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Expression of *Notch* Pathway Components in Spermatogonia and Sertoli Cells of Neonatal Mice

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ABSTRACT: Members of the *Notch* gene family have been shown to play an important role in the control of cell fate in many developmental systems. We hypothesized that the fate of the male germ line stem cells may also be mediated through the Notch signaling pathway. We therefore sought to determine whether the components of the Notch pathway are expressed in the mouse testis. Western blot analysis revealed the expression of three Notch receptors (Notch 1, Notch 2, and Notch 3), Notch ligands (Jagged 1, Jagged 2, and Delta 1), and presenilin 1 (PS1) in neonatal mouse testis. We then examined their cellular localization by immunohistochemical analysis of cocultures of spermatogonia and Sertoli cells. The 3 Notch receptors were found to be expressed in spermatogonia. Sertoli cells expressed only Notch 2 receptor. Among the Notch ligands, Delta 1 and Jagged 1 were localized exclusively in spermatogonia and Sertoli cells, respectively. PS1 was apparent in both spermatogonia and Sertoli cells. The presence of Notch receptors and Notch ligands in spermatogonia and Sertoli cells indicates that these cells are capable of responding to and eliciting Notch signaling during the process of spermatogenesis.

Key words: Cell fate, delta, jagged, presenilin, spermatogenesis.

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Spermatogenesis is a process that begins with the proliferation of spermatogonial stem cells and culminates with the production of spermatozoa (Russell et al, 1990). Although it is generally accepted that the principal stimuli for vertebrate spermatogenesis are gonadotropins and androgens (Steinberger, 1971; Hansson et al, 1976), the mechanisms by which these hormones regulate spermatogenesis are still elusive. Because type A spermatogonial stem cells are devoid of gonadotropin and androgen receptors (Hansson et al, 1976), their fate (renewal, differentiation, or apoptosis) is likely to be determined by local factors that probably involve interactions between germ cells and Sertoli cells.

The highly conserved Notch signaling pathway, which was originally characterized in *Drosophila*, mediates cell-cell communications that are required for a variety of cell fate decisions in invertebrate and vertebrate developmental processes (Artavanis-Tsakonas et al, 1999). Numerous studies have demonstrated that Notch is involved in the regulation of proliferation (Go et al, 1998), differentiation (Mitsiadis et al, 1998), and apoptotic (Shelly et al, 1999) processes. Four Notch genes (Notch 1, Notch 2, Notch 3, and Notch 4) have been identified in vertebrates (Egan et al, 1998). Notch receptors are initially synthesized as single transmembrane proteins of approximately 300 kDa, and proteolysis, which involves presenilins and other proteases, has been shown to be necessary for their maturation and function (Schroeter et al, 1998). Genes that encode Notch ligands and that have been characterized include Delta and Serrate in *Drosophila* (Bishop et al, 1999); LAG-2 in *Caenorhabditis elegans* (Tax et al, 1994); Delta 1 in *Xenopus* (Chitnis et al, 1995); Delta 1 and Serrate 1 in avians (Henrique et al, 1995; Stone and Rubel, 1999); and Delta 1, Jagged 1, and Jagged 2 in mouse (Mitsiadis et al, 1997; Valsecchi et al, 1997; Beckers et al, 1999), rat (Lindsell et al, 1995; Shawber et al, 1996; Mitsiadis et al, 1999), and human (Gray et al, 1999). After ligand binding, the intracellular domain of Notch enters the nucleus and participates in the transcriptional control of downstream target genes (Weinmaster, 1997). It is generally believed that Jagged and Delta act as transmembrane proteins that interact with Notch receptors expressed on adjacent cells; however, there is also strong evidence to support the role of soluble forms of these ligands in Notch signaling (Varnum-Finney et al, 1998; Qi et al, 1999).

Although some of the Notch system genes have been found to be expressed in rodent testis (Lardelli et al, 1994; Lee et al, 1996), the cellular localization and the possible role of Notch in spermatogenesis have not been addressed. The only information available to date has come from *C elegans* (Crittenden et al, 1994; Arduengo et al, 1998). Given the evolutionary conservation of Notch function, we hypothesized that Notch may be involved in mediating cell fate decisions during mouse spermatogen-

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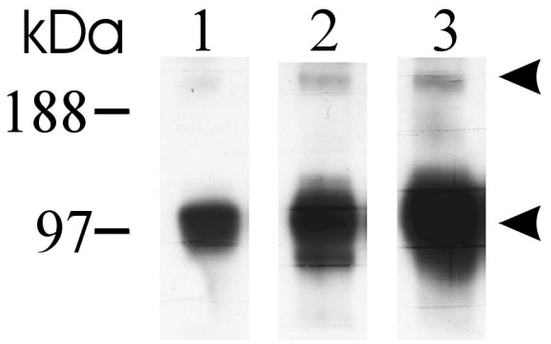

Figure 1. Western blot analysis of immunoprecipitates of testicular extracts from neonatal mice. Lanes 1–3 were probed with antibodies raised against the intracellular domain of Notch receptors. Lane 1 (Notch 1), lane 2 (Notch 2), and lane 3 (Notch 3). Molecular weight markers are indicated to the left of the blots. Arrowheads indicate position of the specific bands.

esis. Therefore, we investigated the expression and cellular localization of the components of the Notch signaling pathway, including Notch receptors (Notch 1, Notch 2, and Notch 3), Notch ligands (Jagged 1, Jagged 2, and Delta 1), and presenilin 1 (PS1) in mouse testis. Here, we demonstrate that the components of the Notch signaling system are present in spermatogonia and Sertoli cells of the neonatal mouse testis, suggesting a potential role for Notch in the regulation of the fate of spermatogonial stem cells during the process of spermatogenesis.

Materials and Methods

Animals

BALB/c mice were purchased from the Charles River Breeding Laboratories, Inc (Wilmington, Mass). The animals were maintained in the animal care facility at Georgetown University Medical Center, Washington, DC, on a 12-hour light:dark cycle and were given access to food (rodent laboratory chow 5001; Ralston-Purina, St Louis, Mo) and water ad libitum. All animal care procedures were carried out according to the National Research Council's *Guide for the Care and Use of Laboratory Animals*, and were approved by the Animal Care and Use Committee of Georgetown University.

Primary Cell Cultures

Sertoli cells were isolated from neonatal (6-day-old) BALB/c mice as described previously (Dirami et al, 1995). Briefly, decapsulated testes were minced for a short period and suspended in Dulbecco modified Eagle medium (DMEM) containing 500 U/mL collagenase (CLS II) and 5 μg/mL DNAse (Type I) for 10 minutes in a shaking water bath (90 cycles/min) at 34°C. The DMEM contained 1.2 mM calcium and 0.8 mM magnesium. The mixture was allowed to settle and the supernatant was discarded. The sediment, which for the most part consisted of long seminiferous cord fragments, was washed twice with DMEM and resuspended in DMEM containing 1.5 mg/mL collagenase, 5 μg/mL DNAse, and 1 mg/mL hyaluronidase (Type III) for 20–30 minutes in a shaking water bath at 34°C. The digestion was terminated when the peritubular myoid layer was observed to have

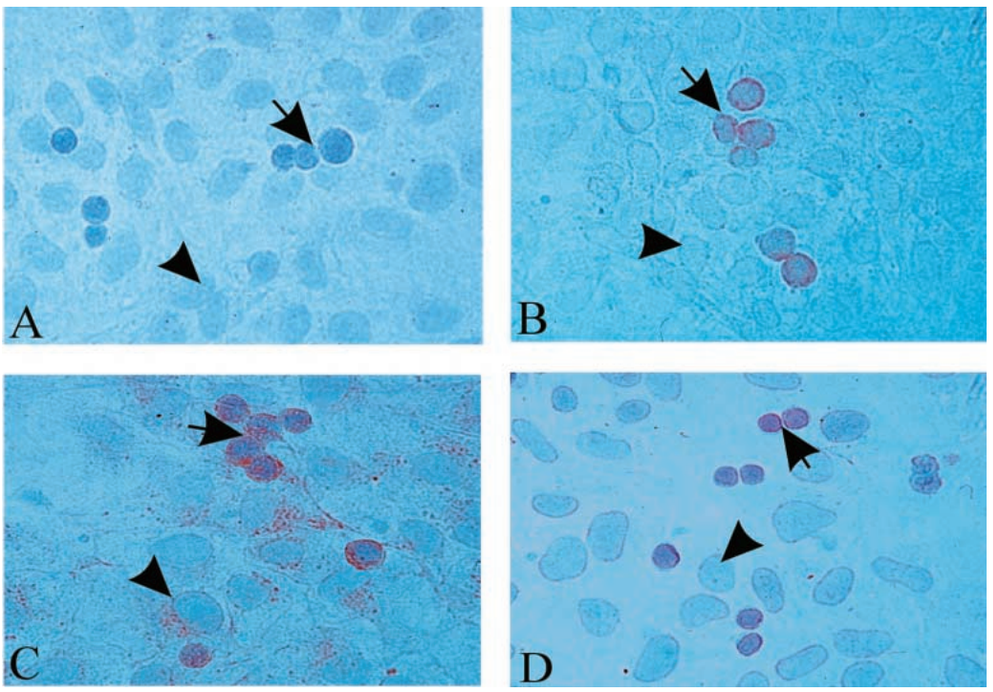

Figure 2. Immunohistochemical localization of Notch receptors in cocultures of Sertoli cells and spermatogonia from neonatal mouse testis. **(B)** Notch 1 is expressed in spermatogonia (arrow); **(C)** Notch 2 in spermatogonia (arrow) and Sertoli cells (arrowhead); and **(D)** Notch 3 in spermatogonia (arrow). The controls **(A)** did not show any immunoreactivity. Magnification, 414×.

fallen away from the base of the Sertoli cells. The seminiferous cord fragments, which were now much smaller in size, were washed twice with DMEM and subjected to a third digestion for approximately 15–20 minutes with collagenase (500 U/mL), hyaluronidase (1 mg/mL), and DNAse (5 μg/mL) until small clumps of 10–50 Sertoli cells were obtained. The clumps were pelleted at low speed centrifugation, washed twice in DMEM, and resuspended in DMEM/F-12 medium supplemented with ITS (insulin, 5 μg/mL; transferrin, 5 μg/mL; and selenium, 5 ng/mL) and 100 ng/mL follicle-stimulating hormone. The purity of each preparation was established by staining for smooth muscle α-actin (a marker for contaminating peritubular myoid cells), *c-kit* (a marker for contaminating type A spermatogonial cells), and 3β-hydroxysteroid dehydrogenase (a marker for contaminating Leydig cells). The purity of Sertoli cells used in the studies ranged between 96% and 98%. The yield of Sertoli cells from 40 6-day-old mice was approximately 100 million. The cells were seeded on laminin-coated coverslips at a density of ~5×10^5 cells per well in 24-well plates, and incubated at 34°C for 3 days.

Type A spermatogonia were isolated from neonatal (6-day-old) BALB/c mice as described previously (Dym et al, 1995). Briefly, the decapsulated testes were suspended in DMEM/F-12 containing collagenase (1.5 mg/mL) and DNAse (1 μg/mL), and incubated at 34°C for 15 minutes in a shaking water bath operated at 100 cycles/min. After 2 washes in DMEM/F-12 medium, seminiferous cord fragments, mostly devoid of interstitial cells, were incubated in DMEM/F-12 medium containing collagenase (1.5 mg/mL), hyaluronidase (1.5 mg/mL), trypsin (0.5 mg/mL), and DNAse (1 μg/mL) for 20–30 minutes using the conditions described above. The dispersed cells were washed twice with medium and filtered through 80 μm and 40 μm nylon mesh (Tetco Inc, Briarcliff Manor, NY), successively. The cells of the dissociated epithelium were then separated by sedimentation velocity at unit gravity at 4°C, with use of a 2%–4% bovine serum albumin (BSA) gradient in DMEM/F-12 medium. The cells were bottom-loaded into an SP-120 chamber in a volume of 30 mL, and a BSA gradient was generated using 275 mL of 2% and 4% BSA. The cells were allowed to sediment for a standard period of 2.5 hours, and then 35 fractions of 15-mL volumes were collected at 90-second intervals. The cells in each fraction were examined under a phase-contrast microscope, and fractions containing cells of similar size and morphology were pooled and spun down by low-speed centrifugation, and then resuspended in DMEM/F-12 medium. Type A spermatogonia were further purified from contaminating Sertoli cells by differential plating in the presence of 5% horse serum for 4 hours (~95%–99% pure). The purity of the cells was established by immunostaining for *c-kit*, smooth muscle α-actin, and 3β-hydroxysteroid dehydrogenase. Approximately 10 million spermatogonia were obtained from 80 6-day-old mice. The cells were added on top of the cultured Sertoli cells and cocultured for a further 48 hours.

Immunocytochemistry

Spermatogonia and Sertoli cell cultures, grown on coverslips in 24 well-plates, were fixed in ice-cold methanol for 4 minutes. Following fixation, the cells were subjected to streptavidin-biotin peroxidase immunostaining using Histostain-SP kits (Zymed Laboratories, Burlingame, Calif) according to the manufacturer's instructions, with the primary antibodies used at a dilution of 1:100. The negative control samples were processed without primary antibodies. The antibodies used in this study were affinity-purified goat polyclonal antisera, generated against a peptide mapping at the carboxy terminus of Notch 1, 2, and 3; Jagged 1 and 2; Delta 1; or PS1 (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif). The cells were counterstained for 3 minutes with hematoxylin and then examined with a Zeiss Axiophot light microscope fitted with a Planapo 63× objective. A positive reaction was characterized by the deposition of a reddish-brown reaction product at the site of the antibody-antigen reaction. The images of positive and control reactions were obtained with a Zeiss 35-mm camera using Kodak color print film. The film was developed and printed using a Fuji automatic developer and printer. The magnification of each photograph was derived as follows: $63 \times 2.5 \times$ area of the print (size of the picture) in millimeters divided by 35 mm (size of the print film). Each experiment was repeated at least 3 times, with similar findings, and representative results are presented.

Preparation of Lysates and Immunoprecipitates

Decapsulated testes from neonatal mouse testis were homogenized with a Dounce homogenizer in ice-cold modified radioimmuno-precipitation assay buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM ethylenediamine tetraacetic acid, 1 mM sodium orthovanadate, 1 mM sodium fluoride, and 1% NP40) containing proteinase inhibitors, including 1 mM phenylmethylsulfonyl fluoride, aprotinin (1 μg/mL), pepstatin (1 μg/mL), and leupeptin (1 μg/mL). The lysates were kept on ice for 15 minutes, then sequentially triturated using 18- and 25-gauge needles attached to a 1-mL syringe. After a 15-minute incubation on ice, the lysates were centrifuged at $14\,000 \times g$ at 4°C for 15 minutes to remove insoluble material. Protein concentration was determined using the Bio-Rad protein assay system (Bio-Rad Laboratories, Hercules, Calif) with BSA as a standard. Notch 1, Notch 2, Notch 3, Jagged 1, Jagged 2, and Delta 1 proteins were immunoprecipitated from whole cell lysates essentially as described in the protocol provided by Santa Cruz Biotechnology. The lysates (100 μg total protein) were first cleared by incubation with Protein G^+ agarose beads (20 μL; Santa Cruz Biotechnology) in 1 mL of lysis buffer at 4°C for 30 minutes and centrifugation at $1000 \times g$ for 5 minutes at 4°C. The supernatants were incubated with the respective primary antibodies (1 μg/mL) for 1 hour at 4°C, followed by addition of Protein G^+ agarose beads (20 μL) and an overnight incubation at 4°C. The immunoprecipitates were collected by centrifugation at $1000 \times g$ for 5 minutes at 4°C and washed in lysis buffer prior to electrophoresis.

Western Blotting

Total lysates (100 μg total protein; PS1 only) and immunoprecipitates from total lysates (100 μg total protein; Notch 1, 2, and 3; Jagged 1 and 2; and Delta 1) were solubilized in 1× NuPAGE LDS sample buffer (Novex, San Diego, Calif), boiled for 3 minutes, centrifuged at $14\,000 \times g$ for 3 minutes, then subjected to electrophoresis on 4%–12% NuPAGE Bis-Tris gels (Novex) using NuPAGE *N*-morpholino propanesulfonic acid (MOPS)/sodium dodecyl sulfate running buffer (Novex). The proteins were

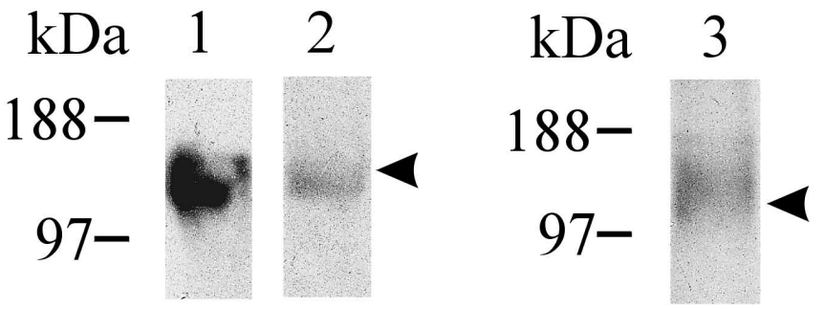

Figure 3. Western blot analysis of immunoprecipitates of testicular extracts from neonatal mice. Lanes 1–3 were probed with antibodies raised against the intracellular domain of Notch ligands. Lane 1 (Jagged 1), lane 2 (Jagged 2), and lane 3 (Delta 1). Molecular weight markers are indicated to the left of the blots. Arrowheads indicate position of the specific bands.

then transferred to a polyvinylidene difluoride membrane (Millipore Co, Bedford, Mass). Blots were blocked with 4% nonfat dry milk in 10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1% Tween 20 [TBST]) for 2 hours at room temperature and incubated with the respective primary antibodies (goat polyclonal antibodies against Notch 1, Notch 2, Notch 3, Jagged 1, Jagged 2, Delta 1, and PS1; 1:100 in TBST) overnight at 4°C. Subsequently, the blots were washed in TBST and incubated with peroxidase-conjugated anti-goat secondary antibody (Santa Cruz Biotechnology), diluted 1:5000 in TBST for 1 hour at room temperature. The proteins were detected using an enhanced chemiluminescence Western blot detection system according to the instructions provided by the manufacturer (Amersham-Pharmacia Biotech Inc, Piscataway, NJ). Each experiment was repeated at least 3 times using extracts isolated from testes of different mice with similar blots, and representative results are presented.

Results

Expression of Notch Receptor Proteins in Neonatal Mouse Testis

Western blot analysis was conducted prior to the immunocytochemical studies to determine whether the Notch receptors (Notch 1, Notch 2, and Notch 3) are expressed in 6-day-old mouse testis. Figure 1 shows immunoblots of immunoprecipitates using specific antibodies directed against the *C*-terminal domain of Notch 1 (lane 1), Notch 2 (lane 2), and Notch 3 (lane 3). The figure also reveals a ~300 kd band (full length) and a more prominent, broader band at ~70–120 kd (the transmembrane domain; Struhl and Adachi, 1998). Notch 3 protein appeared to be expressed at higher levels compared with Notch 1 and Notch 2.

Cellular Localization of Notch Receptor Proteins in Neonatal Mouse Testis

Cocultures of spermatogonia and Sertoli cells isolated from 6-day-old mouse testes were subjected to immunocytochemistry to determine the precise cellular localization of Notch receptors. The negative controls for the 3 receptors did not exhibit any immunoreactivity (Figure 2A). The three Notch receptors were expressed in spermatogonia (Figure 2B, C, and D, respectively), whereas only Notch 2 was apparent in Sertoli cells (Figure 2C). The 3 Notch receptors appear to be specifically localized to the cytoplasm in spermatogonia, whereas a characteristic punctate type of immunoreactivity was seen in Sertoli cells.

Expression of Notch Ligand Proteins in Neonatal Mouse Testis

Western blotting analysis for Notch ligands in 6-day-old mouse testis (Figure 3) revealed the presence of Jagged 1 (lane 1), Jagged 2 (lane 2), and Delta 1 (lane 3). The expression of Jagged 1 was relatively stronger in comparison with Jagged 2 and Delta 1. Jagged 1 and Jagged 2 appeared within the molecular weight range of 150–170 kd (Schroeter et al, 1998; Struhl and Adachi, 1998) and Delta 1 appeared at ~110 kd (Fehon et al, 1990).

Cellular Localization of Notch Ligand Proteins in Neonatal Mouse Testis

The negative controls for Jagged 1, Jagged 2, and Delta 1 did not show any immunoreactivity (Figure 4A). In the spermatogonia/Sertoli cell coculture system, specific localization of Jagged 1 was observed exclusively in Sertoli cells (Figure 4B), the immunoreactivity being localized to the cytoplasm. The cells that stained for Jagged 2 did not exhibit any immunostaining in Sertoli cells (data not shown). However, another Notch ligand, Delta 1, which was detected in the whole testis by Western blot analysis, was specifically localized to spermatogonial cells only (Figure 4C). The immunostaining of spermatogonia for Delta 1 appeared to be heterogeneous and weak. There was no evidence of Delta 1 immunostaining in Sertoli cells.

Distribution of Presenilin 1 in Neonatal Mouse Testis

PS1 immunoblots of lysates from neonatal mouse testis revealed 2 bands at approximately 20 kd and 50 kd that

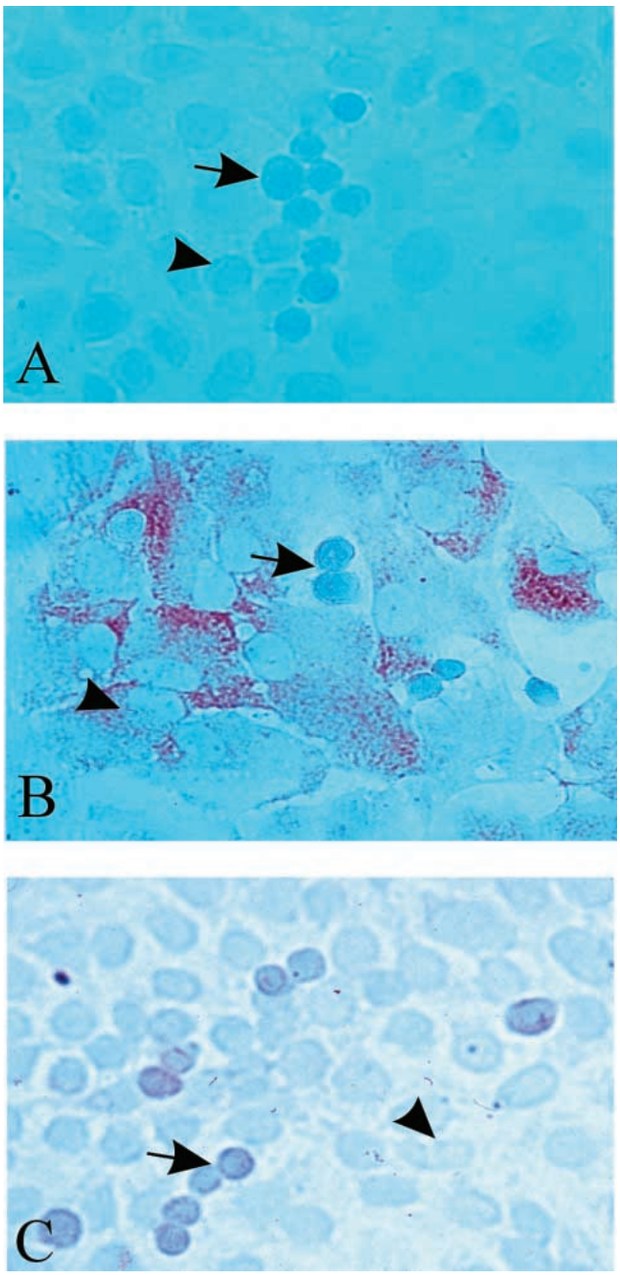

Figure 4. Immunocytochemical localization of Notch ligands in cocultures of Sertoli cells and spermatogonia from neonatal mouse testis. Jagged 1 **(B)** and Delta 1 **(C)** were exclusively expressed in Sertoli cells (arrowhead) and spermatogonia (arrow), respectively. The controls **(A)** did not show any immunoreactivity. Magnification, 414×.

correspond to the *C*-terminal domain and full-length PS1, respectively (Figure 5), consistent with the sizes reported in previous studies (Thinakaran et al, 1996; Yamaguchi et al, 2000). Other bands of higher molecular weights, which may constitute the aggregated forms of PS1 (Yamaguchi et al, 2000), were also apparent.

Cocultures of isolated spermatogonia and Sertoli cells

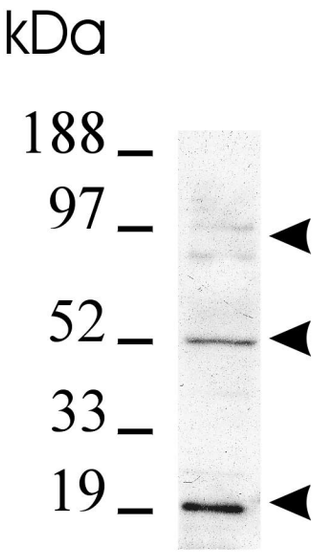

Figure 5. Western blot analysis of testicular extract from neonatal mice. The blot was probed with an antibody raised against the intracellular domain of PS1. Molecular weight markers are indicated to the left of the blots. Arrowheads indicate the position of specific bands.

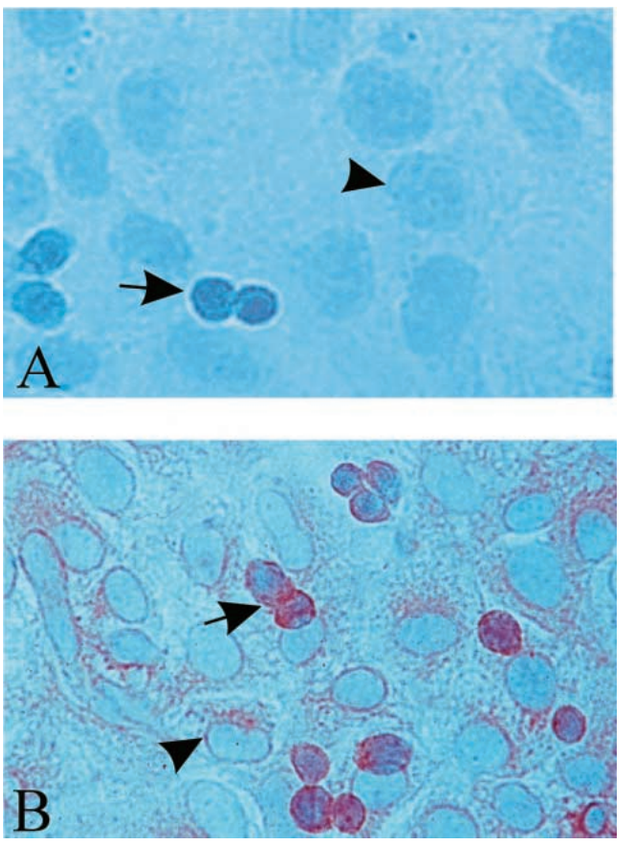

Figure 6. Immunocytochemical localization of PS1 in cocultures of Sertoli cells and spermatogonia from neonatal mouse testis. Both spermatogonia (arrow) and Sertoli cells (arrowhead) exhibited immunoreactivity for PS1 **(B)**. The controls **(A)** did not show any immunostaining (A). Magnification, 414×.

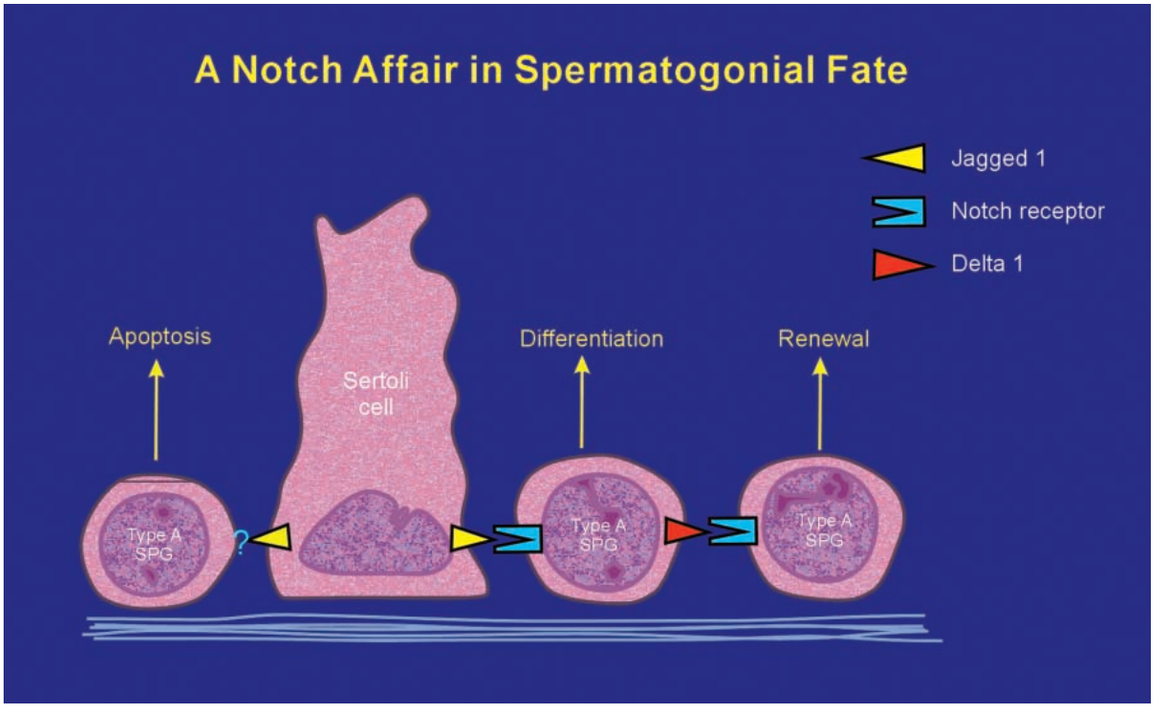

Figure 7. Schematic drawing of a hypothetical model for the role of the Notch system in the fate of type A spermatogonial stem cells (SPG).

from neonatal mouse testes, subjected to immunocytochemistry, revealed the expression of PS1 in both spermatogonia and Sertoli cells (Figure 6B). The immunoreactive deposits appear to be strongly localized in the cytoplasm of spermatogonia compared with their localization in the cytoplasm of Sertoli cells. The negative controls did not show any immunoreactivity (Figure 6A).

Discussion

Defining the cellular mechanisms responsible for the differentiation and self-renewal of spermatogonial stem cells is central to achieving a complete understanding of the process of spermatogenesis. Spermatogonial stem cells must continuously undergo proliferation and differentiation, while also maintaining a pool of uncommitted stem cells to support spermatogenesis. Despite various attempts aimed at depicting the factors that initiate spermatogenesis, the mechanisms involved in the determination of cell fate decisions during spermatogenesis remain obscure. Notch is a general regulator of cell fate determination during development in a wide range of tissues and organs throughout the animal kingdom (Varnum-Finney et al, 1998; Artavanis-Tsakonas et al, 1999). In this study, we demonstrated for the first time that Notch signaling protein components are present in the mouse testis, suggesting an important role for Notch in the regulation of spermatogenesis.

The 3 Notch receptor proteins (Notch 1, Notch 2, and Notch 3) examined were detected in neonatal mouse testes. Their cellular expression patterns overlap in spermatogonia in which the 3 receptors were colocalized. In contrast, a distinct expression pattern was apparent in Sertoli cells that stained only for Notch 2. This is consistent with previous observations that demonstrated complementary and combinatorial patterns of Notch 1, 2, and 3 gene expression during mouse embryogenesis (Lardelli et al, 1994; Williams et al, 1995). The presence of Notch receptors in spermatogonia indicates that Notch may play a major role in determining the fate of these cells.

Although the Notch receptor mutant mouse models demonstrated a crucial role for the Notch system in the development of various organs, the role of the system in spermatogenesis has not been addressed, probably due to their embryonic lethality (Swiatek et al, 1994; Hamada et al, 1999). The pattern of expression of different Notch molecules may permit distinct functions within the same tissue, as suggested by Notch 1, 2, and 3 expression in developing teeth (Mitsiadis et al, 1995). Structural differences in the extracellular and intracellular domains of the Notch receptors may also define distinct activities (Egan et al, 1998; Hicks et al, 2000). Coexpression of several Notch receptors in a given cell type (eg, spermatogonia)

may not necessarily result in potentiation if the Notch receptors are activated simultaneously; a suppressive effect may occur, as was recently reported by Beatus et al (1999), who demonstrated that the intracellular domain of Notch 3 represses Notch 1 activity by competing with the Notch 1 intracellular domain for access to Notch signaling molecules (RBP-JK and a common coactivator). The presence of Notch receptors in both germ cells and somatic cells indicates that the Notch signaling pathway may be involved in the regulation of differentiation of germ cells as well as Sertoli cells. The presence of the basic helix-loop-helix E47 in differentiating Sertoli cells (Chaudhary and Skinner, 1999) and its inhibition by activated Notch 1 and Notch 2 (Ordentlich et al, 1998) supports a role for Notch in Sertoli cell differentiation.

Notch signaling can occur either among a group of equivalent cells (homotypic interactions) or between nonequivalent cells (heterotypic interactions) (Artavanis-Tsakonas et al, 1999). In the present study, we found that the Notch ligands, Jagged 1 and Delta 1, are present in immature mouse testis. Although Jagged 1 was restricted to Sertoli cells, Delta 1 was localized exclusively in spermatogonia. The initiation of spermatogenesis may involve homotypic interactions among spermatogonia mediated by Delta 1 (Figure 7). Notch activation through this type of cellular interaction results in what has been termed *lateral inhibition* (Artavanis-Tsakonas et al, 1995). The apparent differential expression of Delta 1 in the spermatogonial population suggests that such a process may occur in the testis to determine the fate of spermatogonial stem cells. The spermatogonial cells with high levels of Delta 1, which are committed to differentiation, may activate Notch in the neighboring cells to inhibit their differentiation and thus allow stem cell renewal (Henrique et al, 1995; Stone and Rubel, 1999). Jagged 1, which is expressed only in Sertoli cells, may mediate heterotypic interactions between Sertoli cells and spermatogonia (Figure 7). The fate of spermatogonia may be determined by Jagged 1, produced by Sertoli cells, which has been shown to increase the proliferation of murine bone marrow precursor cells (Varnum-Finney et al, 1998) and inhibit oligodendrocyte differentiation (Wang et al, 1998). In addition, Jagged 1 may regulate apoptosis, which has been shown to be inhibited as a result of Notch receptor activation (Shelly et al, 1999). Because Jagged 1 is a ligand for multiple Notch receptors (Shimizu et al, 1999), the Jagged 1 produced by Sertoli cells has the capacity to activate Notch in spermatogonia, where the 3 receptors (Notch 1, 2, and 3) are found. However, Jagged 1 may exert specific effects on these cells, depending on the types and levels of other local factors in their vicinity (Beatus et al, 1999; Walker et al, 1999).

Notch maturation and signaling involve the proteolytic processing of the Notch receptor (Logeat et al, 1998; Schroeter et al, 1998). Full-length Notch protein is first cleaved by a furin-like protease in the trans-Golgi network during trafficking of Notch to the cell surface (Schroeter et al, 1998), resulting in the formation of the mature Notch receptor, which consists of a Notch transmembrane domain/Notch extracellular domain heterodimer found at the plasma membrane (Blaumueller et al, 1997). Ligand binding at the cell surface triggers cleavage at the second extracellular site by a disintegrin-metaloprotease tumor necrosis factor-α converting enzyme (Brou et al, 2000) followed by proteolysis at the transmembrane/intracellular site, involving a γ secretase, to release Notch intracellular domain, which translocates to the nucleus and modifies transcription of target genes and thereby affects cell-fate choices (Egan et al, 1998; Schroeter et al, 1998; Bishop et al, 1999). Membrane proteins with multiple transmembrane domains (Nakai et al, 1999) were found to be expressed ubiquitously in most mammalian tissues (Lee et al, 1996; Suzuki et al, 1996; Lah et al, 1997). Findings in *Drosophila*, *C elegans*, and mouse indicate a functional link between the presenilins and Notch signaling (Levitan and Greenwald, 1998; Nakai et al, 1999). There is also evidence of physical contact between Notch and presenilin 1 (Ray et al, 1999).

The present study provides Western blot and immunocytochemical analysis of PS1 in neonatal mouse testis using a polyclonal antibody specific for the *C*-terminal domain of PS1. PS1 protein was present in neonatal mouse testis, and both spermatogonia and Sertoli cells exhibited immunoreactivity for PS1. The Western blot analysis demonstrated that mouse testicular PS1 undergoes proteolytic processing, which is consistent with results obtained in rat (Yamaguchi et al, 2000) and monkey (Lah et al, 1997) testis. Cleavage of PS1 is known to result in a tightly regulated accumulation of *N*-terminal and *C*-terminal derivatives that remain associated and are considered to be the biologically active form of the protein (Thinakaran et al, 1996). The *N*-terminal fragment (~30 kd), which was not detected in the present study because the antibody used is specific for the *C*-terminal domain, was found to be expressed in monkey testis (Lah et al, 1997). The full-length PS1 has been detected mainly in cell lines or transgenic mice that overexpress PS1 (Thinakaran et al, 1996; Lah et al, 1997; Yamaguchi et al, 2000) and was found to be unstable (Kim et al, 1997). Therefore, the ~50 kd band detected in the present study may represent an aggregated form of PS1 rather than the full-length molecule. The PS1 *N*-terminal and *C*-terminal fragments are known to form a heteromeric complex and interact with target proteins (Sisodia et al, 1999). The mechanism involved in the proteolytic cleavage of Notch is unclear, but PS1 may directly mediate the proteolytic release of Notch intracellular domain by acting as a catalytic or regulatory component of the protease that

cleaves Notch upon ligand binding (Wolfe et al, 1999) or exert an indirect effect by mediating the transit of Notch and γ secretase to an as-yet-undefined subcellular compartment where cleavage occurs (Seugnet et al, 1997).

Future studies focusing on the role of Notch signaling in the fate of spermatogonial stem cells will pave the way for understanding the process of spermatogenesis.

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