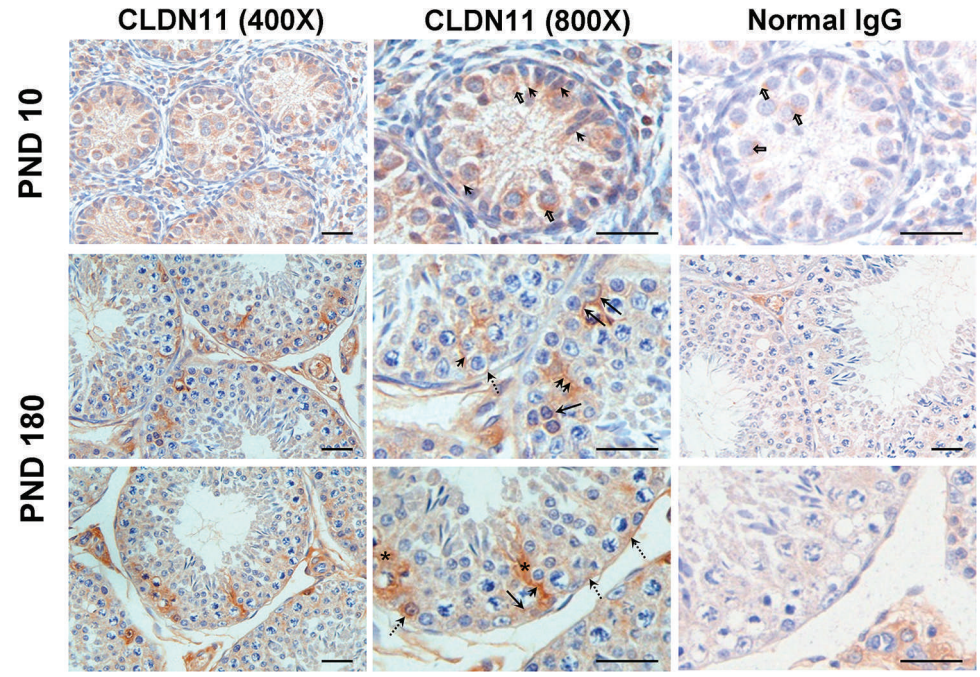

Figure 5. Immunohistochemical localization of CLDN11 in the rabbit testes. On postnatal day (PND) 10, weak claudin-11 (CLDN11) immunoreactivity was found at the basolateral contacts between adjacent Sertoli cells and between Sertoli cells and gonocytes (small arrows). On PND 180, CLDN11 immunoreactivity was either parallel (large arrows) or perpendicular (small arrowheads) to the basal lamina in the basal parts of Sertoli cells. CLDN11 immunoreactivity was also found in the Sertoli cell cytoplasm (asterisks) which harbored elongating/elongated spermatids and at the basal interface between Sertoli cells and the basal lamina (dotted arrows). In the negative control, in which normal rabbit immunoglobulin G (IgG) was used in place of primary antibody, nonspecific reactivity was found in the germ cells (open arrows). Scale bar = 30 μm. Color figure available online at www.andrologyjournal.org.

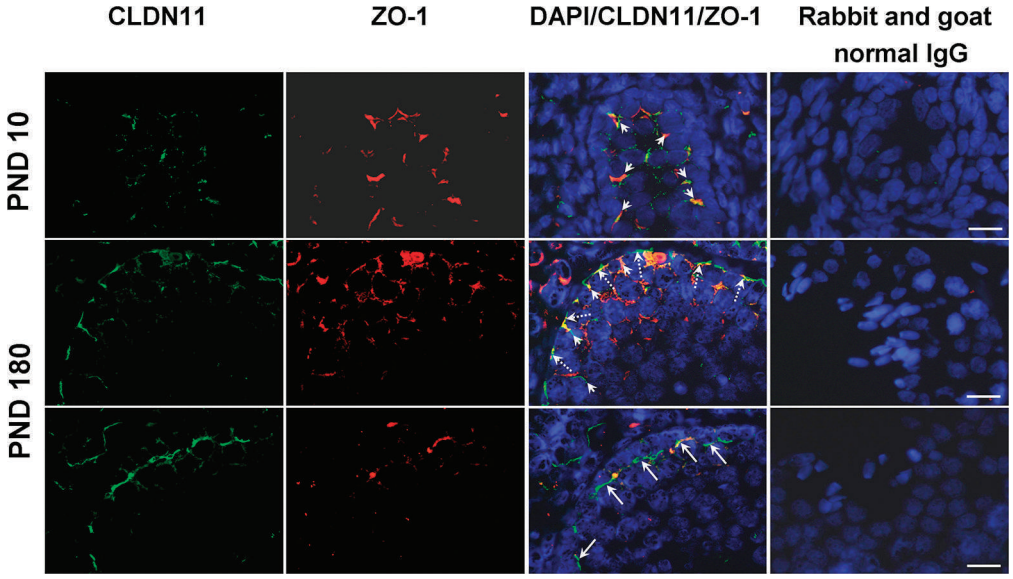

Figure 6. Immunofluorescent localization of claudin-11 (CLDN11) and zonula occluden 1 (ZO-1) in rabbit testes. On postnatal day (PND) 10, CLDN11 (green) and ZO-1 (red) were found at the contacts between adjacent Sertoli cells (arrows), perpendicular to the basal lamina. In the merged picture, CLDN11 was colocalized with ZO-1 (green to yellow). On PND 180, CLDN11 and ZO-1 immunoreactivities were close to the basal lamina (dotted arrows), perpendicular to the basal lamina (small arrows), or wavy and parallel (large arrows) to the basal lamina, delineating pachytene spermatocytes from an earlier stage of germ cells in the basal part of the seminiferous epithelium. 4′,6-diamidino-2-phenylindole (DAPI) for nuclear staining (blue). In the negative controls, in which rabbit and goat normal immunoglobulin G (IgG) replaced the primary antibodies, no specific signal was observed. Scale bar = 20 μm. Color figure available online at www.andrologyjournal.org.

Table 1. *Localization of CLDN11 in rabbit testes, efferent duct, and epididymides*

Postnatal Day	Testis	Epididymis				Efferent Duct	Initial Segment
		Caput	Corpus	Cauda	Vas		
10							
	SS^{+}	A^{++}	A^{++}	A^{++}	A^{++}	...	...
	SC^{+}	L^{++}	L^{++}	L^{++}	L^{++}	...	...
	SL^{-}	C^{++a}	C^{++a}	C^{++a}	C^{++a}	...	...
180							
	SS^{+++}	A^{+}	$A^{+/-}$	$A^{+/-}$	A^{+}	A^{++}	A^{+}
	SC^{+++}	$L^{+/-}$	L^{+}	$L^{+/-}$	L^{+}	L^{++}	L^{+b}
	SL^{++}	$EB^{+/-}$	EB^{+}	$EB^{+/-}$	$EB^{+/-}$	$CC^{+/-}$	$EB^{+/-}$
	...	$C^{+/-}$	C^{+}	$C^{+/-}$	$C^{+/-}$	NC^{++}	$C^{+/-}$

Abbreviations: −, absent; +/−, marginal; +, weak; ++, moderate; +++, strong. A, apical contacts between epithelial cells; C, epithelial cell cytoplasm; CC, ciliated cells; CLDN11, claudin-11; EB, contacts between principal cells and basal cells; L, lateral contacts between epithelial cells; NC, nonciliated cells; SC, Sertoli cell cytoplasm; SL, Sertoli cell–basal lamina contacts; SS, inter–Sertoli cell contacts; Vas, vas deferens.

[a] Apical.

[b] Narrow cells.

PND 180, the subcellular localization of CLDN11 in the epithelium differed along the excurrent duct system. In the efferent duct, CLDN11 was largely found at the apicolateral contacts between nonciliated cells and adjacent ciliated cells. In the initial segment, weak CLDN11 immunoreactivity was found in narrow cells. In the caput, CLDN11 immunoreactivity at the contacts between adjacent epithelial cells was negligible in the proximal part but was evident in the distal part. In the corpus epididymis, weak CLDN11 immunoreactivity was found in the cytoplasm of the principal cells and basal cells, and CLDN11 immunoreactivity was negligible in the epithelium of the proximal cauda and was increased throughout the vas deferens. In the epididymis on PND 10, and less so in the adults, cytoplasmic CLDN11 immunostaining was observed. In the negative control, where rabbit normal IgG was used instead of the primary antibody, nonspecific reactivity was negligible (Figure 7). Localization of CLDN11 in the efferent duct and regions of the epididymis is summarized in Table 1.

Discussion

Developmental Changes in CLDN11 Expression in Rabbit Testes

The CLDN11 mRNA and protein levels were lower in the immature rabbit testis compared with those of the adult testis, indicating that transcription of the CLDN11 gene is not fully active in the Sertoli cells of the rabbit testis during the early postnatal period. Light microscopic analysis revealed CLDN11 immunoreactivity at the lateral contacts between apposing Sertoli cells. In the double-immunofluorescence labeling experiment, CLDN11 immunoreactivity was largely perpendicular to the basal lamina and was colocalized with ZO-1 at the apicolateral contacts between adjacent Sertoli cells in PND 10 testis. Similarly, in rodent and mink testes, short and blind-ended TJ strands were found scattered around the lateral membranes of the Sertoli cells during the early prepubertal period, and a continuous network of TJ strands was found to gradually develop in the basal region (Nagano and Suzuki, 1976; Meyer et al, 1977; Pelletier, 1988). In mice, an impermeable BTB is established when Sertoli cells cease to proliferate and begin differentiation (Nagano and Suzuki, 1976). In the rabbit testis, Sertoli cells actively proliferate postnatally, which essentially ceases by the seventh week of life, when differentiation begins and the meiotic spermatocytes proliferate within the seminiferous tubule (Gondos and Byskov, 1981). The cellular mechanism through which CLDN11 is assembled at the basal inter–Sertoli TJs is not active in Sertoli cells during the early postnatal period in the rabbit testis. Taking into account that the BTB develops when the meiotic spermatocytes proliferate within the seminiferous tubule, CLDN11 may not actively participate in the formation of the BTB but may function as a mode of cellular adhesion between adjacent Sertoli cells in the immature rabbit testis.

In the adult rabbit testis on PND 180, CLDN11 mRNA levels markedly increased. Similarly, CLDN11 mRNA levels increased during the prepubertal period (Hellani et al, 2000). In rodent Sertoli cells, transcription of the CLDN11 gene is activated by androgens and by the bindings of GATA, nuclear factor YA (NF-YA), and cAMP response element–binding protein to the GATA/NF-Y region of the CLDN11 gene promoter (Gye, 2003a; Florin et al, 2005; Tan et al, 2005; Kaitu'u-Lino et al, 2007; Lui et al, 2007). Therefore, adulthood increases in CLDN11 mRNA may be attributable to the

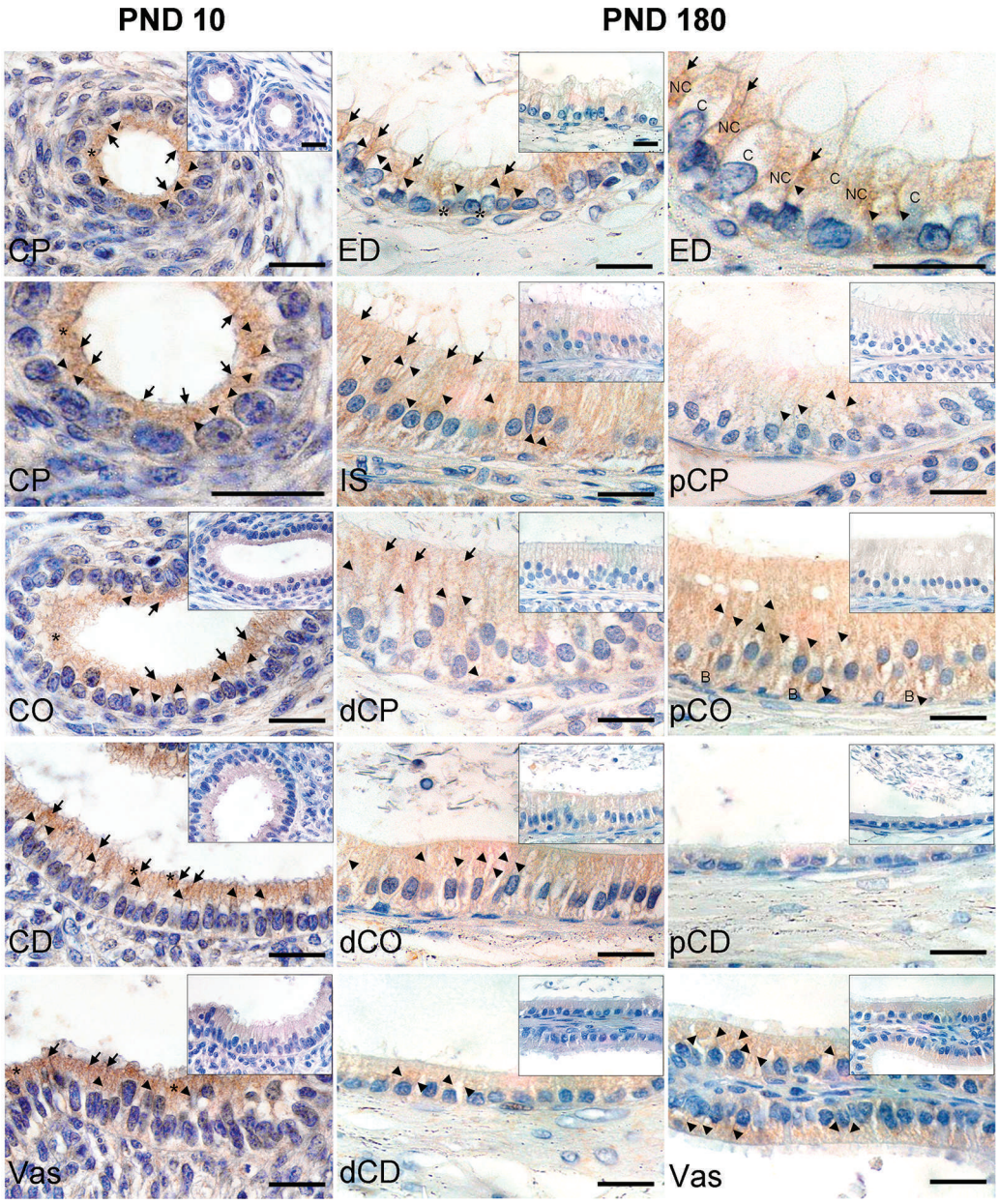

Figure 7. Immunohistochemical localizations of claudin-11 (CLDN11) in the rabbit efferent duct (ED), epididymis, and vas deferens (Vas). On postnatal day (PND) 10, CLDN11 was found in the apical (arrows) and lateral (arrowheads) contacts between epithelial cells and in the apical cytoplasm of epithelial cells (asterisks). On PND 180, CLDN11 was found in the efferent duct at the apicolateral contacts (arrows) between nonciliated cells (NC) and ciliated cells (C). In the initial segment (IS) and caput (CP) epididymis, apical (arrows) and lateral contacts (arrowheads) between epithelial cells were weakly positive for CLDN11. In other segments, CLDN11 immunoreactivity was largely localized at the lateral contacts between adjacent epithelial cells (arrowheads). Of note, CLDN11 was most readily found in the corpus (CO) and cauda (CD) epididymis at the contacts (arrowheads) between principal cells and basal cells (B). In the negative control, in which rabbit normal immunoglobulin G was used instead of primary antibody, nonspecific reactivity was not observed. Scale bar = 20 μm. Please see Figure 1 caption for further explanation of abbreviations. Color figure available online at www.andrologyjournal.org.

increase in androgens and the activation of CLDN11 gene transcription factors in differentiated Sertoli cells. In the adult rabbit testis, CLDN11 protein demonstrated differential subcellular localization in the seminiferous epithelia according to the spermatogenic cycle. This was similar to the localization pattern of CLDN11 in mouse and hamster testes (Hellani et al, 2000; Tarulli et al, 2008; Komljenovic et al, 2009). In most of the seminiferous epithelia, spermatogonia and early spermatocytes were outside of the region of CLDN11 immunoreactivity, which had a wavy appearance and was located parallel to the basal lamina. In addition, the double-immunofluorescence labeling experiment revealed colocalization of CLDN11 and ZO-1 at the basal

part of the seminiferus epithelium, indicating the active assembly of CLDN11 at the basal inter–Sertoli TJs in the adult rabbit testis. CLDN11 was also found at the basolateral cell contacts between Sertoli cells and germ cells perpendicular to the basal lamina, suggesting the participation of CLDN11 in the adhesion between the cells. The BTB in the basal part of the seminiferous epithelium is composed of inter–Sertoli TJs, basal ectoplasmic specialization (ES), basal tubulobulbar complexes (TBCs), and desmosomelike junctions between apposing Sertoli cells. Of these, basal TBCs and basal ES mediate the anchoring junction between Sertoli cells and germ cells (Lui and Cheng, 2007). Therefore, CLDN11 at the basal TBC and basal ES may also support the BTB in the adult rabbit testis. Of note, CLDN11 immunoreactivity was also found with ZO-1 in the basal parts of Sertoli cells adjacent to the basal lamina in the adult rabbit testis. Similarly, in mouse hamster testes, CLDN11 was also found at the basal interface between Sertoli cells and basal lamina (Hellani et al, 2000; Tarulli et al, 2008; Komljenovic et al, 2009), suggesting that CLDN11 may also participate in the newly formed basal TJs. Taking into account the CLDN11 interaction with β1 integrin in oligodendrocytes (Tiwari-Woodruff et al, 2001), CLDN11 may participate in a cell matrix type of anchoring junction in Sertoli cells, which may be important for the structural integrity of the seminiferous epithelium.

Developmental Changes in CLDN11 Expressions in the Rabbit Efferent Duct and Epididymis

CLDN11 mRNA levels were relatively high in all epididymal segments in the immature rabbit on PND 10. Although the CLDN11 mRNA level in the caput epididymis (including the efferent duct and initial segment) was significantly higher than those of the corpus and cauda epididymis and vas deferens, the subcellular localization of CLDN11 in the epithelium was not largely different among the epididymal segments on PND 10; CLDN11 was found in the apicolateral contacts between epithelial cells, indicating that CLDN11 is important for TJs and cell adhesion in the immature rabbit epididymis. This also suggests a low level of segmental differentiation in the rabbit epididymis during the early postnatal period due to an insufficiency of androgens or other testicular factors. Similarly, in the immature rat epididymis, CLDN4, CLDN7, occludin, and ZO-1 were not localized exclusively to apical TJs, but rather were all along the lateral plasma membrane of the epithelium (DeBellefeuille et al, 2003; Gregory and Cyr, 2006; Inai et al, 2007). Epithelial TJ strands were continuous and impermeable to lanthanum nitrate in the rat epididymis on PND 7 (Guan et al, 2005). Therefore, CLDN11 may participate in the TJs between epithelial cells, which function as a paracellular barrier in the epididymis even before active spermatogenesis and transport of spermatozoa into the epididymis on PND 10 in rabbit. Of note, CLDN11 immunoreactivity was found in the apical cytoplasm of epithelial cells. CLDNs are palmitoylated and oligomerized in the Golgi apparatus and are internalized by endocytosis together with other TJ proteins (Findley and Koval, 2009). Therefore, cytoplasmic CLDN11 may be a recycled form in the Golgi apparatus in epithelial cells.

The CLDN11 mRNA level was markedly decreased in the adult epididymis compared with those of immature animals, indicating down-regulation of CLDN11 gene expression during the adult differentiation of these organs. The CLDN11 mRNA level was the highest in the efferent duct, followed by those of the distal cauda, vas deferens, distal cauda epididymis, and proximal corpus. The CLDN11 mRNA level was low in the initial segment, caput, distal corpus, and proximal cauda. This indicates differential control of CLDN11 gene activation along the length of the male excurrent duct. Rat epididymal cDNA microarray data revealed vast differences in gene expression among the first 4 segments of the epididymis (Turner et al, 2007). In the mouse epididymis, the initial segment (segment 1) has a transcription profile that distinguishes it from subsequent segments (Johnston et al, 2005; Jelinsky et al, 2007). Therefore, the marked differences in CLDN11 mRNA expression among the segments of the head of the rabbit epididymis are not an uncommon occurrence in mammals. In the efferent duct epithelium composed of ciliated and nonciliated cells (Ilio and Hess, 1994), CLDN11 immunoreactivity was found at the apicolateral surface, indicating that CLDN11 may participate in the apical TJs as well as cell adhesion in the efferent duct epithelium, where it contributes to structural integrity and water absorption. Although the initial segment is important for the reabsorption of water and ions from the testicular fluid (Clulow et al, 1998), the CLDN11 mRNA level was low, and weak CLDN11 immunoreactivity was found in narrow cells that play an important role in luminal acidification (Shum et al, 2009), suggesting that CLDN11 in the narrow cell TJs may contribute to the luminal acidification in these segments. Similarly, in the caput epididymis, there were basal levels of CLDN11 mRNA, and only weak CLDN11 immunoreactivity was found at the apicolateral contacts between principal cells. In rats, CLDN1, CLDN3, and CLDN4 are expressed in the epithelial cells in the head of the epididymis (Gregory et al, 2001; Gregory and Cyr, 2006), and other TJ proteins may compensate for the lack or insufficiency of CLDN11 TJs between adjacent epithelial cells. In the corpus epididymis,

weak CLDN11 immunoreactivity was found in the basal cells, which sense the luminal environment via transepithelial projections in the epididymis (Shum et al, 2008, 2009). Given that TJs do not develop between principal cells and basal cells (Cyr et al, 1995), CLDN11 may serve as an adhesion molecule in the corpus epididymis at contacts between principal cells and basal cells. Taking into account that CLDN11 expression was low in the principal cells, the basal cell seems to be the major contributor for CLDN11 in the distal part of the epididymis.

The zonulae occludentes domain, the principal structural component of the BEB, differed in permeability, width, and strand number along the duct of the epididymis. A greater number of strands of occluding junctions correlates well with decreased junction permeability, especially in the cauda epididymis (Lopez et al, 1997). In the adult rabbit epididymis, the CLDN11 mRNA level was relatively low in the distal corpus through the proximal cauda. Although the CLDN11 mRNA level increased little in the distal cauda, CLDN11 immunoreactivity was weak to negligible in the apical contacts between epithelial cells. Similarly, in the rat, the CLDN11 mRNA level was decreased in the distal part of the epididymis, where there was no detectable CLDN11 protein (Guan et al, 2005; Gregory and Cyr, 2006). These data suggest that CLDN11 may not directly participate in the principal cell TJs in the distal part of epididymis. Other TJ proteins may compensate for the lack or insufficiency of apical CLDN11 TJs in the distal part of the epididymis and vas deferens. In the vas deferens, the CLDN11 mRNA level was significantly higher than those of the distal corpus and proximal cauda epididymis, and weak CLDN11 immunoreactivity was found at the lateral contacts between epithelial cells. This suggests that CLDN11 may participate in the creation of the luminal environment via cell adhesion in the vas deferens epithelium.

In summary, CLDN11 may function as a structural component in the BTB and anchoring junctions between Sertoli cells and gonocytes and between Sertoli cells and the basal lamina in the rabbit testis. In the excurrent duct system, CLDN11 may participate in the epithelial TJs and cell adhesion during immaturity. In adults, CLDN11 may participate in cell adhesion in nonciliated cells in the efferent duct and in the narrow cells and basal cells in the epididymal segments, contributing to the structural integrity of the luminal epithelium and supporting the creation of a luminal environment for sperm maturation and storage.

References

Bielli A, Gastel T, Castrillejo A, Morana A, Rodriguez-Martinez H. Pubertal development of intersertoli cell junctions in the testis of corriedale ram lambs. *Acta Vet Scand*. 1995;36:543–551.

Cambrosio Mann M, Friess AE, Stoffel MH. Blood-tissue barriers in the male reproductive tract of the dog: a morphological study using lanthanum nitrate as an electron-opaque tracer. *Cells Tissues Organs*. 2003;174:162–169.

Cavicchia JC, Sacerdote FL, Ortiz L. The human blood-testis barrier in impaired spermatogenesis. *Ultrastruct Pathol*. 1996;20:211–218.

Clulow J, Jones RC, Hansen LA, Man SY. Fluid and electrolyte reabsorption in the ductuli efferentes testis. *J Reprod Fertil Suppl*. 1998;53:1–14.

Connell CJ. A freeze-fracture and lanthanum tracer study of the complex junction between Sertoli cells of the canine testis. *J Cell Biol*. 1978;76:57–75.

Cyr DG, Hermo L, Egenberger N, Mertineit C, Trasler JM, Laird DW. Cellular immunolocalization of occludin during embryonic and postnatal development of the mouse testis and epididymis. *Endocrinology*. 1999;140:3815–3825.

Cyr DG, Robaire B, Hermo L. Structure and turnover of junctional complexes between principal cells of the rat epididymis. *Microsc Res Tech*. 1995;30:54–66.

DeBellefeuille S, Hermo L, Gregory M, Dufresne J, Cyr DG. Catenins in the rat epididymis: their expression and regulation in adulthood and during postnatal development. *Endocrinology*. 2003;144:5040–5049.

Dubé E, Chan PT, Hermo L, Cyr DG. Gene expression profiling and its relevance to the blood-epididymal barrier in the human epididymis. *Biol Reprod*. 2007;76:1034–1044.

Dubé E, Dufresne J, Chan PT, Hermo L, Cyr DG. Assessing the role of claudins in maintaining the integrity of epididymal tight junctions using novel human epididymal cell lines. *Biol Reprod*. 2010;82:1119–1128.

Florin A, Maire M, Bozec A, Hellani A, Chater S, Bars R, Chuzel F, Benahmed M. Androgens and postmeiotic germ cells regulate claudin-11 expression in rat Sertoli cells. *Endocrinology*. 2005;146:1532–1540.

Findley MK, Koval M. Regulation and roles for claudin-family tight junction proteins. *IUBMB Life*. 2009;61:431–437.

Gondos B, Byskov AG. Germ cell kinetics in the neonatal rabbit testis. *Cell Tissue Res*. 1981;215:143–151.

Gow A, Southwood CM, Li JS, Pariali M, Riordan GP, Brodie SE, Danias J, Bronstein JM, Kachar B, Lazzarini RA. CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. *Cell*. 1999;99:649–659.

Gregory M, Cyr DG. Identification of multiple claudins in the rat epididymis. *Mol Reprod Dev*. 2006;73:580–588.

Gregory M, Dufresne J, Hermo L, Cyr D. Claudin-1 is not restricted to tight junctions in the rat epididymis. *Endocrinology*. 2001;142:854–863.

Guan X, Inai T, Shibata Y. Segment-specific expression of tight junction proteins, claudin-2 and -10, in the rat epididymal epithelium. *Arch Histol Cytol*. 2005;68:213–225.

Gye MC. Changes in the expression of claudins and transepithelial electrical resistance of mouse Sertoli cells by Leydig cells coculture. *Int J Androl*. 2003a;26:271–278.

Gye MC. Expression of claudin-1 in mouse testis. *Arch Androl*. 2003b;49:271–279.

Gye MC. Expression of tight junction proteins in dog testis and epididymis. *Reprod Dom Anim*. 2004;39:43–47.

Gye MC, Lee YH, Kim C, Kim MK, Lee H. Expression of zonnula occludens-1 in mouse testis. *Dev Reprod*. 2000;4:37–43.

Hellani A, Ji J, Mauduit C, Deschildre C, Tabone E, Benahmed M. Developmental and hormonal regulation of the expression of oligodendrocyte-specific protein/claudin 11 in mouse testis. *Endocrinology*. 2000;141:3012–3019.

Hermo L, Korah N, Gregory M, Liu LY, Cyr DG, D'Azzo A, Smith CE. Structural alterations of epididymal epithelial cells in cathepsin a deficient mice affect the blood-epididymal barrier and lead to altered sperm motility. *J Androl*. 2007;28:784–797.

Ikenouchi J, Furuse M, Furuse K, Sasaki H, Tsukita SA, Tsukita SH. Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. *J Cell Biol*. 2005;171:939–945.

Ilio KY, Hess RA. Structure and function of the ductuli efferentes: a review. *Microsc Res Tech*. 1994;29:432–467.

Inai T, Sengoku A, Hirose E, Iida H, Shibata Y. Claudin-7 expressed on lateral membrane of rat epididymal epithelium does not form aberrant tight junction strands. *Anat Rec (Hoboken)*. 2007; 290:1431–1438.

Jelinsky SA, Turner TT, Bang HJ, Finger JN, Solarz MK, Wilson E, Brown EL, Kopf GS, Johnston DS. The rat epididymal transcriptome: comparison of segmental gene expression in the rat and mouse epididymides. *Biol Reprod*. 2007;76:561–570.

Johnston DS, Jelinsky SA, Bang HJ, DiCandeloro P, Wilson E, Kopf GS, Turner TT. The mouse epididymal transcriptome: transcriptional profiling of segmental gene expression in the epididymis. *Biol Reprod*. 2005;73:404–413.

Kaitu'u-Lino TJ, Sluka P, Foo CF, Stanton PG. Claudin-11 expression and localisation is regulated by androgens in rat Sertoli cells in vitro. *Reproduction*. 2007;133:1169–1179.

Komljenovic D, Sandhoff R, Teigler A, Heid H, Just WW, Gorgas K. Disruption of blood-testis barrier dynamics in ether-lipid-deficient mice. *Cell Tissue Res*. 2009;337:281–299.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*. 1970;227:680–685.

Landon GV, Pryor JP. The blood-testis barrier in men of diverse fertility status: an ultrastructural study. *Virchows Arch Pathol Anat*. 1981;392:355–364.

Lopez ML, Fuentes P, Retamal C, De Souza W. Regional differentiation of the blood-epididymis barrier in stallion (*Equus caballus*). *J Submicrosc Cytol Pathol*. 1997;29:353–363.

Lui WY, Cheng CY. Regulation of cell junction dynamics by cytokines in the testis: a molecular and biochemical perspective. *Cytokine Growth Factor Rev*. 2007;18:299–311.

Lui WY, Wong EW, Guan Y, Lee WM. Dual transcriptional control of claudin-11 via an overlapping GATA/NF-Y motif: positive regulation through the interaction of GATA, NF-YA, and CREB and negative regulation through the interaction of Smad, HDAC1, and mSin3A. *J Cell Physiol*. 2007;211:638–648.

Meng J, Holdcraft RW, Shima JE, Griswold MD, Braun RE. Androgens regulate the permeability of the blood-testis barrier. *Proc Natl Acad Sci U S A*. 2005;102:16696–16700.

Meyer R, Posalaky Z, McGinley D. Intercellular junction development in maturing rat seminiferous tubules. *J Ultrastruct Res*. 1977; 61:271–283.

Mitic LL, Van Itallie CM, Anderson JM. Molecular physiology and pathophysiology of tight junctions. I. Tight junction structure and function: lessons from mutant animals and proteins. *Am J Physiol Gastrointest Liver Physiol*. 2000;279:G250–G254.

Morita K, Sasaki H, Fujimoto K, Furuse M, Tsukita S. Claudin-11/ OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis. *J Cell Biol*. 1999;145:579–588.

Moroi S, Saitou M, Fujimoto K, Sakakibara A, Furuse M, Yoshida O, Tsukita S. Occludin is concentrated at tight junctions of mouse/rat but not human/guinea pig Sertoli cells in testes. *Am J Physiol*. 1998;274:C1708–C1717.

Morrow CM, Tyagi G, Simon L, Carnes K, Murphy KM, Cooke PS, Hofmann MC, Hess RA. Claudin 5 expression in mouse seminiferous epithelium is dependent upon the transcription factor ets variant 5 and contributes to blood-testis barrier function. *Biol Reprod*. 2009;81:871–879.

Nagano T, Suzuki F. The postnatal development of the junctional complexes of the mouse Sertoli cells as revealed by freeze-fracture. *Anat Rec*. 1976;185:403–417.

Orgebin-Crist MC. Studies on the function of the epididymis. *Biol Reprod*. 1969;(suppl 1):155–175.

Pelletier RM. Cyclic modulation of Sertoli cell junctional complexes in a seasonal breeder: the mink (*Mustela vison*). *Am J Anat*. 1988; 183:68–102.

Russell LD, Bartke A, Goh JC. Postnatal development of the Sertoli cell barrier, tubular lumen, and cytoskeleton of Sertoli and myoid cells in the rat, and their relationship to tubular fluid secretion and flow. *Am J Anat*. 1989;184:179–189.

Russell LD, Peterson RN. Sertoli cell junctions: morphological and functional correlates. *Int Rev Cytol*. 1985;94:177–211.

Shum WW, Da Silva N, Brown D, Breton S. Regulation of luminal acidification in the male reproductive tract via cell-cell crosstalk. *J Exp Biol*. 2009;212:1753–1761.

Shum WW, Da Silva N, McKee M, Smith PJ, Brown D, Breton S. Transepithelial projections from basal cells are luminal sensors in pseudostratified epithelia. *Cell*. 2008;135:1108–1117.

Tan KA, De Gendt K, Atanassova N, Walker M, Sharpe RM, Saunders PT, Denolet E, Verhoeven G. The role of androgens in Sertoli cell proliferation and functional maturation: studies in mice with total or Sertoli cell-selective ablation of the androgen receptor. *Endocrinology*. 2005;146:2674–2683.

Tarulli GA, Meachem SJ, Schlatt S, Stanton PG. Regulation of testicular tight junctions by gonadotrophins in the adult Djungarian hamster in vivo. *Reproduction*. 2008;135:867–877.

Tiwari-Woodruff SK, Buznikov AG, Vu TQ, Micevych PE, Chen K, Kornblum HI, Bronstein JM. OSP/claudin-11 forms a complex with a novel member of the tetraspanin super family and beta1 integrin and regulates proliferation and migration of oligodendrocytes. *J Cell Biol*. 2001;153:295–305.

Tsukita S, Furuse M, Itoh M. Structural and signalling molecules come together at tight junctions. *Curr Opin Cell Biol*. 1999;11: 628–633.

Tsukita S, Yamazaki Y, Katsuno T, Tamura A, Tsukita S. Tight junction-based epithelial microenvironment and cell proliferation. *Oncogene*. 2008;27:6930–6938.

Turner TT, Johnston DS, Finger JN, Jelinsky SA. Differential gene expression among the proximal segments of the rat epididymis is lost after efferent duct ligation. *Biol Reprod*. 2007;77:165–171.

Van Itallie CM, Anderson JM. Claudins and epithelial paracellular transport. *Annu Rev Physiol*. 2006;68:403–429.

Wang RS, Yeh S, Chen LM, Lin HY, Zhang C, Ni J, Wu CC, di Sant'Agnese PA, deMesy-Bentley KL, Tzeng CR, Chang C. Androgen receptor in sertoli cell is essential for germ cell nursery and junctional complex formation in mouse testes. *Endocrinology*. 2006;147:5624–5633.

Yoon SI, Park CJ, Nah WH, Gye MC. Expression of occludin in testis and epididymis of wild rabbits, *Lepus sinensis coreanus. Reprod Dom Anim*. 2009;44:745–750.