Biochem. J. (1991) 279, 75–80 (Printed in Great Britain)

Secretion of latent type IV procollagenase and active type IV collagenase by testicular cells in culture

Menachem AILENBERG,* William G. STETLER-STEVENSON† and Irving B. FRITZ*‡
*Banting and Best Department of Medical Research, C. H. Best Institute, 112 College Street, University of Toronto, Toronto, Ontario M5G 1L6, Canada, †Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, U.S.A., and ‡Department of Molecular Physiology, A.F.R.C. Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, U.K.

Testicular peritubular myoid cells, which have properties similar to those of vascular smooth-muscle cells, secrete a variety of metalloproteinases when maintained in culture in a chemically defined medium. The predominant metalloproteinases secreted were identified as latent type IV procollagenases having molecular masses of 72 kDa and 75 kDa, as detected in Western immunoblots with specific antibodies against type IV procollagenase. When peritubular cells were stimulated by dibutyryl cyclic AMP, forskolin or cholera toxin, they secreted increased amounts of type IV procollagenase. However, little if any of the active type IV collagenase, having a lower molecular mass of 66 kDa, could be detected under these conditions. Addition of low concentrations of cytochalasin D to peritubular cells in monoculture resulted in conversion of the latent type IV collagenase into its active form, assessed with antibody-specificity studies and by the appearance of the 66 kDa protein. In contrast, Sertoli cells in culture did not manifest an increased conversion of type IV procollagenase into type IV collagenase in the presence of cytochalasin D, even though cytochalasin D addition invariably resulted in a disruption of the microfilament assembly in each of these gonadal somatic cell populations. When peritubular cells were co-cultured with Sertoli cells, addition of cytochalasin D no longer resulted in formation of increased amounts of the active form of type IV collagenase. Sertoli cells and peritubular cells each secreted a tissue inhibitor of metalloproteinase type 2, detected with a specific antibody in a Western immunoblot to have a molecular mass of 21 kDa. We conclude that cytochalasin D acts on mesenchymal-type peritubular cells, but not on epithelial-type Sertoli cells, to enhance the conversion of latent type IV procollagenase into active type IV collagenase. This conversion of type IV procollagenase into type IV collagenase by peritubular cells was inhibited by factor(s) secreted by Sertoli cells. Interactions between Sertoli cells and peritubular cells are postulated to modulate net proteinase activities in discrete regions of the testis.

INTRODUCTION

Extracellular proteolysis is implicated in tissue restructuring, tumour invasion, tissue repair and morphogenesis (for reviews see refs. [1] and [2]). Several extracellular proteinases are secreted in their latent proenzyme form, and must be activated in order to exert their catalytic action. For example, specific proplasminogen activators are secreted that must themselves be activated before the enzyme can specifically cleave the Arg–Val peptide bond in plasminogen required for the generation of plasmin, a proteinase with proteolytic activity similar to that of trypsin (for review see ref. [3]).

Testicular somatic Sertoli cells secrete plasminogen activators [4], and peritubular cells secrete plasminogen activator inhibitor type 1 [5]. Sertoli cells have been shown to secrete metalloproteinases, one of which has been characterized as type IV procollagenase [6]. Among metalloproteinases secreted by different populations of cells, type IV procollagenase is of special interest because type IV collagenase has been implicated in basement-membrane degradation during tumour metastasis [7]. Type IV procollagenase can be chemically converted into the type IV collagenase by treatment *in vitro* with organomercurial compounds such as *p*-aminophenylmercuric acetate (APMA) [8]. The possible chemical basis for this activation has been elucidated [9,10]. However, the physiological mechanisms by which type IV procollagenase is converted into its active form *in vivo* remain unknown. Since disruption of microfilaments results in increased collagenase activity [11,12], the cytoskeleton seems likely to play a potential role.

In the present study, we demonstrate that testicular peritubular cells, which have some of the characteristics of vascular smooth-muscle cells [13], secrete several metalloproteinases when maintained in culture in a chemically defined medium, and that latent type IV procollagenase is the predominant metalloproteinase detected. Treatment with cytochalasin D results in the conversion of type IV procollagenase into its active form by peritubular cells maintained in monoculture. Other evidence to be presented indicates that interactions among somatic cells in the testis are involved in modulating the activation of type IV procollagenase.

MATERIALS AND METHODS

Cell preparation and culture

Sertoli-cell-enriched and peritubular-cell-enriched preparations were isolated from testes of 20-day-old Wistar-strain rats by using sequential enzymic digestion by procedures previously described [13–15]. Sertoli-cell pellets diluted 10-fold with Eagle's minimal essential medium (MEM) were plated in 100 mm × 20 mm Petri dishes (1.4×10^5 cells/cm^2). When Sertoli cells were to be co-cultured with peritubular cells, subconfluent cultures of primary peritubular cells initially were prepared and maintained for 1 week in MEM containing 10% (v/v) calf serum. The peritubular cells were washed for 30 min in serum-free MEM, and Sertoli cells were then plated on to the peritubular-cell monolayer (8.5×10^5 cells/cm^2) in culture in serum-free MEM. Additions to the medium were made im-

Abbreviations used: APMA, *p*-aminophenylmercuric acetate; BCIP, 5-bromo-4-chloroindol-3-yl phosphate *p*-toluidine salt; dbcAMP, dibutyryl cyclic AMP; Me_2SO, dimethyl sulphoxide; NBT, Nitro Blue Tetrazolium; TIMP-2, tissue inhibitor of metalloproteinase type 2; MEM, Eagle's minimal essential medium.

mediately after Sertoli-cell plating. Cultured cells were kept for 48 h at 32 °C in a humidified atmosphere of air/CO_2 (19:1).

For investigations of peritubular cells and Sertoli cells in co-culture in two-chambered assemblies, cells were maintained by procedures previously described [16–19]. Briefly, 12 mm Millipore millicell-CM chambers (Millipore, Bedford, MA, U.S.A.) were coated with 1:3-diluted cold Matrigel (Collaborative Research, Bedford, MA, U.S.A.), applied to the filter surface facing the inner portion of the inner chamber [16]. Excess Matrigel was removed, and remaining Matrigel was allowed to gel for 30 min at 37 °C. Secondary cultures of peritubular cells were plated on the uncoated reverse side of the filters at an initial density of 7.5×10^4 cells/cm^2 in MEM containing 10% calf serum. After 3 h, Sertoli cells were plated on the Matrigel-coated surface of the filter to yield a Sertoli cell density of 1.25×10^6 cells/cm^2. Outer chambers initially contained 470 μl and inner chambers 200 μl of MEM containing 10% calf serum. At 48 h after the Sertoli cells had been plated, the chambers were washed with serum-free MEM, and fresh serum-free MEM was added to both compartments. Other components were added to MEM in the outer chamber, as described in the Results section. After 48 h, media were collected for analysis. Viability of cells was assessed by determining Trypan Blue dye exclusion. None of the treatments utilized changed the viability of either peritubular cells or Sertoli cells.

Detection of activities of metalloproteinases secreted into the medium

Media collected were in some cases subjected directly to SDS/PAGE or they were initially concentrated. In the latter case, portions were dialysed against distilled water, freeze-dried and taken up to the desired final concentrations in distilled water. For chemical activation of latent type IV procollagenase, portions of peritubular-cell-conditioned medium were incubated with 1 mM-APMA (Sigma Chemical Co., St. Louis, MO, U.S.A.) for 6 h at 37 °C. Samples were diluted with non-reducing sample buffer, and applied to 10% (w/v) gels co-polymerized with 0.2% (w/v) gelatin [20]. Gels were run overnight at a current of 7.5 mA/gel. They were then washed for 2 h in 2.5% Triton X-100 to remove SDS, rinsed three times with distilled water, and then incubated overnight in 50 mM-Tris at pH 8.0 in the presence of 5 mM-$CaCl_2$. Gels were stained for 1 h in 0.05% Coomassie Brilliant Blue R-250 in water/methanol/acetic acid (6:3:1, by vol.) and destained for another 1 h in the same solution. They were photographed, sandwiched between two cellophan membranes (Bio-Rad Laboratories, Richmond, CA, U.S.A.), placed on top of double 3 mm filter paper and dried *in vacuo* at 60 °C.

Detection of latent and active type IV collagenases and tissue inhibitor of metalloproteinase type 2 (TIMP-2) by Western immunoblot procedures

Peritubular-cell-conditioned medium was concentrated as described above, and then subjected to SDS/PAGE under non-reducing or reducing conditions, with the use of 10% gels for collagenases and 12% gels for TIMP-2 by the procedures of Laemmli [21]. Proteins were transferred electrophoretically (20 mA) to 0.45 μm nitrocellulose paper (Schleicher and Schuell, Keene, NH, U.S.A.) overnight with cooling. Non-specific binding was blocked with 5% non-fat milk. Affinity-purified antibodies raised in rabbits against synthetic peptides from the latent or active portions of type IV collagenase [8], or against human TIMP-2 [22], were used in a dilution of 1:200, 1:100 and 1:200 respectively. Anti-(type IV procollagenase) antibody (designated 'H1') recognizes specifically latent type IV procollagenase, whereas anti-(type IV collagenase) antibody (designated 'H2') recognizes both active and latent forms of type IV collagenase [8]. Anti-TIMP-2 polyclonal antibodies were raised against human TIMP-2 purified from A2058 human melanoma cells [22]. Proteins were detected with a second antibody, consisting of goat anti-(rabbit IgG) antibody conjugated to alkaline phosphatase (1:5000; Zymed, San Francisco, CA, U.S.A.), stained by Nitro Blue Tetrazolium (NBT) and 5-bromo-4-chloroindol-3-yl phosphate *p*-toluidine salt (BCIP) as the dye reagents (Sigma Chemical Co.), by using previously published procedures [23].

Detection of plasminogen activator activity

Plasminogen-dependent degradation by proteins separated by SDS/PAGE, in gels co-polymerized with gelatin and plasminogen, was measured by the same procedures as those previously described [20,24], with the use of Ca^{2+}-free buffers.

RESULTS

We measured proteinase activities in conditioned medium, in which Sertoli cells or peritubular cells had been in culture, by determining the degradation of gelatin in the presence and in the absence of Ca^{2+} in co-polymerized gels by proteins in conditioned medium separated by SDS/PAGE. In confirmation of previously published results [17,24], Sertoli-cell- and peritubular-cell-conditioned media contained no detectable proteinase activity in the absence of added plasminogen in a Ca^{2+}-free metal-free buffer (Fig. 1, EGTA lane). In the presence of added Ca^{2+}, however, several plasminogen-independent proteinases readily became detectable in both media (Fig. 1, control lanes). We then

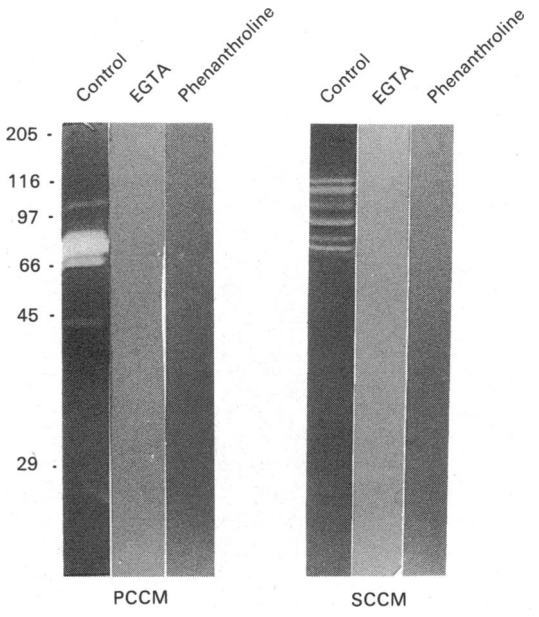

Fig. 1. SDS/PAGE zymography of 30-fold concentrated peritubular-cell-conditioned medium (PCCM) and 50-fold concentrated Sertoli-cell-conditioned medium (SCCM)

Conditioned medium was preincubated for 3 h with EGTA (5 mM) or 1,10-phenanthroline (50 μM). Control medium was incubated under comparable conditions without additions. Portions (100 μl) of conditioned medium plus 100 μl of sample buffer were applied to polyacrylamide gels co-polymerized with gelatin. After dye had reached the bottom of the gel after electrophoresis, gels were incubated for 2 h in Triton X-100 (2.5%) in water, followed by overnight incubation in 50 mM-Tris buffer, pH 8.0, containing Ca^{2+} (5 mM) or with the same buffer containing EGTA or 1,10-phenanthroline. Standard proteins of known molecular masses were run in parallel, and their positions are indicated on the left, expressed as kDa.

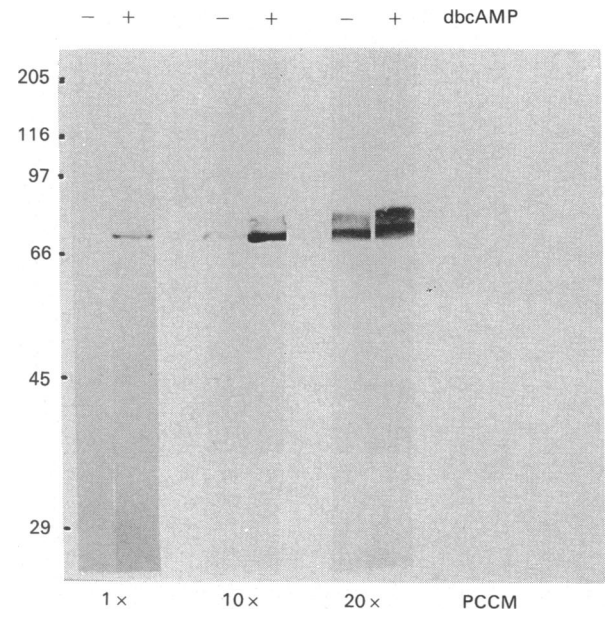

Fig. 2. Western immunoblots of peritubular-cell-conditioned medium (PCCM) at concentrations indicated (1×, 10×, 20×)

The medium was obtained after culture of cells for 48 h in the absence or in the presence of dbcAMP (0.2 mM). Samples were applied to gels under non-reducing conditions, and at the end of the run the resolved proteins were transferred to nitrocellulose paper. The nitrocellulose paper was incubated with anti-(type IV procollagenase) (H1) rabbit antibody (dilution 1:200), and the type-IV-procollagenase-reactive protein was detected by treatment with alkaline-phosphatase-conjugated goat anti-(rabbit IgG) antibody, followed by the addition of NBT plus BCIP, as defined in the Materials and methods section. Positions of molecular-mass standards are indicated on the left, expressed as kDa.

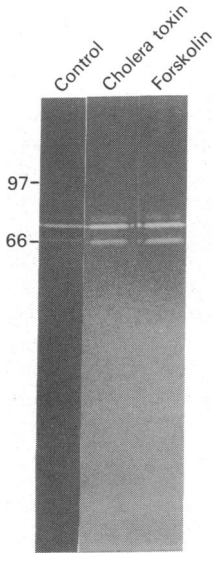

Fig. 3. SDS/PAGE zymography of non-concentrated peritubular-cell-conditioned medium obtained after cells had been in culture for 48 h in the presence or in the absence of cholera toxin (100 ng/ml) or forskolin (50 μM)

Positions of molecular-mass standards are indicated on the left, expressed as kDa. Additional details are described in the Materials and methods section.

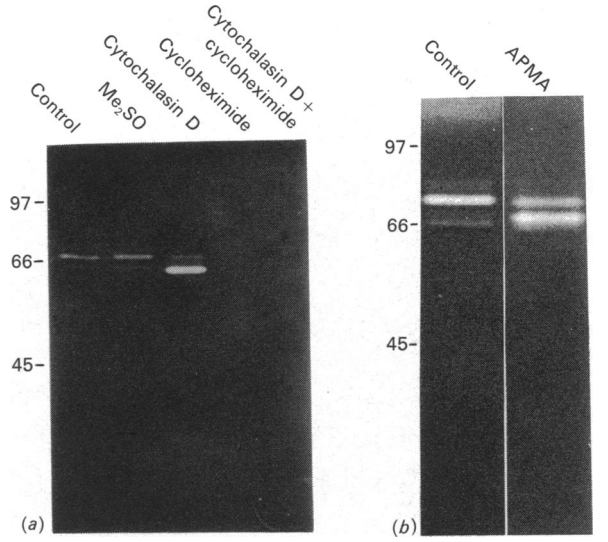

Fig. 4. SDS/PAGE zymography of non-concentrated peritubular-cell-conditioned medium obtained after cells had been in culture for 48 h in the presence or in the absence of Me_2SO (1%), cytochalasin D (5 μg/ml), cycloheximide (25 μg/ml) or cytochalasin D plus cycloheximide in panel (*a*)

In experiments shown in panel (*b*) peritubular cells were incubated with dbcAMP (0.2 mM). In the gel shown in lane 2 of (*b*), the peritubular-cell-conditioned medium was incubated for 6 h at 37 °C in the presence of 1 mM-APMA before SDS/PAGE analysis. Positions of molecular-mass standards are indicated on the left, expressed as kDa.

determined the influences of EGTA, which chelates Ca^{2+}, on proteinases secreted by Sertoli cells and peritubular cells. Inhibition by EGTA of all detectable proteinase activities in both media (Fig. 1) demonstrated that both Sertoli cells and peritubular cells secrete different patterns of readily detectable plasminogen-independent Ca^{2+}-dependent metalloproteinases, ranging in molecular masses from around 45 kDa to nearly 160 kDa (Fig. 1). Other metalloproteinases were present with weaker activity, ranging in molecular mass from 20 kDa to 200 kDa (results not shown). In the absence of Ca^{2+}, proteinase activity could not be restored by the addition of 5 mM-Mg^{2+}, -Ba^{2+} or -Zn^{2+} (results not shown). A Zn^{2+} chelator (50 μM-1,10-phenanthroline) also inhibited the activity of metalloproteinases, even when Ca^{2+} was present (Fig. 1).

Since metalloproteinases are known to include collagenases of various types, we examined the reactivity of proteinases in Sertoli-cell- and peritubular-cell-conditioned media with antibodies specific against the latent and active forms of collagenase IV. An antibody (H1) against an epitope of the *N*-terminus (residues 1–17) specific to procollagenase IV is not reactive with the active form of collagenase IV [8]. This antibody immunostained bands having molecular masses of 72 kDa and 75 kDa in samples of peritubular-cell-conditioned medium subjected to gel electrophoresis (Fig. 2). In peritubular cells cultured in basal MEM in the absence of dbcAMP, only slight procollagenase IV activity was detected in unconcentrated medium or in medium concentrated 10-fold (Fig. 2). In contrast, in peritubular cells stimulated by 0.2 mM-dbcAMP during culture, procollagenase activity was secreted in sufficiently large amounts to reveal prominent bands stained by H1 antibody in the non-concentrated medium (Fig. 2). This increase in procollagenase IV secreted by peritubular cells treated with dbcAMP (Fig. 2) was associated with increases in activities of other metalloproteinases in conditioned medium (results not shown). We next determined

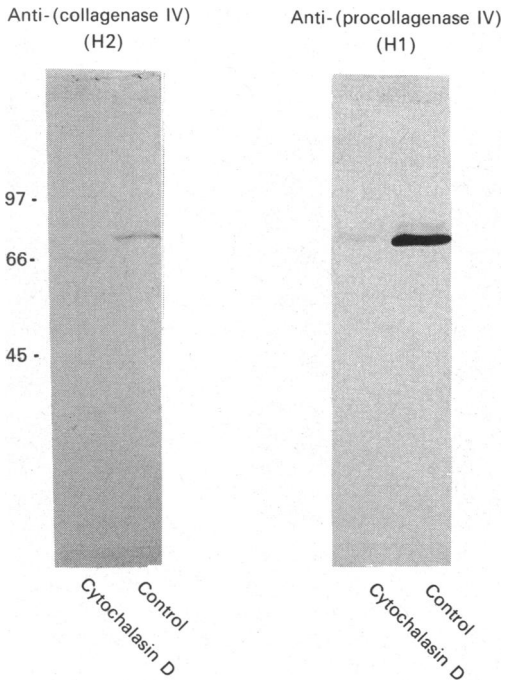

Fig. 5. Western immunoblots of 30-fold concentrated peritubular-cell-conditioned medium obtained after cells had been in culture for 48 h in the absence or in the presence of cytochalasin D (5 μg/ml)

Samples were subjected to 10% SDS/PAGE and transferred to nitrocellulose paper. Sheets were incubated with anti-(procollagenase antibody) (H1) at 1:200 dilution or with anti-collagenase antibody (H2) at 1:100 dilution. For a description of specific epitopes recognized by these antibodies see ref. [8] and the Materials and methods section. Positions of molecular-mass standards are indicated on the left, expressed as kDa.

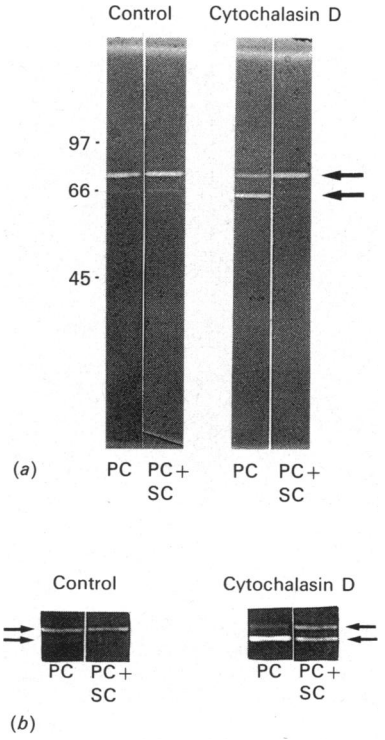

Fig. 6. SDS/PAGE zymography of non-concentrated conditioned medium from peritubular cells (PC) obtained after cells had been in monoculture for 48 h, or in co-culture with Sertoli cells (SC), in the absence or in the presence of cytochalasin D (5 μg/ml)

(*a*) Cells were cultured in conventional culture dishes. (*b*) Cells were cultured in a two-chambered assembly. Upper arrow indicates activity corresponding to the 72 kDa latent procollagenase, and the lower arrow indicates activity corresponding to the 66 kDa active type IV collagenase. Positions of molecular-mass standards are indicated on the left, expressed as kDa.

possible influences of agents known to enhance adenylate cyclase activity, namely forskolin and cholera toxin. Stimulation of peritubular cells by addition of 50 μM-forskolin or 100 ng of cholera toxin/ml to the medium increased amounts of 75 kDa, 72 kDa and 66 kDa metalloproteinases secreted, in a manner comparable with that observed in dbcAMP-stimulated cells (Fig. 3).

Addition of dbcAMP or cytochalasin D is known to result in disruption of the microfilament assembly in Sertoli cells, and also in peritubular cells [25,26]. We therefore determined whether cytochalasin D also affects the activities of metalloproteinases secreted by peritubular cells. Addition of various concentrations of cytochalasin D to these cells in culture did indeed enhance metalloproteinase activities in conditioned medium, with a threshold response at a concentration of 0.3 μg/ml, and maximal effects at a concentration of 5 μg of cytochalasin D/ml (results not shown). Proteinase activity in the 72 kDa protein was diminished in peritubular cells treated with cytochalasin D, which was associated with a concomitant increase in the activity of the 66 kDa protein (Fig. 4*a*). Addition of cytochalasin D to peritubular-cell-conditioned medium in the absence of cells had no effect on patterns of proteinase activities detected (results not shown), and the solvent used for cytochalasin D had no effects (Me_2SO lane in Fig. 4*a*). The influences of cytochalasin D on the activities of metalloproteinases secreted by peritubular cells were abolished if the cells were incubated in the presence of 25 μg of cycloheximide/ml (Fig. 4*a*, last lane). Conversion of the relatively higher-molecular-mass procollagenase IV into the lower-molecular-mass active form of collagenase IV has been reported to take place after reaction of procollagenase with the organo-mercurial reagent APMA [8]. In peritubular-cell-conditioned medium treated with 1 mM-APMA for 6 h at 37 °C, proteinase activity was shifted from the 72 kDa protein to the 66 kDa protein (Fig. 4*b*), indicating that the predicted chemical conversion of procollagenase IV into the active form had occurred. Since comparable shifts in activities of proteins with these molecular masses took place in peritubular cells rounded up by cytochalasin D treatment during culture (Figs. 4*a* and 5), we infer that cytochalasin D stimulated peritubular cells to convert the latent form of collagenase IV into its active form.

As indicated above, Western immunoblots with the antibody H1 against procollagenase IV revealed the presence of the latent forms of collagenase IV in peritubular-cell-conditioned medium, having molecular masses of 72 kDa and 75 kDa (Fig. 2). In peritubular cells incubated with cytochalasin D, the pro-collagenase IV bands reactive with H1 were diminished (Fig. 5). The antibody H2 reacts with the epitope of an internal domain near the *C*-terminus (residues 472–490) present in both pro-collagenase IV and the active form of the enzyme, but with lower affinity [8]. Immunostaining with antibody H2 consistently revealed the presence of collagenase IV of molecular mass of 66 kDa in peritubular-cell-conditioned medium obtained from peritubular cells treated with cytochalasin D. In contrast, conditioned medium from cells maintained under basal conditions reacted with H2 to reveal the 72 kDa procollagenase IV, but no 66 kDa protein was detectable (Fig. 5).

The patterns of Ca^{2+}-dependent metalloproteinases secreted by Sertoli cells in culture were different from those in peritubular-

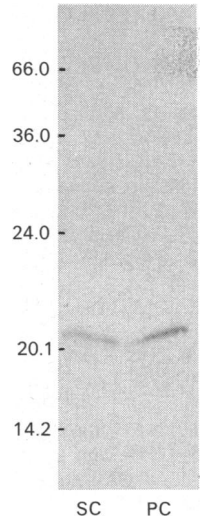

Fig. 7. Western immunoblot of 30-fold and 50-fold concentrated peritubular-cell (PC)- or Sertoli-cell (SC)-conditioned medium respectively after cells had been in culture for 48 h in MEM

Samples were subjected to 12% SDS/PAGE, and transferred to nitrocellulose paper. Sheets were incubated with anti-TIMP-2 antibody at 1:200 dilution. Positions of molecular-mass standards are indicated on the left, expressed as kDa.

cell-conditioned medium (Fig. 1). Western immunoblots with H1 indicated the presence of procollagenase IV in the Sertoli-cell-conditioned medium. However, treatment with cytochalasin D did not result in the expected formation of detectable collagenase IV (results not shown). Sertoli cells in direct contact with peritubular cells inhibited the conversion by the latter of the 72 kDa procollagenase IV into the 66 kDa active collagenase IV, a process otherwise elicited by the addition of cytochalasin D (Fig. 6*a*). The inhibition by Sertoli cells of conversion by peritubular cells was nearly total when the two types of cell were in direct contact in conventional culture dishes (Fig. 6*a*), but only partial when they were separated from each other by a filter in two-chambered vessels (Fig. 6*b*). To determine whether TIMP-2 is involved in the control of procollagenase IV activity, we searched for components in conditioned media from the two types of cell that might react with a specific anti-TIMP-2 antibody in Western immunoblots. TIMP-2, with a molecular mass of 21 kDa, was detected in products secreted by both peritubular and Sertoli cells (Fig. 7).

DISCUSSION

The data presented demonstrate that peritubular cells secrete a variety of metalloproteinases. Type IV collagenase is of special interest because it has been implicated in tumour metastasis [7], and it degrades a major component (type IV collagen) present in all basement membranes [27]. The primary metalloproteinase secreted by peritubular cells under basal conditions is the latent type IV procollagenase. Disruption of the cytoskeleton in these cells by cytochalasin D was followed by increases in activities of several metalloproteinases, and by activation of latent type IV collagenase to its active form. The decrease in the procollagenase IV of molecular masses 75 kDa and 72 kDa was accompanied by an increase in the 66 kDa active type IV collagenase protein, as demonstrated by both Western immunoblotting and zymography. Interestingly, H-*ras*-oncogene-transformed human bronchial epithelial cells secrete a 72 kDa latent type IV procollagenase that can be chemically activated to the 66 kDa active collagenase type IV [28].

The cytoskeleton appears to be involved in signal transduction [29–31]. Several reports [11,12,32–36] have shown that treatment of synovial fibroblasts with agents that elicit disruption of actin microfilament assemblies, causing a rounding up of cells, is accompanied by increased activities of metalloproteinases. The disruption of actin microfilaments precedes the increase in collagenase activity. These data suggest that the cytoskeleton may be required for mediating an extracellular stimulus that modulates the control of gene expression for collagenases [37]. In partial support of this conjecture, Werb *et al.* [38] showed that treatment of cells with antibody against the fibronectin receptor resulted in failure of cells to adhere to the substratum, to rounding up of the cells and to increased expression of genes encoding for collagenase and stromelysin. Our observations, demonstrating enhanced activity of the 66 kDa collagenase secreted by peritubular cells after disruption of the cytoskeleton by cytochalasin D (Fig. 4), offer further support to the above interpretations of Werb *et al.* [38].

It has been shown that human melanoma cells and transformed lung fibroblasts secrete a specific inhibitor of the 72 kDa procollagenase, an inhibitor called TIMP-2 [39,40]. Activation of the 72 kDa procollagenase by APMA could be inhibited by TIMP-2 in a dose-dependent manner [40]. TIMP-2 could be involved in the regulation of activation of procollagenase IV in peritubular cells, since the 21 kDa TIMP-2 is secreted by Sertoli cells, and also by peritubular cells, in culture (Fig. 7). Further studies are required to evaluate the possibility that cytochalasin D may affect amounts of TIMP-2 secreted, and thereby influence the activation processes.

Co-culture of Sertoli cells with peritubular cells unexpectedly resulted in decreased activities of metalloproteinases in the medium. Surprisingly, cytochalasin D failed to elicit any detectable changes in activities of metalloproteinases secreted by Sertoli cells, even though the microfilament assembly in these cells was disrupted. Furthermore, the presence of Sertoli cells inhibited the cytochalasin D-induced activation of type IV collagenase by peritubular cells. Inhibition of the conversion of procollagenase IV into its active form also occurred, but to a lesser extent, during co-culture in a two-chambered assembly in which the two cell types were separated by a filter (Fig. 6*b*). These data suggest that Sertoli cells inhibited the influences of cytochalasin D on peritubular cells by secretion of paracrine factor(s) that modulate the conversion of type IV procollagenase into its active form. The possible role in these processes of TIMP-2, synthesized by Sertoli cells, remains to be evaluated. Other kinds of interactions between the two cell types have been shown to influence net plasminogen activator activity (for review see ref. [41]). A specific inhibitor of plasminogen activator (type 1) secreted by peritubular cells decreases the activities of urokinase and tissue-type plasminogen activators secreted by Sertoli cells [17,41]. By analogy, it seems possible that paracrine component(s) secreted by Sertoli cells may influence activities of collagenases synthesized by peritubular cells.

Peritubular myoid cells are separated from Sertoli cells *in vivo* by a basal lamina rich in laminin, type IV collagen and various proteoglycans [42,43]. This basal lamina is formed co-operatively by both cell types in co-culture, with each population of cells contributing unique extracellular-matrix components [42,44]. Deposition of secreted soluble extracellular-matrix components to form an organized basal lamella requires direct contact between the two cell types [42,45]. Other types of metabolic co-operativity between peritubular cells and Sertoli cells have been documented (for reviews see refs. [42] and [46]). Interactions in the modulation of metalloproteinase activities demonstrated in this paper add additional examples of ways in which Sertoli cells and peritubular myoid cells can influence each other *in vitro*, and

presumably *in vivo*. Sang *et al.* [47] recently reported that activities of several metalloproteinases secreted by Sertoli cells in culture are modulated in a complex manner by the presence of testicular peritubular cells. In addition, a fibronectin-degrading proteinase, with putative molecular mass of 50 kDa, was reported to be present in the conditioned medium of co-cultures of Sertoli cells and peritubular cells treated with follicle-stimulating hormone, but not in the absence of this hormone, or in conditioned medium of monocultures of Sertoli cells or peritubular cells incubated in the presence or in the absence of the hormone [47].

Changes in plasminogen activator activities in seminiferous tubules have been correlated with the restructuring that occurs during specific stages of spermatogenesis [5,42]. These and other proteinases, including metalloproteinases in specific regions of the tubule, could potentially modulate tight junctional complex degradation and formation, and thereby influence the integrity of the seminiferous tubule barrier (for discussion and review see refs. [41] and [42]).

We thank Kathy Wong and Krystyna Burdzy for excellent technical assistance, and Linda Miller and Rosa Jara for skilful preparation of the typescript. The work was supported by Grant MT 3292 from the Canadian Medical Research Council.

REFERENCES

1. Reich, E. (1978) in Molecular Basis of Biological Degradative Processes (Herman, H., Lepow, J. H. & Tanzer, J. M., eds.), pp. 155–169, Academic Press, New York
2. Saksela, O. & Rifkin, D. B. (1988) Annu. Rev. Cell Biol. **4**, 93–126
3. Dano, K., Andreasen, P. A., Grondhal-Hansen, J., Kristensen, P., Nielsen, L. S. & Shriver, L. (1985) Adv. Cancer Res. **44**, 140–266
4. Lacroix, M., Smith, F. E. & Fritz, I. B. (1977) Mol. Cell. Endocrinol. **9**, 227–236
5. Hettle, J. A., Balekjian, E., Tung, P. S. & Fritz, I. B. (1988) Biol. Reprod. **38**, 359–371
6. Sang, Q.-X., Stetler-Stevenson, W. G., Liotta, L. A. & Byers, S. W. (1990) Biol. Reprod. **43**, 956–964
7. Liotta, L. A., Tryggvason, K., Garbisa, S., Hart, I., Foltz, C. M. & Shafie, S. (1980) Nature (London) **284**, 67–68
8. Stetler-Stevenson, W. G., Krutzsch, H. C., Wacher, M. P., Margulies, I. M. K. & Liotta, L. A. (1989) J. Biol. Chem. **264**, 1353–1356
9. Springman, E. B., Angleton, E. L., Brinkedal-Hansen, H. & Van Wart, H. E. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 364–368
10. Van Wart, H. E. & Brinkedal-Hansen, H. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 5578–5582
11. Harris, E. D., Jr., Reynolds, J. J. & Werb, Z. (1975) Nature (London) **257**, 243–244
12. Unemori, E. N. & Werb, Z. (1988) J. Biol. Chem. **263**, 16252–16259
13. Tung, P. S. & Fritz, I. B. (1990) Biol. Reprod. **42**, 351–365
14. Tung, P. S., Dorrington, J. H. & Fritz, I. B. (1975) Proc. Natl. Acad. Sci. U.S.A. **72**, 1838–1842
15. Tung, P. S., Skinner, M. K. & Fritz, I. B. (1984) Biol. Reprod. **30**, 199–211
16. Ailenberg, M., Tung, P. S., Pelletier, M. & Fritz, I. B. (1988) Endocrinology (Baltimore) **122**, 2604–2612
17. Ailenberg, M. & Fritz, I. B. (1988) Endocrinology (Baltimore) **122**, 2613–2618
18. Ailenberg, M. & Fritz, I. B. (1989) Endocrinology (Baltimore) **124**, 1399–1407
19. Ailenberg, M., McCabe, D. & Fritz, I. B. (1990) Endocrinology (Baltimore) **126**, 1561–1568
20. Heussen, C. & Dowdle, E. B. (1980) Anal. Biochem. **102**, 196–202
21. Laemmli, U. K. (1970) Nature (London) **227**, 680–685
22. Stetler-Stevenson, W. G., Brown, P. D., Onisto, M., Levy, A. T. & Liotta, L. A. (1989) J. Biol. Chem. **265**, 13933–13938
23. Towbin, H., Staehlin, T. & Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354
24. Hettle, J. A., Waller, E. K. & Fritz, I. B. (1986) Biol. Reprod. **34**, 895–904
25. Tung, P. S. & Fritz, I. B. (1987) Dev. Biol. **120**, 139–153
26. Ailenberg, M., Tung, P. S. & Fritz, I. B. (1990) Biol. Reprod. **42**, 499–509
27. Linsenmayer, T. F. (1981) in Cell Biology of the Extracellular Matrix (Hay, E. D., ed.), pp. 5–37, Plenum Press, New York and London
28. Collier, I. E., Wilhelm, S. M., Eissen, A. Z., Marmer, B. L., Grant, G. A., Seltzer, J. L., Kronberger, A., He, C., Bauer, E. A. & Goldberg, G. I. (1988) J. Biol. Chem. **263**, 6579–6587
29. Ingber, D. E. & Folkman, J. (1989) in Cell Shape Determinants, Regulation and Regulatory Role (Stein, W. D. & Bronner, F., eds.), pp. 3–31, Academic Press, New York
30. Ben-Ze'ev, A. (1989) in Cell Shape Determinants, Regulation and Regulatory Role (Stein, W. D. & Bronner, F., eds.), pp. 95–119, Academic Press, New York
31. Hay, E. D. & Svoboda, K. K. (1989) in Cell Shape Determinants, Regulation and Regulatory Role (Stein, W. D. & Bronner, F., eds.), pp. 147–172, Academic Press, New York
32. Werb, Z. & Aggeler, J. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 1839–1843
33. Aggeler, J., Frisch, S. M. & Werb, Z. (1984) J. Cell Biol. **98**, 1656–1661
34. Aggeler, J., Frisch, S. M. & Werb, Z. (1984) J. Cell Biol. **98**, 1662–1671
35. Unemori, E. N. & Werb, Z. (1986) J. Cell Biol. **103**, 1021–1031
36. Werb, Z., Hemberg, R. M., Murphy, G. & Aggeler, J. (1986) J. Cell Biol. **102**, 697–702
37. Frisch, S. M., Clark, E. J. & Werb, Z. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 2600–2604
38. Werb, Z., Tremble, P. M., Behrendtsen, O., Crowley, E. & Damsky, C. H. (1989) J. Cell Biol. **109**, 877–889
39. Stetler-Stevenson, W. G., Krutzsch, H. C. & Liotta, L. A. (1989) J. Biol. Chem. **264**, 17374–17378
40. Goldberg, G. I., Mormer, B. L., Grant, G. A., Eisen, A. Z., Wilhelm, S. & Chenshi, H. (1989) Proc. Soc. Natl. Acad. Sci. U.S.A. **86**, 8207–8211
41. Fritz, I. B., Lacroix, M., Smith, F., Hettle, A., Nargolwalla, C., Tung, P. & Ailenberg, M. (1989) in Development and Function of the Reproductive Organs, vol. 3 (Josso, N., ed.), pp. 133–152, Ares-Serono Symposia, Rome
42. Fritz, I. B. & Tung, P. S. (1986) in Gametogenesis and the Early Embryo (Gall, J., ed.), pp. 151–173, Alan R. Liss, New York
43. Hadley, M. A. & Dym, M. (1987) Biol. Reprod. **37**, 1283–1289
44. Tung, P. S., Skinner, M. S. & Fritz, I. B. (1984) Ann. N.Y. Acad. Sci. **438**, 435–446
45. Tung, P. S. & Fritz, I. B. (1986) Dev. Biol. **115**, 155–170
46. Fritz, I. B. & Tung, P. S. (1989) in Cellular and Molecular Events in Spermiogenesis (Hamilton, D. W. & Waites, G. M. H., eds.), pp. 177–192, Cambridge University Press, Cambridge
47. Sang, Q.-X., Dym, M. & Byers, S. W. (1990) Biol. Reprod. **43**, 946–955

Received 10 January 1991/25 March 1991; accepted 12 April 1991