

# Serine proteases and serine protease inhibitors in testicular physiology: the plasminogen activation system

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## Abstract

**The testis is an organ in which a series of radical remodeling events occurs during development and in adult life. These events likely rely on a sophisticated network of proteases and complementary inhibitors, including the plasminogen activation system. This review summarizes our current knowledge on the testicular occurrence and expression pattern of members of the plasminogen activation system. The various predicted functions for these molecules in the establishment and maintenance of the testicular architecture and in the process of spermatogenesis are presented.**

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## Introduction

The testis is a highly dynamic organ not only in the fetal stage but also during postnatal development and in adult life. It is composed of two major compartments: the interstitium with the steroidogenic Leydig cells, and the seminiferous tubules. The seminiferous tubules are surrounded by peritubular cells. They are composed of Sertoli cells and germ cells at different developmental stages. Sertoli cells play key roles in spermatogenesis. They are target cells for follicle stimulating hormone (FSH) and testosterone, responsible for the initiation and maintenance of spermatogenesis. They form the tubules and provide structural and nutritional support for the developing germ cells (Russell 1980, Griswold 1998).

The gonads emerge as an outgrowth and will develop either as a testis or an ovary, depending on the presence of the *Sry* gene located on the Y chromosome. In response to *Sry*, Sertoli cells differentiate. They synthesize the Müllerian-inhibiting substance, and they aggregate to form the cords together with peritubular cells originating from the mesonephros. Subsequently, Leydig cells differentiate in the interstitial milieu and start producing testosterone (Wilhelm *et al.* 2007). At puberty, dynamic changes are associated with the transformation of the cords into tubules and initiation of spermatogenesis. In adult life, germ cells migrate from the base to the apex of the tubule epithelium while differentiating further. Finally, spermatids are released from the apex of the seminiferous epithelium into the tubular lumen, becoming spermatozoa (Russell 1980).

Previous reports suggested that various proteinases and their cognate inhibitors were involved in this spatiotemporal and highly orchestrated process both during testis development and in adult life (Fritz *et al.* 1993, Charron & Wright 2005). This review describes our current knowledge on the plasminogen activation system in the testis, and its predicted functions in the establishment and maintenance of the testicular architecture and in the process of spermatogenesis.

## General aspects of proteases and protease inhibitors

A number of important processes that regulate the activity and fate of many proteins are strictly dependent on proteolytic events. For example, proteases are involved in the ectodomain shedding of cell surface proteins; the activation or inactivation of cytokines, hormones, and growth factors; the exposure of cryptic neoproteins exhibiting functional roles distinct from the parent molecule; and degradation of multiple extracellular matrix (ECM) components facilitating cell migration and invasion. Accordingly, proteases are fundamental in nearly all complex processes of tissue maintenance, repair, growth and development, and alterations in the structure and expression patterns of proteases underlie many pathological processes including cancer, arthritis, osteoporosis, neurodegenerative disorders, and cardiovascular diseases. The completion of the human genome sequence has allowed to determine that more than 2% of all human genes are proteases or protease inhibitors, reflecting the importance of proteolysis in human biology (Puente & Lopez-Otin 2004).

The activity of proteases is regulated at multiple levels including the level of production, the activation of the protease generally synthesized in an inactive pro-form, and the production of specific inhibitors. Proteases catalyze the hydrolysis of peptide bonds in proteins. The exopeptidases attack only peptide bonds localized at/or near the amino- or carboxy-terminal portion of peptide chains. The endopeptidases, also named the proteinases, catalyze the hydrolysis of internal bonds in polypeptides. They are divided into five classes, i.e., aspartic, metzincins, cysteine, serine, and threonine proteases, depending on their catalytic sites. Analysis of the full repertoire of proteases present in the human, mouse, and rat genomes indicates that serine proteases represent one-third of the proteolytic enzymes in rat (221 out of 626), mouse (227 out of 641), and human (178 out of 561; Puente & Lopez-Otin 2004).

### Serine proteases and serine protease inhibitors (SERPINs)

The serine protease family is one of the earliest characterized and largest multigene proteolytic families. It has well-characterized roles including blood coagulation, platelet activation, fibrinolysis, and thrombolysis. It can be subdivided into 16 families including the plasminogen activators (PAs), activated protein C, and the kallikreins.

### Plasminogen activators

In mammals, two major types of PA have been identified, urokinase type (uPA) and tissue type (tPA). Even though both types of PA catalyze the activation of plasminogen, the currently established functions of uPA-dependent plasminogen activation are mainly within physiological and pathological tissue remodeling processes, whereas tPA is mainly involved in thrombolysis and neurobiology. However, it has been observed in gene-deficient mice that PAs could substitute to each other (Dano *et al.* 2005). Both PAs are released from cells as single chains with no (uPA) or low (tPA) activity, with cleavage of a polypeptide bond leading to the fully active two-chain forms. The most important feature of this system is the amplification loop achieved by the reciprocal activation of pro-PAs and plasminogen on the cell surface. In addition, although tPA and uPA are secreted proteases, both can bind to the cell surface via specific cell surface receptors, being thus protected from the inhibitory actions of the abundant plasma inhibitors (Dano *et al.* 2005).

At least eight distinct plasmin/plasminogen-binding proteins have been proposed, including  $\alpha$ -enolase, amphoterin, and annexin II. Annexin II is a 36 kDa, calcium-dependent, phospholipid-binding protein that exhibits specific saturable binding for both plasminogen and tPA. It independently binds tPA (but not uPA) and

plasminogen, anchoring them with high affinity in close proximity to each other on the cell surface, thus providing an environment in which plasmin production is greatly increased (Kim & Hajjar 2002).

The receptor for uPA (uPAR) is a cysteine-rich, highly glycosylated protein, attached to the cell surface by a COOH-terminal glycosylphosphatidylinositol anchor. Both the inactive single chain and the active two-chain uPA can bind to uPAR with high affinity. The receptor uPAR can also bind the serum and extracellular matrix protein vitronectin, an interaction that requires uPA. In contrast, plasminogen does not bind to uPAR. Although lacking a cytosolic domain, uPAR activates multiple intracellular signaling molecules through a connection with integrins (for example, vitronectin is a ligand of  $\alpha v \beta 3$  integrin), G-protein-coupled receptors, and caveolin. These include cytosolic kinase pathways with the activation of intracellular tyrosine kinases, the focal adhesion kinase pathway leading to cytoskeletal reorganization, and intracellular calcium mobilization. In addition, both uPA and uPAR exhibit growth activities independent of their proteolytic activities (Blasi & Carmeliet 2002).

### SERPINS

The SERPINs are a superfamily of proteins that fold into a conserved tertiary structural domain, with full-length coding sequences known or predicted for about half of a total of 500. The name SERPIN derives from the fact that most of the first identified SERPINs were inhibitors of serine proteinases. SERPINs are classified into clades based on phylogenetic relationships. PA inhibitors (PAIs) belong to clades A, B, and E. SERPINA5 also known as protein C inhibitor (PCI) or PAI-3 binds retinoic acid and targets activated Protein C and the two PAs. SERPINB2 or PAI-2 inhibits uPA and weakly inhibits tPA. SERPINE1 is PAI-1 and SERPINE2 is proteinase nexin-1. They both inhibit the two PAs (Law *et al.* 2006).

SERPINS targeting serine proteinases have a unique suicide-substrate mechanism through an interaction with proteinases to form covalent complexes that are not dissociable when boiling in SDS but are sensitive to nucleophiles. Such a mechanism is based on a dramatic conformational change in the SERPIN. Thus, the trapped complex is irreversible in nature. In addition, several SERPINs including the SERPINs A5, E1, and E2 are activated by binding to negatively charged glycosaminoglycans. The resulting enhancement in the rates of proteinase inhibition can be up to several 1000-fold suggesting that glycosaminoglycans are rate-limiting factors at sites of SERPIN action. In the case of the three aforementioned SERPINs, the mechanism involves bridging in which glycosaminoglycans bind both SERPIN and proteinase to bring them into an appropriate interaction (Pike *et al.* 2005).

## An overview of the repertoire of the plasminogen/plasmin system in the testis

PAs were the first serine proteases identified within the testis. Plasminogen is also synthesized within the testis. Originally, it was described that Sertoli cells were the site of synthesis of the two PAs, and FSH stimulates tPA while reducing uPA levels in the rat testis. In addition to a hormonal level of regulation, PAs are highly regulated by a complex network of locally produced cytokines including at least fibroblast growth factor 2 (FGF2), interleukin 1 $\alpha$  (IL1 $\alpha$ ), and tumour necrosis factor- $\alpha$  (TNF $\alpha$ ; Fig. 1). PAs are also positively regulated by germ cells both in coculture or through the addition of germ cell-conditioned media. However, the simultaneous addition of anti-FGF2 or anti-TNF $\alpha$  antibodies together with the germ cell-conditioned media does not abolish the effects, indicating that the factors active in stimulating PAs are neither FGF2 nor TNF $\alpha$  (Fig. 1 and unpublished data).

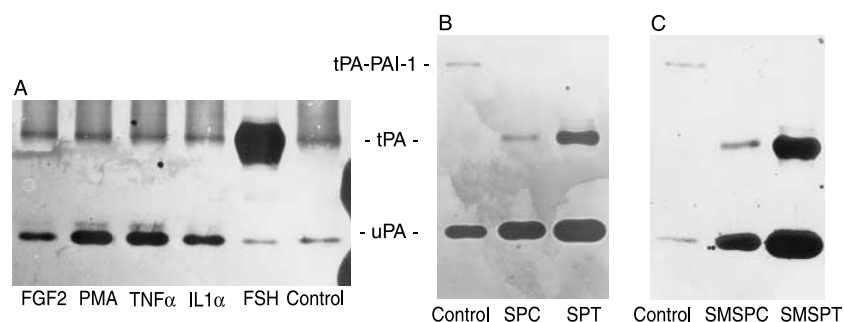
Interestingly, pachytene and diakinetoc spermatocytes exhibit immunoreactivity for tPA, indicating that a tPA proteolytic event may occur at the spermatocyte surface level. It would be interesting to determine whether the immunoreactivity corresponded to a tPA-binding protein or a tPA receptor present on the germ cells. Annexin II is a good candidate, because it acts as a receptor for tPA and its mRNA is represented in a testis cDNA library (Ref. in Fritz *et al.* 1993, Charron & Wright 2005). By contrast, the receptor for urokinase has been identified on both Leydig cells and at Sertoli cell-germ cell contacts and/or germ cells (Odet *et al.* 2004), indicating that proteolysis involving plasminogen may occur in the vicinity of Sertoli and germ cells and at the Leydig cell membrane.

The binding of uPA to its receptor promotes cell adhesion by increasing the affinity of uPAR for vitronectin (Dellas & Loskutoff 2005, Lijnen 2005). It is thus of interest that vitronectin has been identified in early spermatids (Fig. 2) and that PAI-1 is a Sertoli and a peritubular cell

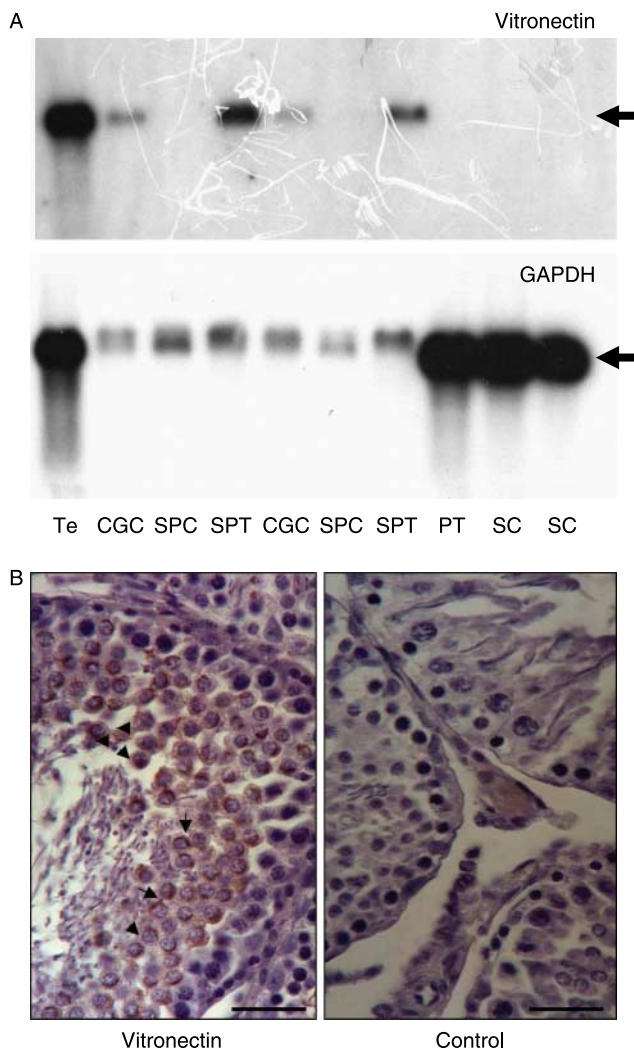
product (Fritz *et al.* 1993, Le Magueresse-Battistoni *et al.* 1998). Indeed, Sertoli cell PAI-1 might regulate spermatid adhesion through competing with uPAR in binding to vitronectin. As for the PAs, PAI-1 is highly regulated by FSH (negatively) and locally produced cytokines (positively by transforming growth factor- $\beta$ 1 (TGF $\beta$ 1), FGF2, and TNF $\alpha$ ; Fritz *et al.* 1993, Le Magueresse-Battistoni *et al.* 1997, 1998, Charron & Wright 2005). In contrast, PAI-3 (SERPINA5) is up-regulated by FSH and testosterone (Anway *et al.* 2005, Meachem *et al.* 2005, Denolet *et al.* 2006). It is noteworthy that the serine proteinases eppin and the SERPINS A3n and A12n are also up-regulated by androgens (Denolet *et al.* 2006).

Germ cells are a source of various serine proteases and inhibitors, including the activated protein C and its inhibitor (SERPINA5; Odet *et al.* 2004) and the hepatocyte growth factor activator and its two specific inhibitors, the HAIs (Odet *et al.* 2006). However, it is not known whether these proteases act within the seminiferous epithelium or later in gamete recognition as shown with most A disintegrin and metalloproteases (ADAMs), and anticipated as well for the uPA receptor and vitronectin (Bronson *et al.* 2000, Blasi & Carmeliet 2002, Rubinstein *et al.* 2006).

Few studies have explored the contribution of Leydig cells to the testicular protease repertoire. It has been reported that Leydig cells express various serine proteases and complementary inhibitors. For some of them, Leydig cells are the unique site of expression in the testis, i.e., the neurotrypsin and kallikreins 21, 24, and 27 (Matsui & Takahashi 2001, Puente & Lopez-Otin 2004). Interestingly, luteinizing hormone (LH)-hCG regulates several serine proteases and inhibitors identified in Leydig cells (including urokinase, matriptase-2, kallikrein-21, HAI-2, and PCI; Odet *et al.* 2006), indicating that common transcriptional signals may drive the expression of these molecules. Furthermore, kallikreins are regulated by testosterone and estradiol (Matsui & Takahashi 2001, Eacker *et al.* 2007).



**Figure 1** Regulation of tPA and uPA in cultured rat Sertoli cells. Sertoli cells were isolated from 20-day-old rat testes and maintained in culture for 48 h prior to stimulation for 48 h (A) with FSH phorbol 12-myristate 13-acetate (PMA) or various cytokines (FGF2, TNF $\alpha$ , IL1 $\alpha$ ). Control, control cells. Culture media were collected and assayed for plasminogen zymography. (B) Sertoli cells were cocultured for 48 h with pachytene spermatocytes (SPC; 2.10<sup>6</sup> per well) or early spermatids (SPT; 8.10<sup>6</sup> per well). Control, control cells. (C) Sertoli cells were stimulated with media conditioned (spent media, SM) by pachytene spermatocytes (SMSPC) or early spermatids (SMSPT). Control, control cells. SPC and SPT were recovered from adult rats by centrifugal elutriation (enrichment >80%), and coculture experiments and preparation of SM were as described in Le Magueresse & Jégou (1988a, 1988b). Plasminogen zymography was performed as described in Le Magueresse-Battistoni *et al.* (1998) and Sigillo *et al.* (1998).



**Figure 2** Testicular localization of vitronectin. (A) Northern blot analysis of vitronectin (upper signal at 1700 kb) and GAPDH (lower signal) in adult testis (Te), crude germ cells (CGC), pachytene spermatocytes (SPC), early spermatids (SPT) (all from adult rat testis), peritubular cells (PT), and Sertoli cells (SC) (both from 20-day-old rat testes). These cells were prepared as described in Le Magueresse-Battistoni *et al.* (1998). Northern blot analysis was performed as described in Le Magueresse-Battistoni *et al.* (1994). The probe used for hybridization was a 600 bp *Pst*I fragment of mouse vitronectin cDNA kindly provided by D J Loskutoff (The Scripps Research Institute, La Jolla, CA, USA). (B) Immunohistochemical localization of vitronectin in adult rat testis. Immunohistochemistry was performed as described in Longin *et al.* (2001), using a rabbit anti-human vitronectin polyclonal antibody (Chemicon International, Temecula, CA, USA) at a 1:200 dilution. Immunostaining (arrows) is concentrated in early spermatids within the acrosomal region. No immunostaining is evident in the tubules in control testicular sections (omission of the primary antibody); bar, 50  $\mu$ m.

### What potential functions in testicular physiology?

The plasminogen activation system is largely present in testis where it is regulated both by the tropic hormones and various locally produced cytokines, suggesting that it may exert multiple roles of physiological importance in testis development and in adult life.

### Growth factor or receptor activation, receptor shedding, and proteinase activation

Testicular proteases and antiproteases probably display a unique function in delivering growth factors trapped in the ECM, activating growth factors or growth factor receptors, or (and this remains to be demonstrated) in the shedding of transmembrane receptors, generating soluble forms that would act as dominant negatives and impede the normal transducing pathway following ligand binding to its receptor. For example, FGF2, which is deeply involved in testicular physiology (see Ref. in El Ramy *et al.* 2005), does not contain a sequence signal for secretion, and it has been proposed that following environmental stimuli, FGF2 is released from the ECM through the action of proteases, allowing it to bind to specific transmembrane FGF receptors and transduce a signal (Powers *et al.* 2000). It is also shown that uPA activates (at least *in tubo*) pro-TGF- $\beta$  and pro-HGF, two decisive growth factors in testicular physiology (Catizone *et al.* 2001, Itman *et al.* 2006). Additionally, an extensive network of proteases and inhibitors are influenced by the PA system, the largest group being the matrix metalloproteinases (MMPs) and their respective inhibitors the tissue inhibitors of MMPs (TIMPs; Page-McCaw *et al.* 2007). Several of them are produced in the testis, including the gelatinases MMP2 and MMP9. Interestingly, MMPs 2 and 9 are regulated strongly by cytokines and weakly (MMP2) or not regulated (MMP9) by hormones (Fritz *et al.* 1993, Longin *et al.* 2001, Charron & Wright 2005, Wong & Cheng 2005), indicating that their activity may be secondary to PA activation.

### ECM matrix synthesis and remodeling

In the testis, the importance of ECM was evidenced with the finding that male infertility is associated with abnormal thickening of the basement membrane surrounding the seminiferous tubules (also found in aged testes and in Klinefelter patients; de Kretser *et al.* 1975). Indeed, the basement membrane is the structural basis of testis cord organization in the developing gonad; and in adult life it is essential for the maintenance of the differentiated functions of Sertoli cells (Dym 1994, Griswold 1998). Conversely, much less is known of the ECM matrix surrounding Leydig cells (Kuopio & Pelliniemi 1989).

### Testis cord formation

Originally, the genital ridge is composed of primordial germ cells and a thickened layer of coelomic epithelium. When the indifferent gonad has an XY genotype, SRY induces a cascade of gene expression which results initially in the migration of mesenchymal cells as well as endothelial cells from the adjacent mesonephros, and the formation of a basement membrane between the

epithelializing Sertoli cells and the mesenchymal peritubular cells. No migration occurs in case of an XX gonad (Brennan & Capel 2004). Such a migration is accompanied by extensive restructuring. Accordingly, major sex-related differences in the distribution of ECM components and the expression of proteases and inhibitors have been reported, including the SERPINs E2 and A5 (Nef *et al.* 2005, Wilhelm *et al.* 2007), and TIMP-1 (Guyot *et al.* 2003).

#### *Testis growth and lumen formation*

The prepubertal period is characterized by a rapid growth of the testis, the transformation of the seminiferous cords into tubules, and the initiation of spermatogenesis. Specifically, tight junctions are formed between neighboring Sertoli cells, thus creating the blood–testis barrier, and cords develop a lumen, becoming tubules. Accordingly, Sertoli cells accommodate their cytoskeleton to support additional spermatogenic cell types as spermatogenesis is initiated, and tubules increase in diameter as well as in length. Strong arguments have been given that not only tPA (Fritz *et al.* 1993) and the growth factors HGF and FGF2, but also basement membrane components (specifically laminin; Skinner 2005), are critically involved in these substantial prepubertal changes. In addition, recent data illustrated that ECM components regulate the expression of tight junction proteins and the formation of a lumen in concert with MMP9 and its inhibitor TIMP-1 (Wong & Cheng 2005). Inasmuch as both HGF and FGF2 must be activated (see above), that PAs activate pro-MMPs including MMPs 2 and 9, and that both MMPs and PAs degrade laminin, fibronectin, and collagen IV – i.e., the major basement membrane components – the plasminogen activation system occupies a central position in assisting remodeling necessary to support the rapid and extensive growth of the prepubertal testis.

#### ***Spermatogenesis and the apical migration of germ cells towards the lumen***

Different authors have been interested in the understanding of the dynamics of spermatogenesis, which relies on the passage of the blood–testis barrier (translocation) and the release of the elongated spermatids at the apex (spermiation). The description of the testis barrier is beyond the scope of this review and has been treated recently (Wong & Cheng 2005). However, it is noteworthy that the testis barrier is unique when compared with other blood–tissue barriers (e.g., blood–brain and blood–retina barriers), as it is composed of gap junctions, desmosomes, tight junctions, and ectoplasmic specializations, precluding that the passage of germ cells requires a finely tuned process not disturbing the integrity of the testis barrier, which would provoke a

pathological arrest of spermatogenesis. Since this situation is reminiscent of cell migration across the ECM, different authors have concentrated their efforts in determining the composition of the junctions, most specifically those that are restricted to testis, i.e., the ectoplasmic specializations, and the way junctional proteins are transcriptionally and post-transcriptionally regulated. It was also reasonable to think that proteases which act like scissors would help germ cells in migrating along Sertoli cell membranes, and that specific inhibitors would restrict the activity of the proteases in a finely tuned regulatory fashion to preserve homeostasis. Therefore, a list of the cytokines, proteases, and inhibitors present at the right time and in the right place has been tentatively established (Charron & Wright 2005, Xia *et al.* 2005).

First evidence came from the demonstration that the PAs were expressed as a function of the stages of the seminiferous epithelium, and an increased PA activity was found at the time of translocation and spermiation at stages VII and VIII (Fritz *et al.* 1993). Interestingly, immunostaining of  $\alpha$ 2-macroglobulin (a protease inhibitor with a large spectrum of inhibitory activities against proteinases) concentrated at stages I–VI, thus prior spermiation indicating that  $\alpha$ 2-macroglobulin may protect the integrity of the seminiferous epithelium against excessive proteolysis (Wong & Cheng 2005). In addition, the enhancement of Sertoli PA activity (and of the cysteine protease cathepsin L; Charron & Wright 2005) was evidenced in cocultures of Sertoli cells and germ cells (Fig. 1B and C), and this correlated in time with the dynamics of assembly/disassembly of the *de novo* adherent junctions forming between the cultured Sertoli cells. Furthermore, the expression of not only  $\alpha$ 2-macroglobulin but also cystatin (a cathepsin L inhibitor) in the coculture model was consistent with the idea that proteases and their corresponding inhibitors were working synergistically, supporting the evidence that they may be involved in the adherence of germ cells to Sertoli cells and the subsequent formation of intercellular junctions (Charron & Wright 2005, Wong & Cheng 2005). These data are also in line with previous findings reporting that protease-sensitive elements of unknown nature hold spermatids and Sertoli cells together (Russell 1980).

Spermiation, i.e., extrusion of elongated spermatids in the lumen, is the alternate major event that occurs during stages VII and VIII. It is followed by the phagocytosis of the cytoplasts shed from the elongated spermatids, which are called the residual bodies (Russell 1980). An *in vitro* model has been established in the past where residual bodies (recovered by elutriation of a mixed germ cell preparation) are phagocytosed by Sertoli cells with kinetics comparable to the *in vivo* situation. Using such an *in vitro* model, it was demonstrated that phagocytosis of residual bodies resulted in an interleukin 1 $\alpha$ -dependent enhancement of Sertoli cell PA expression and activity (Sigillo *et al.* 1998).

Interestingly, interleukin 1 $\alpha$  production is enhanced upon phagocytosis of residual bodies (Jégou *et al.* 1995), and interleukin 1 $\alpha$  is known to stimulate DNA synthesis in spermatogonia and spermatocytes. Thus, residual bodies upon phagocytosis could trigger the induction of Sertoli interleukin 1 $\alpha$ , which would lead to the enhancement of the PA activity facilitating germ cell migration in the adluminal compartment and their entry into meiosis, and in the stimulation of germ cell proliferation preceding the initiation of a new wave of spermatogenesis. Therefore, the synchronization process of the spermatogenesis cycle may depend on a proteolytic message (as summarized in Fig. 3), revisiting the pioneering hypothesis of Regaud and Roosen-Runge (referenced in Jégou *et al.* 1995, Sigillo *et al.* 1998). Two other cytokines have proven to be essential at least in the passage of the testis barrier by preleptotene spermatocytes. These are TGF $\beta$ 3 and TNF $\alpha$ , and TNF $\alpha$  enhances uPA dramatically (Fig. 1A). Recent reviews have focused on these cytokines in the testis (Wong & Cheng 2005, Xia *et al.* 2005).

Interestingly, stages VII and VIII are highly testosterone dependent as demonstrated in models with testosterone deficiency in which a premature detachment of germ cells in the lumens of the tubules is described (Denolet *et al.* 2006, Tsai *et al.* 2006, Eacker *et al.* 2007). To date, no MMPs have been shown to be under androgen dependency. Conversely, androgens inhibit Sertoli cell PA activity *in vitro* (Fritz *et al.* 1993), and PA expression is altered in a model of Sertoli cell androgen receptor deficiency (Denolet *et al.* 2006). Thus, SERPINA5 is of tremendous interest because it is up-regulated by testosterone (Anway *et al.* 2005, Denolet *et al.* 2006), it opposes PA activity, and deficient mice develop male sterility (Uhrin *et al.* 2000). Specifically, lumens are filled with immature germ cells because of an unopposed proteolytic activity of the urokinase type (Uhrin *et al.* 2000). Such a testicular phenotype is reminiscent of the testicular phenotype described in mice deficient for claudin 11 (Gow *et al.* 1999). Claudin 11 and claudins 1 and 3 are essential components of the testis barrier, and they are under testosterone control (Gow *et al.* 1999, Florin *et al.* 2005, Meng *et al.* 2005). In addition, claudins contribute – together with MMP-14 and TIMP-2 – to activating MMP-2 secreted as a pro-form (Miyamori *et al.* 2001). MMPs may also be activated by uPA. Thus, the germ cell enhancement of MMP-2 activity (Longin *et al.* 2001) may, in part, result from the increase in the activity of the PAs observed in Sertoli cell–germ cell cocultures (Wong & Cheng 2005; Fig. 1B and C). It remains to be determined whether claudins are substrates for either PAs or MMPs, and what is their expression level in the SERPINA5-deficient testes.

Therefore and collectively, it appears that germ cells that do not bear classic characteristics of migrating cells regulate their own progression within the seminiferous epithelium, through a modulation of the expression pattern of the proteases and inhibitors

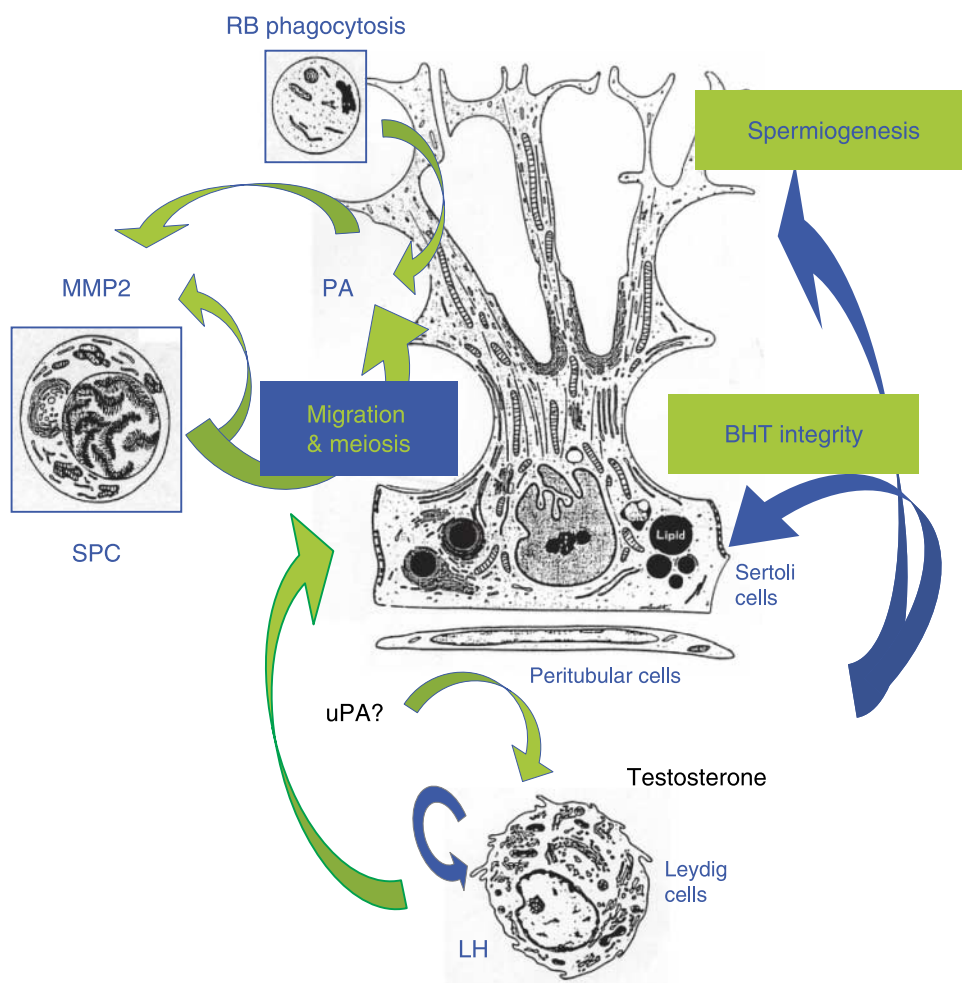
produced by Sertoli cells as exposed in Fig. 3, supporting the hypothesis that Sertoli cells act as facilitators of migration adding a new testosterone-dependent function to these nurse cells.

### **Proteolysis and steroidogenesis**

Different arguments emphasize a role of ECM in the capacity of Leydig cells to respond to LH–hCG *in vitro*, and thus indirectly of a role of proteases and inhibitors. For instance, it was shown that fibronectin and collagen IV induce down-regulation of the steroidogenic response to gonadotropins (Diaz *et al.* 2005). Furthermore, TGF $\beta$ , known to cause augmented fibronectin deposition and to elicit cytoskeletal changes in Leydig cells similar to those evidenced when these cells are cultured on plates pre-coated with fibronectin, antagonizes gonadotropin steroidogenic action in Leydig cells (Dickson *et al.* 2002). Finally, we recently demonstrated that Leydig cells exhibit two immediate responses upon LH stimulation: an increased expression of StAR and an increased expression of uPA followed by an increased expression of SERPINB2 and tPA (Odet *et al.* 2006). Thus, two hypotheses (Fig. 3) may be raised. Either the uPA peak signals the immediate matrix environment altering Leydig cell responsiveness to LH or uPA is part of the dialog between the interstitial compartment and the seminiferous epithelium.

### **Conclusions and future directions**

A series of evidence has been provided, highlighting that proteases may be active partners in establishing and maintaining testicular architecture, and in facilitating germ cell migration throughout the spermatogenic developmental process. However, very few knockout mouse models have, to date, contributed to our understanding of their roles in testicular function. One of the reasons may be because proteases and inhibitors are extremely abundant and redundant in their spectrum of actions. For example, male mice deficient in uPA, tPA, both PAs, or PAI-1 still reproduce (Carmeliet & Collen 1995), although mice deficient for both PAs suffered reduced weight, shortened lifespan, and increased fibrin deposition (Carmeliet *et al.* 1994). Nonetheless, it should be stated that most of the time no systematic analysis of the testes of the deficient mice had been undertaken unless the authors experienced reproductive difficulties as seen with male sterility in mice deficient for SERPINA5 (Uhrin *et al.* 2000). For instance, the morphological analysis of the seminiferous epithelium of cathepsin L-deficient mice has demonstrated that, although a lack of this proteinase may not cause infertility, it is required for quantitatively normal spermatogenesis (Wright *et al.* 2003). Therefore, it may be worthwhile to revisit the phenotypes of transgenic male mice deficient for a proteinase or an inhibitor that



**Figure 3** Schematic drawing illustrating that coordination of the seminiferous epithelial cycle may be dependent on a proteolytic signal. We predict that proteases – specifically the enzymes of the plasminogen activation system – are involved in concert with androgens. Indeed, on the one hand, androgens of Leydig origin act on both peritubular cells and Sertoli cells, regulate the integrity of the BHT, and facilitate spermiogenesis. On the other hand, proteases and specifically the enzymes of the plasminogen activation system of both Sertoli and Leydig origin are under hormonal regulation, and they may assist germ cells in migrating along Sertoli cell membranes. In turn, germ cells (and among them, spermatocytes, SPC) and residual bodies (RB) would contribute to the regulation of the proteolytic balance.

has been proven to be expressed at the time of translocation and/or spermiation.

The use of *in vitro* models coupled with the SiRNA strategy to specifically knock down a protease or its inhibitor should also constitute an elegant means to link morphogen cytokines, ECM components, restructuring events, and proteases. Furthermore, inasmuch as various proteases, inhibitors, and junctional components (e.g., claudins) are under a complex hormonal control via gonadotropins and/or testosterone, and local regulatory control involving cytokines and growth factors, models with reduced testosterone bioavailability or with limited FSH or LH action coupled with microarray studies, such as those recently published (Meachem *et al.* 2005, Denolet *et al.* 2006, Tsai *et al.* 2006, Eacker *et al.* 2007), should be of tremendous benefit to fully understand the mechanisms that underpin the role of proteases and inhibitors in testis development and function. A challenge for the future will be to identify the full complement of proteases and their regulatory mechanisms. This will enable the design of additional studies to define precisely the role and relative

importance of each in the complex steps of testis development and spermatogenesis. Then, the phenotypic effects in gene knockout experiments can be interpreted with the knowledge of their integrated roles and potential for compensatory action.

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