

Plasminogen Activators in Tissue Remodeling and Invasion: mRNA Localization in Mouse Ovaries and Implanting Embryos

André-Pascal Sappino,* Joaquin Huarte, Dominique Belin,‡ and Jean-Dominique Vassalli

* Division of Onco-Haematology, ‡ Department of Pathology, and Institute of Histology and Embryology, University of Geneva, Medical School, 1211 Geneva 4, Switzerland

Abstract. To assess *in vivo* the postulated participation of urokinase-type (u-PA) and tissue-type (t-PA) plasminogen activators in processes involving tissue remodeling and cell migration, we have studied the cellular distribution of u-PA and t-PA mRNAs during mouse oogenesis and embryo implantation. By *in situ* hybridizations, we detected t-PA mRNA in oocytes and u-PA mRNA in granulosa and thecal cells from preovulatory follicles. These findings are compatible with a role for plasminogen activators in oogenesis and follicular disruption. We demonstrated the presence of u-PA mRNA in the invasive and migrating trophoblast cells of 5.5- and 6.5-d-old embryos. At 7.5 days, u-PA mRNA was predominantly localized to

trophoblast cells that had reached the deep layers of the uterine wall, while the peripheral trophoblast cells surrounding the presomite stage embryo were devoid of specific signal. In 8.5-d-old embryos abundant u-PA mRNA expression resumed transiently in the giant trophoblast cells at the periphery of the embryo and in the trophoblast cells of the ectoplacental cone, to become undetectable in 10.5-d-old embryos. These observations establish the *in vivo* expression of the u-PA gene by invading and migrating trophoblast cells in a biphasic time pattern; they are in agreement with the proposed involvement of the enzyme in the extracellular proteolysis accompanying embryo implantation.

MAMMALIAN gametogenesis and embryogenesis are associated with extensive tissue remodeling and cellular migrations. Such processes require temporospatially regulated degradation of extracellular matrices and experimental evidence has accumulated suggesting that extracellular proteases are indeed involved in ovulation (Espey, 1974; Beers, 1975; Beers et al., 1975), sperm maturation (Lacroix et al., 1979), fertilization (Stambaugh, 1978; Saling, 1981; Bleil et al., 1981; Moller and Wassarman, 1989), and embryo implantation (Denker, 1972; Strickland et al., 1976; Glass et al., 1983). Though the proteases susceptible of participating in these various conditions have not been fully identified, plasminogen activators (PAs)¹ are known to be secreted during gametogenesis (Beers, 1975; Lacroix et al., 1981; Huarte et al., 1985, 1987a; Vihko et al., 1989) and early embryonic development (Strickland et al., 1976; Marotti et al., 1982; Axelrod, 1985). PAs are serine proteases that catalyze the conversion of plasminogen into plasmin, an active protease of broad specificity capable of degrading, directly or indirectly, i.e., through the activation of metalloprotease zymogens, all components of the extracellular matrix (Reich, 1978; Danø et al., 1985). The two known PAs, urokinase-type (u-PA) and tissue-type (t-PA) plasminogen activators, are the product of two distinct genes; they are synthe-

sized and secreted by a wide spectrum of cell types and are generally thought to play a central role in regulating extracellular proteolysis in physiological and pathological conditions, precisely where tissue disruption and cellular migration are prominent (Danø et al., 1985). In the mouse ovary, u-PA is produced by granulosa cells under the control of gonadotropins (Canipari et al., 1987), while t-PA is the first protease to have been identified in murine oocytes (Huarte et al., 1985); plasmin-mediated proteolysis facilitates follicular disruption (Beers, 1975) and could also be involved in oocyte migration in the oviduct. Trophoblasts are the first cell type to differentiate in mammalian embryos: they are transiently endowed with marked invasive and migrating properties, to achieve embryo implantation via the penetration of the uterine mucosa. Cultured mouse blastocysts and trophoblasts have been shown to produce PA activity, with a timing that corresponds to the period of trophoblastic invasiveness *in vivo* (Strickland et al., 1976). These cumulated observations suggest that PAs may act as catalyzers of the extracellular proteolysis accompanying gametogenesis and embryo implantation; however, they are based on *in vitro* analyses and limited information is presently available concerning PA synthesis *in vivo*.

This prompted us to perform *in situ* hybridizations, using specific cRNA probes for mouse u-PA and t-PA mRNAs to assess the cellular distribution of PA synthesis during oogenesis and early phases of embryo implantation. We report here that cell types engaged in follicular disruption and in uterine

1. Abbreviations used in this paper: PA, plasminogen activator; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator.

invasion, such as granulosa cells of preovulatory follicles and trophoblast cells, express high levels of u-PA mRNA in a time-dependent fashion compatible with the postulated participation of the PA-plasmin system in physiological events of tissue remodeling and cell migration.

Materials and Methods

Materials

Ovaries were collected from adult NMRI virgin females (Kleintierfarm Madorfing, Basel); preovulatory ovaries were obtained from pregnant mare serum primed females, injected 46 h later with human chorionic gonadotropins, and killed 5 h after the last injection (5 IU of pregnant mare serum and human chorionic gonadotropins ip/female); postovulatory ovaries were obtained from females similarly treated and killed 16 h after the last injection, i.e., ~4 h after ovulation. Ovaries were dissected, embedded in Tissu-Tek (Miles Ames Div., Elkhart, IN), frozen down in precooled methylbutane and stored at -70°C . Four to six ovaries were analyzed for each condition.

Adult NMRI female mice were caged with males in the evening and monitored for the appearance of a vaginal plug the following morning. On the 4th day of gestation, blastocysts were flushed from the uteri of NMRI females with F11 medium supplemented with 5% FCS, and then cultured for 4 d in F11 medium, supplemented with 10% FCS. At the end of the culture, the trophoblast outgrowths were collected and pooled in groups of 10 embryos before analysis.

Pregnant mice were sacrificed on the 6th, 7th, 8th, and 11th days of gestation to obtain 5.5-, 6.5-, 7.5-, 8.5-, and 10.5-d-old embryos. Segments of uteri were dissected, embedded, and stored as above. The presumptive stages were confirmed histologically using Theiler's description (1989). 8.5-d-old embryos were at the three to seven somites stage and 10.5 d of development corresponded to the 28–34 somites stage. For each time point, four to six whole embryos were analyzed, and 10–40 tissue sections were prepared per embryo.

Enzymatic Analyses

Protein extractions, immunoprecipitation, gel electrophoresis, and zymography were performed as described (Huarte et al., 1985).

Plasmid Constructions and In Vitro Transcriptions

The u-PA antisense probe pSP64-MU containing the 658-bp Pst I–Hind III fragment of the mouse u-PA cDNA clone pDB29 (Belin et al., 1985), the u-PA sense probe pSP65-MU containing the same fragment in the opposite direction and the t-PA antisense probe pSP64-MT₃ containing the 726-bp Pvu II–Spe I fragment of the mouse t-PA cDNA clone pUC9-A33 (Ricklees et al., 1988) were transcribed in vitro, as previously described (Huarte et al., 1987a,b), using SP6 RNA polymerase, in the presence of 12.5 μM of α -³²P-labeled UTP (400 Ci/mmol; Amersham International, Amersham, UK) for Northern blot analyses, or 30 μM ³H-labeled UTP and 30 μM ³H-labeled CTP (40 and 20 Ci/mmol, respectively; Amersham International) for in situ hybridizations. ³H-labeled RNAs were reduced to an average size of 50–100 nt by mild alkaline hydrolysis, in 50 mM Na₂CO₃ for 30 min (u-PA probes) or 15 min (t-PA probes) at 50°C, neutralized with 1 M HOAc, extracted in phenol chloroform, precipitated in ethanol, and resuspended in a minimal volume of water.

Northern Blot Analyses

Total RNAs from vas deferens, oviduct, and ovaries were extracted as described elsewhere (Busso et al., 1986). Blastocyst RNA was prepared as described (Huarte et al., 1987b). RNAs were denatured with glyoxal, electrophoresed in 1.2% agarose gels, and transferred overnight onto Biodyne nylon membranes (Pall Corp., Glen Cove, NY). Prehybridizations, hybridizations, and posthybridizations washes were performed as previously described (Huarte et al., 1987a,b).

In Situ Hybridizations

5- μm cryostat tissue sections were mounted on poly-L-lysine- (Sigma Chemical Co., St. Louis, MO) coated microscope slides, fixed in 1% paraformaldehyde in PBS for 1–2 min, rinsed in PBS, and stored in 70% ethanol at

4°C until analyzed. Fixed sections were rinsed in 2 \times SSC (1 \times SSC = 150 mM NaCl, 15 mM Na-citrate, pH 7.0), acetylated with 0.25% (vol/vol) acetic anhydride in 0.1 M triethanolamine, pH 8.0, at room temperature for 10 min, incubated in 0.1 M Tris-hydrochloride, pH 7.0, 0.1 M glycine at room temperature for 30 min, and prehybridized in 2 \times SSC, 50% formamide at 50°C for 15 min. 10–20 ng of ³H-labeled RNAs were applied to each section in 20 μl of hybridization mixture (2 \times SSC, 50% formamide, 1 mg/ml BSA (RNase-free; Sigma Chemical Co.), 1 mg/ml *Escherichia coli* rRNA (Boehringer Mannheim GmbH, Mannheim, FRG), 10 mM DTT and 5% dextran sulfate) at 50°C for 3 h. Slides were subsequently washed in 2 \times SSC, 50% formamide and 0.2 \times SSC, 50% formamide, at 50°C for 30 min each. Unhybridized transcripts were digested with 10 $\mu\text{g}/\text{ml}$ ribonuclease A (u-PA probe) or 5 $\mu\text{g}/\text{ml}$ ribonuclease T1 (t-PA probe), at 37°C for 30 min. The slides were washed again in 2 \times SSC, 50% formamide at 50°C for 15 min dehydrated in graded ethanol and air-dried. Finally, they were immersed in NTB-2 emulsion (Eastman Kodak Co., Rochester, NY), diluted 1:1 in deionized water. After 4–5 wk exposure, they were developed in Kodak D-19 developer, fixed in 30% Na thiosulfate, and counterstained in methylene blue.

Microphotographs were taken with a Zeiss photomicroscope (Zeiss, Oberkochen, FRG), equipped with an immersion dark-field condenser, using Kodak Ektachrome 50 color film.

Controls of Specificity

They were performed as previously described (Huarte et al., 1987a) and included the use of sense RNA probes and dot blot analysis under conditions of in situ hybridizations. As compared to our previous report (Huarte et al., 1987a), the stringency conditions of hybridizations and posthybridizations washes were increased, according to results obtained in preliminary experiments comparing the signals obtained with the u-PA and t-PA probes on tissue sections of the vas deferens and the oviduct. These organs have a similar anatomical organization but they contain respectively and predominantly u-PA and t-PA mRNA (see Fig. 3). An optimal distinction between u-PA and t-PA mRNAs was achieved by including a wash at low ionic strength and RNase treatments. In each hybridization, control tissue sections of the vas deferens and the oviduct were included.

Results

t-PA mRNA in Oocytes and u-PA mRNA in Ovarian Granulosa and Thecal Cells

In ovaries from unstimulated females, t-PA mRNA was detected in the cytoplasm of a fraction of the oocytes; the positively labeled oocytes were observed in medium and large size follicles (Fig. 1, A and B). The distribution of t-PA mRNA remained localized to oocytes in preovulatory and postovulatory ovaries (not shown), although, as expected, the number of large follicles was increased in gonadotropin-treated animals. In unstimulated ovaries, moderate amounts of u-PA mRNA were confined to the granulosa cells of large follicles (not shown). In postovulatory ovaries, the u-PA signal was comparable to that obtained in unstimulated ovaries and predominantly localized in the outer part of the follicles (Fig. 1 C). In preovulatory ovaries, the concentration of u-PA mRNA in granulosa cells was greatly increased (Fig. 1 D) and, in addition, some thecal cells were also found to express abundant u-PA mRNA (Fig. 1 E). The exclusive distribution of t-PA mRNA in oocytes and of u-PA mRNA in follicular cells corroborated in vitro observations (Huarte et al., 1987b; Canipari et al., 1987) and confirmed the respective specificities of the t-PA and u-PA cRNA probes.

Synthesis of u-PA by Cultured Trophoblast Cells

Previous studies have demonstrated the secretion of PA enzymatic activity by cultured mouse trophoblasts (Strickland et al., 1976). However, the type of PA has not been reported. To identify the type of PA produced by trophoblast cells, we

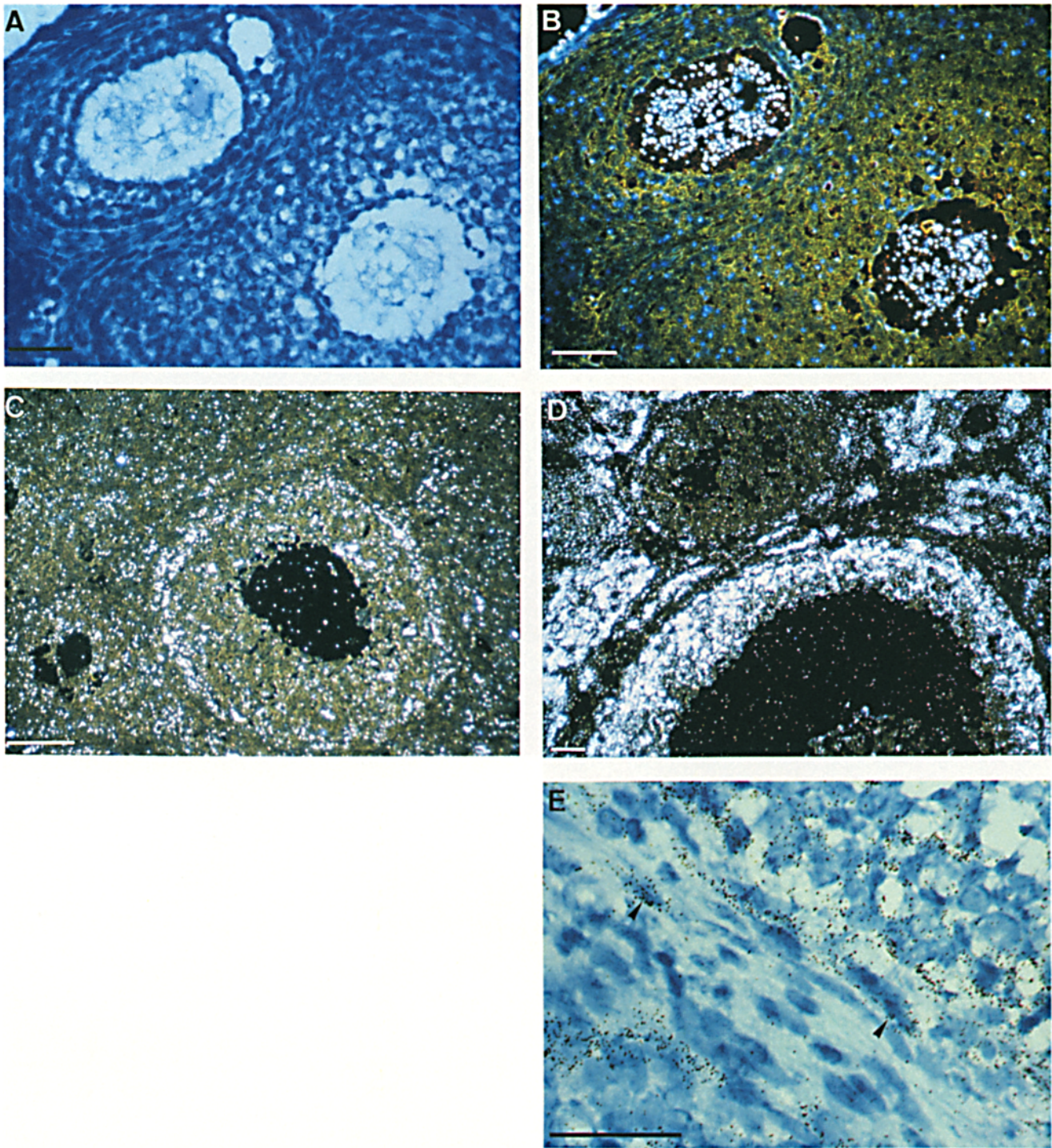


Figure 1. Localization of t-PA (*A* and *B*) and u-PA mRNA (*C*, *D*, and *E*) by in situ hybridizations of ^3H -labeled cRNA probes to frozen sections from ovaries. (*A*, *B*, and *C*) Unstimulated ovaries; (*D* and *E*) preovulatory ovaries. *B*, *C*, and *D* are darkfield micrographs; *A* and *E* are light-field micrographs. Note the absence of grains over the oocyte nucleus in *A* and *B*. Sections shown in *C*, *D*, and *E* were hybridized with the same probe and exposed for the same length of time. Arrowheads in *E* denote labeled thecal cells. Bar, 10 μm .

assayed extracts of isolated trophoblasts from cultured blastocysts for PA activity and mRNA content. One single type of PA was revealed by zymography after SDS-PAGE, and immunoprecipitation with specific antibodies showed that this PA is undistinguishable from the 48,000-*M_r* u-PA (Fig. 2). Preparations of total RNA were analyzed by Northern blot hybridizations. The detection of u-PA mRNA, but not of t-PA

mRNA, confirmed the exclusive synthesis of u-PA by cultured trophoblast cells (Fig. 3, *A* and *B*).

Localization of u-PA mRNA in Invasive and Migrating Trophoblast Cells

To localize u-PA and t-PA mRNAs in vivo, we analyzed implanting embryos by in situ hybridizations. In 5.5-d-old em-

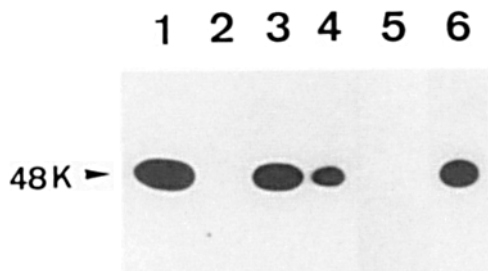


Figure 2. Characterization of the proteolytic activity of trophoblast cells by SDS-PAGE and zymography. Extracts of 10 trophoblast outgrowths were analyzed directly (lane 1) or after immunoprecipitation with anti-murine u-PA IgG (lane 2) or irrelevant IgG (lane 3). Eluates of the *S. aureus*-bound immune complexes formed with anti-murine u-PA IgG (lane 4) and irrelevant IgG (lane 5) were also analyzed. Murine u-PA standard (lane 6). The photograph was taken after 28 h of incubation at 37°C.

bryos, trophoblast cells are polyploid, surround the egg cylinder, and also constitute the ectoplacental cone which projects above the embryonic pole. At that stage, u-PA mRNA was present in cells recognizable by their morphology and their distribution as the peripheral flattened trophoblasts that have begun to erode the uterine mucosa and as ectoplacental cone cells, while the egg cylinder and the deciduous cells were devoid of specific signal (Fig. 4, A and B). In contrast, t-PA mRNA was not detected in any embryonic cell, but in epithelial cells of the numerous glands located at the periphery of the decidua cells (Fig. 4, C and D). t-PA has been isolated from uterine tissue (Rijken et al., 1979; Rijken and Collen, 1981) and our results identify the endometrial glands as the most probable source of the enzyme. The distribution of u-PA and t-PA mRNAs in different cell types provides an internal control for the specificity of their detection; furthermore, no signal was observed when a sense u-PA RNA probe was used (not shown).

During the next day, the trophoblast cells continue to penetrate into the uterine wall and the ectoplacental cone gains contact with the mesometrial blood vessels. In 6.5-d-old embryos, the invasive and migrating trophoblast cells, identified as polyploid cells, contained large amounts of u-PA mRNA, while the trophoblasts located at the periphery of the embryo showed moderate, albeit significant, labeling. The inspection of successive sections showed that the highly labeled giant cells that had infiltrated the uterine mucosa were clearly detached from the ectoplacental cone (Fig. 5, A and B); a similar intense signal was obtained on individual giant cells present at the opposite pole, close to the zone of decaying uterine epithelium, suggesting that trophoblasts are migrating from both poles of the embryo (Fig. 5, C and D). The majority of the uterine glandular structures observed at 5.5 d were no longer visible and no t-PA mRNA could be detected (not shown).

In 7.5-d-old embryos, a number of trophoblast cells have reached the deep layers of the endometrium, whereas a persistent layer of flattened trophoblasts surrounds the parietal endoderm. At that stage, we observed an intense u-PA mRNA signal in a few isolated giant cells that had penetrated into the uterine stroma, while the trophoblast cells surrounding the embryo remained weakly positive or were negative (Fig. 5, E and F). Here again, no detectable t-PA mRNA was observed (not shown).

During the next 24 h, the embryo expands considerably and in 8.5-d-old embryos, corresponding to the three to seven somites stage, the peripheral trophoblast cells enlarge to form a loose network of cells, attached to the maternal tissues and adherent to Reichert's membrane. These giant trophoblast cells contained large amounts of u-PA mRNA (Fig. 6, A and B), a signal comparable in intensity to that observed in the ectoplacental trophoblasts at 5.5 and 6.5 d of development. High levels of u-PA mRNA were also observed in the ectoplacental cone; though they were of smaller size than peripheral trophoblasts, the positively labeled cells had prominent nuclei, were localized predominantly at the front edge of the ectoplacental cone, and contained glycogen granules, indicating they were trophospongium precursors (Fig. 6, C and D).

Finally, in 10.5-d-old specimens, corresponding to the 30-34 somites stage, the giant trophoblast cells surrounding the embryo were not labeled by the u-PA cRNA probe, whereas the numerous maternal venous sinuses were lined

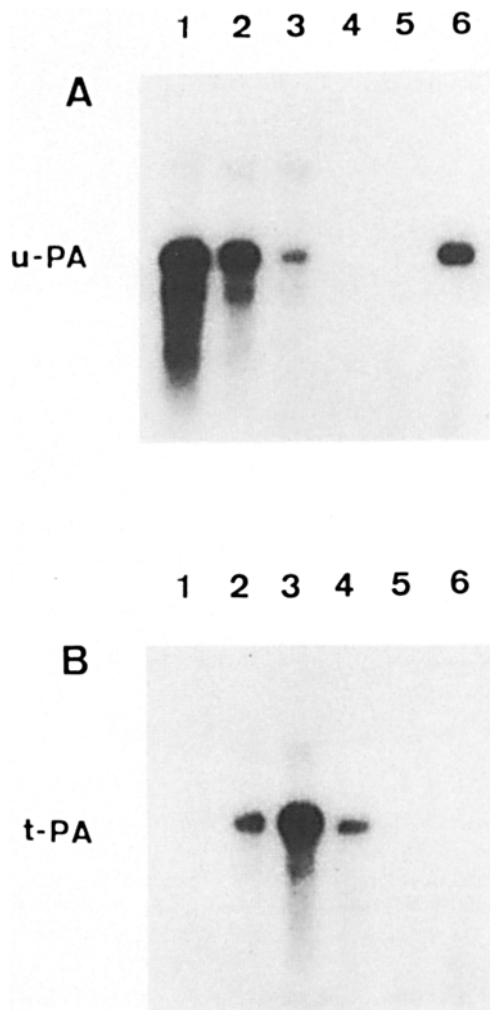


Figure 3. Northern blot analysis of u-PA (A) and t-PA (B) mRNA in adult tissues and cultured blastocysts. 2 μ g of total RNA was prepared from the following tissues: vas deferens (lane 1), ovary (lane 2), and oviduct (lane 3); total RNA was obtained from 30 primary oocytes (lane 4), from the inner cell masses (lane 5), and trophoblast outgrowths (lane 6) of 10 embryos.

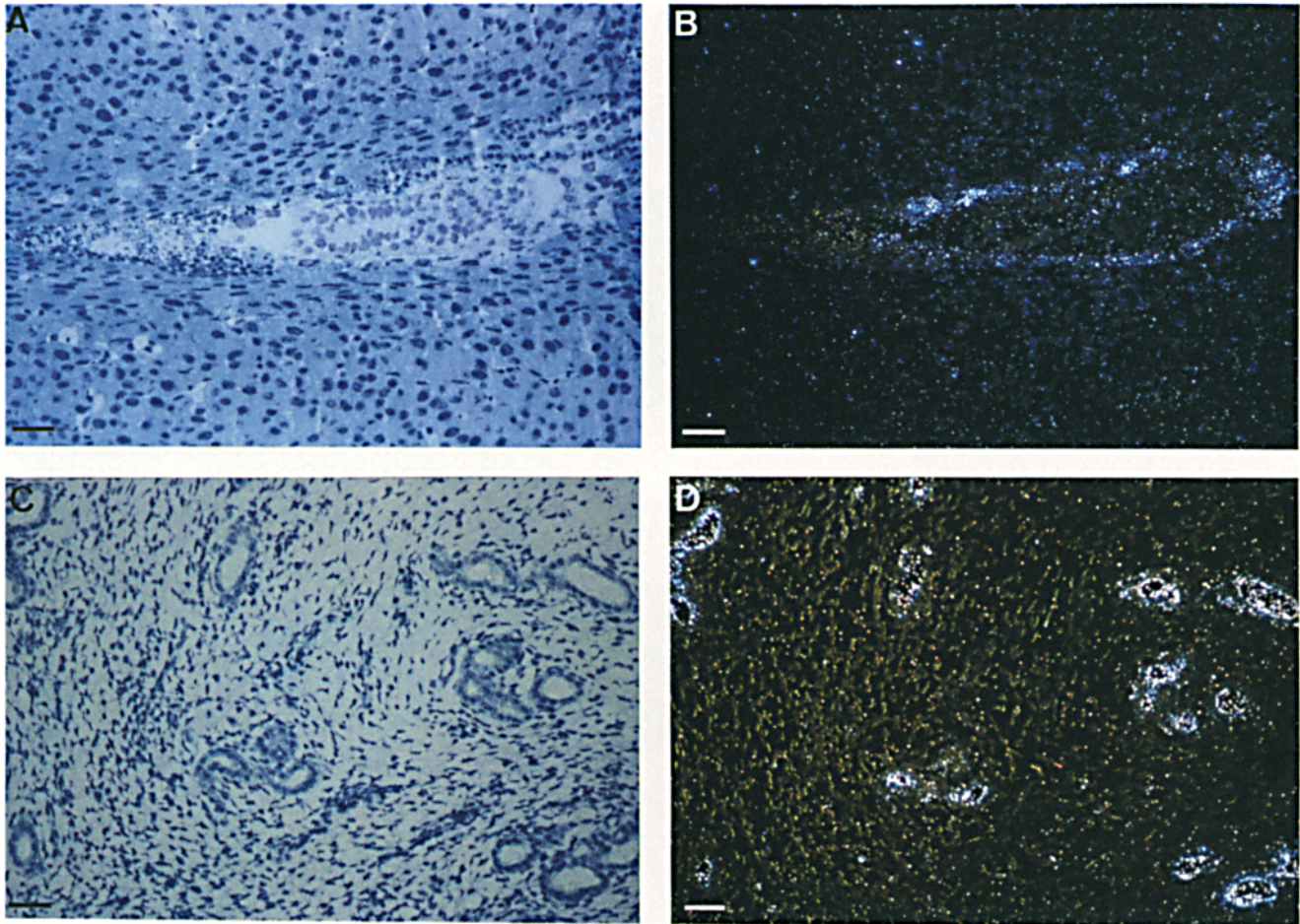


Figure 4. Localization of u-PA mRNA in peripheral and ectoplacental trophoblast cells (*A* and *B*) and of t-PA mRNA in the endometrial glands (*C* and *D*) by in situ hybridizations of ^3H -labeled cRNA probes to frozen sections of a gravid uterus containing a 5.5-d-old embryo. *A* and *C* are light-field micrographs; *B* and *D* are the corresponding dark-field micrographs. Bar, 10 μm .

with cells expressing u-PA mRNA (Fig. 7, *A* and *B*). These cells constitute probably the outer layer of the hemotrichorial murine placenta (Enders, 1965). Hybridizations with the t-PA cRNA probe revealed a weak positive signal over a few cells localized in the labyrinth (not shown). The identity of these cells cannot be ascertained, but they could represent the embryonic endothelial cells.

Discussion

In mammals, considerable experimental evidence has linked production of PAs to normal and pathological conditions involving tissue destruction and cell migration (Reich, 1978; Danø et al., 1985). For example, PAs are thought to play a role in regulating extracellular proteolysis associated with gametogenesis, fertilization, and early embryonic development (Beers, 1975; Lacroix et al., 1979; Strickland et al., 1976; Huarte et al., 1985, 1987a; Vihko et al., 1989). However, most studies have relied on in vitro observations to substantiate the potential implications of PAs in physiological processes of tissue remodeling. Though a few immunohistochemical studies on mouse tissue have been reported for u-PA (Larsson et al., 1984) and t-PA (Kristensen et al., 1985), only very limited information concerning the cellular

sites of PA synthesis in vivo is presently available. Indeed, secondary interactions of these secreted proteins with components of extracellular matrices (Hoylaerts et al., 1982; Salonen et al., 1984), cell surface receptors (Vassalli et al., 1985; Stoppelli et al., 1985), or inhibitors (Baker et al., 1980; Loskutoff et al., 1983; Lecander and Astedt, 1986; Wohlwend et al., 1987) can result in erroneous interpretations of results based on their immunological or enzymatic localization. By using in situ hybridization, a method that allows unambiguous identification of potential cellular sites of synthesis for a given protein, we have localized t-PA and u-PA mRNAs in the mouse ovary and implanting embryo.

t-PA mRNA was present in the cytoplasm of oocytes, in agreement with previous biochemical studies showing that dormant t-PA mRNA accumulates in growing oocytes and is translated in oocytes undergoing meiotic maturation (Huarte et al., 1987b). In contrast, we detected u-PA mRNA in granulosa cells of medium size follicles, confirming recent work reporting the production of u-PA by mouse granulosa cells in vitro (Canipari et al., 1987). We also observed that hormonal stimulation increases u-PA mRNA in both granulosa and thecal cells, whereas in the rat, gonadotropins stimulate production of t-PA by granulosa cells and of u-PA by thecal cells (Canipari and Strickland, 1985). The differential

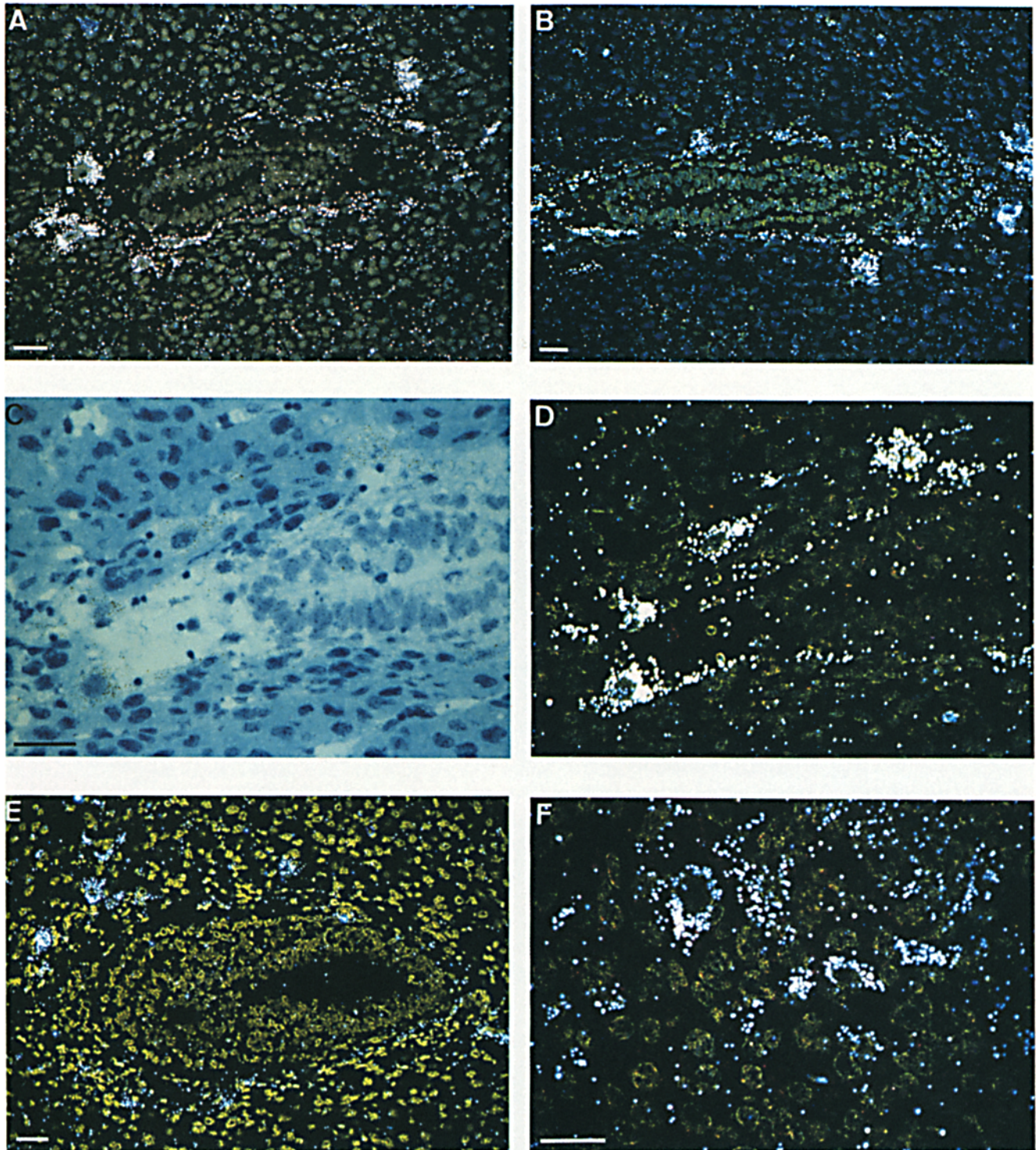


Figure 5. Localization of u-PA mRNA in invasive and migrating trophoblast cells by in situ hybridization of ^3H -labeled cRNA probe to frozen sections of 6.5-d-old (*A*, *B*, *C*, and *D*) and 7.5-d-old (*E* and *F*) embryos. *A*, *B*, *D*, *E*, and *F* are dark-field micrographs; *C* is the corresponding light-field micrograph shown in *D*. The ectoplacental cone is located on the right in *A*, *B*, *C*, and *D*, and on the left in *E* and *F*. *A*, *B*, and *C* correspond to different sections of the same embryo. Bar, 10 μm .

distribution of u-PA and t-PA synthesis in the mouse ovary may be related to the functional differences attributed to these two enzymes. t-PA activity is enhanced in presence of fibrin (Hoylaerts et al., 1982; Suenson et al., 1984), heparin, and proteoglycans (Andrade-Gordon and Strickland, 1986), and t-PA is produced by endothelial cells (Levin, 1983; Kristensen

et al., 1984). The primary function of t-PA could thus be to catalyze fibrinolysis. In this view, t-PA synthesized and released by maturing oocytes might prevent premature clot formation in the ovarian stigma and egg adhesion to fibrin deposits in the oviduct before implantation (Canipari and Strickland, 1985; Huarte et al., 1985). In contrast, u-PA catalyzed pro-

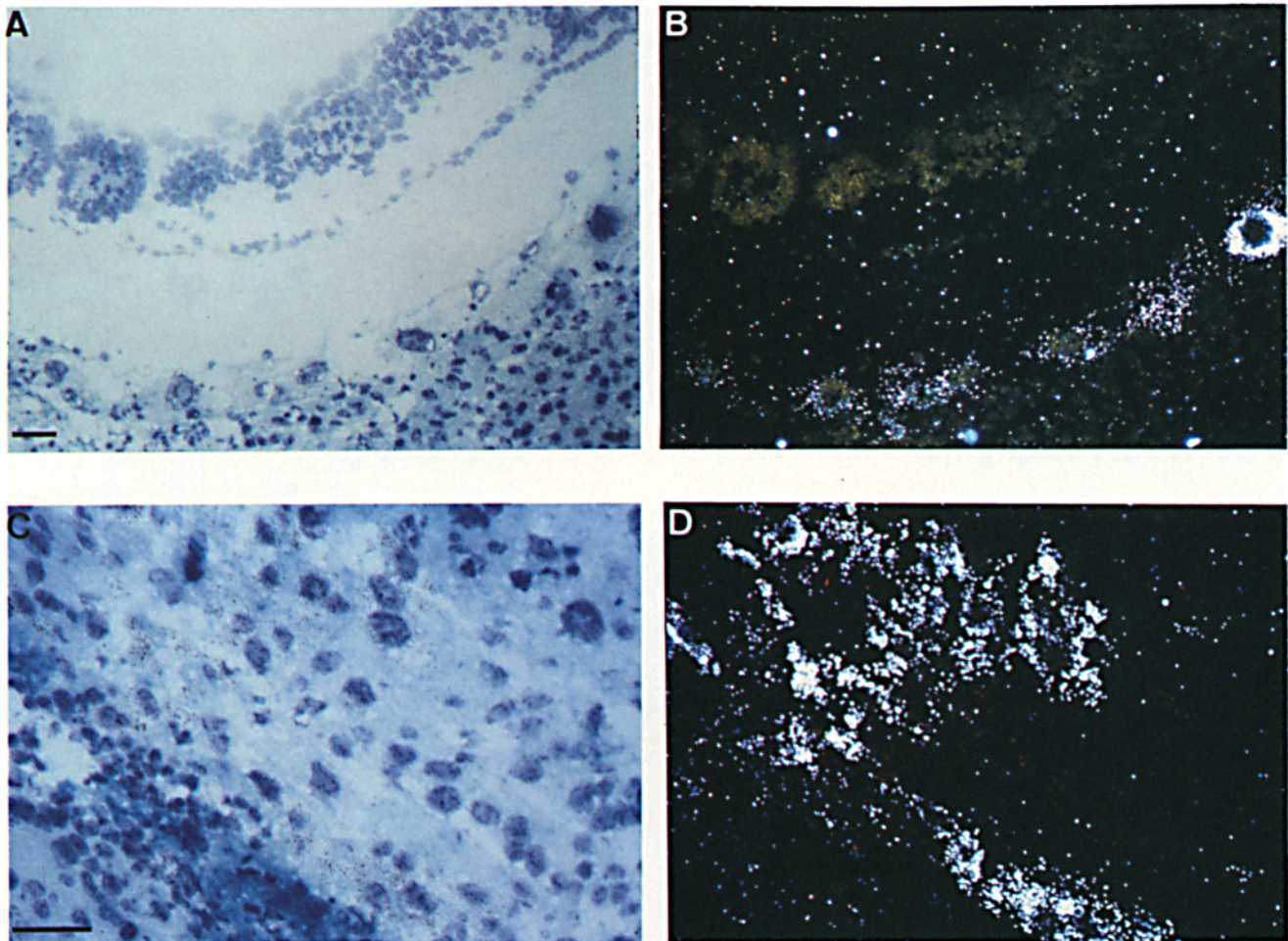


Figure 6. Localization of u-PA mRNA in the trophoblast cells surrounding the embryo (*A* and *B*) and in the trophospongial cells of the ectoplacental cone (*C* and *D*) by in situ hybridizations of ^3H -labeled cRNA probe to frozen sections of 8.5-d-old embryos. *A* and *C* are light-field micrographs; *B* and *D* are the corresponding darkfield micrographs. Bar, 10 μm .

teolysis is generally associated with tissue remodeling and cellular migration processes, including inflammatory reactions and organ involutions (Unkeless et al., 1974; Vassalli et al., 1976; Ossowski et al., 1979). The high levels of u-PA

mRNA in granulosa and thecal cells of preovulatory follicles are compatible with the postulated participation of the enzyme in follicular remodeling. Indeed, maturing follicles are complex structures that display considerable fluctuations in

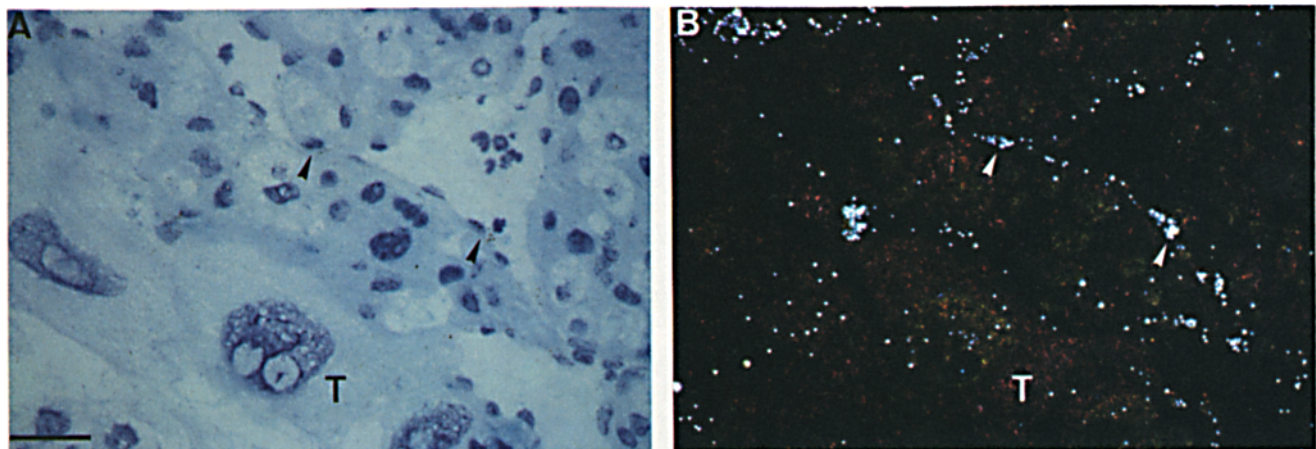


Figure 7. Localization of u-PA mRNA in the cells lining venous sinuses, adjacent to the trophoblast cell layer, by in situ hybridizations of ^3H -labeled cRNA probe to frozen sections of 10.5-d-old embryos. *A* is a light-field micrograph and *B* is the corresponding dark-field micrograph. Cells lining maternal venous sinuses are labeled (*arrowheads*), while a large trophoblast cell is devoid of grains. Bar, 10 μm .

their cellular content, with marked preovulatory cell proliferation, followed by rupture and complete reorganization after ovulation. Our observations support a role for u-PA in the focalized extracellular proteolysis required for follicular rupture and/or the redistribution of granulosa and thecal cells to achieve luteal differentiation, and add to the evidence that u-PA is implicated in physiological conditions characterized by hormonally mediated tissue remodeling (Ossowski et al., 1979).

Trophoblast cells exemplify a cell type capable of major tissue disruption (Schlafke and Enders, 1975); they are the first cells to differentiate in mammalian embryos and are generally considered as playing a pivotal role in embryo implantation, by acquiring invasive and migratory properties. Cultured trophoblast cells are known to produce PA activity, during a time period corresponding to their invasive phase in utero (Strickland et al., 1976). However, the type of PA they secrete had not been characterized; most importantly, numerous cell lines produce PAs in vitro, and it was essential to determine whether trophoblast cells do express PAs genes in vivo. Our investigations identify u-PA as the enzyme produced by mouse trophoblast cells in culture and establish the synthesis of u-PA mRNA by trophoblast cells in vivo. From fertilization to day 4 of development, i.e., before implantation begins, the embryos do not contain detectable u-PA activity (Strickland et al., 1976; Huarte et al., 1985). Trophoblasts display a first wave of high u-PA mRNA content between the 5th and 7th days of gestation, corresponding to the period when they penetrate the uterine wall. After a transient reduction, u-PA mRNA synthesis resumes in the peripheral giant trophoblast cells that surround the embryo at the three to seven somites stage; this second wave of u-PA mRNA production is also limited in time and coincides with a period of major embryonic expansion. These findings give support to the proposed role for u-PA in tissue remodeling: they document a developmental modulation of the u-PA synthetic potential in a highly invasive and migrating cell type. The synthesis of u-PA provides trophoblast cells with an efficient mechanism to trigger the localized production of plasmin, a protease that can catalyze the degradation of all components of the extracellular matrix, such as is needed for the endometrial disruption accompanying implantation and early growth of the embryo. Interestingly, we also localized u-PA mRNA in the cells that are lining the maternal venous sinuses and which are thought to derive from the outer layer of trophoblasts (Enders, 1965); as u-PA mRNA was detected in these cells during the development and enlargement of venous sinuses, the enzyme may play a role in preventing coagulation and in maintaining the patency of these vascular structures.

The production of u-PA by trophoblast cells is in agreement with the concept of shared phenotypic similarities with neoplastic cells (Yagel et al., 1988). Like trophoblasts, malignant cells are endowed with proliferative and migrating properties and have the ability to invade neighboring tissues. Transformed cell lines and tumors frequently produce u-PA (Danø et al., 1985) and experimental evidence suggests that the enzyme is essential to their invasive properties (Ossowski and Reich, 1983); though correlations between enzyme production and metastatic behavior have been found only in some cases, it has been proposed that u-PA may promote the metastatic dissemination of malignant cells via the dissolution of basement membranes (Danø et al., 1985; Sappino et

al., 1987). The presence of u-PA mRNA in implanting trophoblasts in vivo provides novel evidence for a contribution of the enzyme to developmental processes involving tissue destruction and cell migration.

We thank Dr. G. Lazarus for helpful discussions; B. Baumann and D. Ducrest for technical assistance; and P. Rumbeli and M. Baumann for photographic work.

This work was supported by grants 3.736-0.83 and 3.59-0.87 from the Swiss National Science Foundation and by the Georg Waechter Memorial Foundation.

Received for publication 5 May 1989 and in revised form 3 July 1989.

References

- Andrade-Gordon, P., and S. Strickland. 1986. Interaction of heparin with plasminogen activators and plasminogen: effects on the activation of plasminogen. *Biochemistry*. 25:4033-4040.
- Axelrod, H. R. 1985. Altered trophoblast functions in implantation-defective mouse embryos. *Dev. Biol.* 108:185-190.
- Baker, J. B., D. A. Low, R. L. Simmer, and D. D. Cunningham. 1980. Protease-nexin: a cellular component that links thrombin and plasminogen activator and mediates their binding to cells. *Cell*. 21:37-45.
- Beers, W. H. 1975. Follicular plasminogen and plasminogen activator and the effect of plasmin on ovarian follicle wall. *Cell*. 6:379-386.
- Beers, W. H., S. Strickland, and E. Reich. 1975. Ovarian plasminogen activator: relationship to ovulation and hormonal regulation. *Cell*. 6:387-394.
- Belin, D., J. D. Vassalli, C. Combépine, F. Godeau, Y. Nagamine, E. Reich, H. P. Kocher, and R. M. Duvoisin. 1985. Cloning, nucleotide sequencing and expression of cDNAs encoding mouse urokinase-type plasminogen activator. *Eur. J. Biochem.* 148:225-232.
- Bleil, J. D., C. F. Beall, and P. M. Wassarman. 1981. Mammalian sperm-egg interaction: fertilization of mouse eggs triggers modification of the major zona pellucida glycoprotein, ZP2. *Dev. Biol.* 86:189-197.
- Busso, N., D. Belin, C. Faily-Crépin, and J. D. Vassalli. 1986. Plasminogen activators and their inhibitors in a human mammary cell line (HBL-100). Modulation by glucocorticoids. *J. Biol. Chem.* 261:9309-9315.
- Canipari, R., and S. Strickland. 1985. Plasminogen activator in the rat ovary. Production and gonadotropin regulation of the enzyme in granulosa and thecal cells. *J. Biol. Chem.* 260:5121-5125.
- Canipari, R., M. L. O'Connell, G. Meyer, and S. Strickland. 1987. Mouse ovarian granulosa cells produce urokinase-type plasminogen activator, whereas the corresponding rat cells produce tissue-type plasminogen activator. *J. Cell Biol.* 105:977-981.
- Danø, K., P. A. Andreasen, J. Grøndahl-Hansen, P. Kristensen, L. S. Nielsen, and L. Skriver. 1985. Plasminogen activators, tissue degradation, and cancer. *Adv. Cancer Res.* 44:139-266.
- Denker, H. W. 1972. Blastocyst protease and implantation: effect of ovariectomy and progesterone substitution in the rabbit. *Acta Endocrinol.* 70:591-602.
- Enders, A. C. 1965. A comparative study of the fine structure of the trophoblast in several hemochorial placentas. *Am. J. Anat.* 116:29-68.
- Espey, L. L. 1974. Ovarian proteolytic enzymes and ovulation. *Biol. Reprod.* 10:216-235.
- Glass, R. H., J. Aggeler, A. Spindle, R. A. Pedersen, and Z. Werb. 1983. Degradation of extracellular matrix by mouse trophoblast outgrowths: a model for implantation. *J. Cell Biol.* 96:1108-1116.
- Hoylaerts, M., D. C. Rijken, H. R. Lijnen, and D. Collen. 1982. Kinetics of the activation of plasminogen by human tissue plasminogen activator. *J. Biol. Chem.* 257:2912-2919.
- Huarte, J., D. Belin, and J. D. Vassalli. 1985. Plasminogen activator in mouse and rat oocytes: induction during meiotic maturation. *Cell*. 43:551-558.
- Huarte, J., D. Belin, D. Bosco, A. P. Sappino, and J. D. Vassalli. 1987a. Plasminogen activator and mouse spermatozoa: urokinase synthesis in the male genital tract and binding of the enzyme to the sperm cell surface. *J. Cell Biol.* 104:1281-1289.
- Huarte, J., D. Belin, A. Vassalli, S. Strickland, and J. D. Vassalli. 1987b. Meiotic maturation of mouse oocytes triggers the translation and polyadenylation of dormant tissue-type plasminogen activator mRNA. *Genes & Dev.* 1:1201-1211.
- Kristensen, P., L. I. Larsson, L. S. Nielsen, J. Grøndahl-Hansen, P. A. Andreasen, and K. Danø. 1984. Human endothelial cells contain one type of plasminogen activator. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 168:33-37.
- Kristensen, P., L. S. Nielsen, J. Grøndahl-Hansen, P. B. Andreasen, L. I. Larsson, and K. Danø. 1985. Immunocytochemical demonstration of tissue-type plasminogen activator in endocrine cells of the rat pituitary gland. *J. Cell Biol.* 101:305-311.
- Lacroix, M., F. Smith, and I. B. Fritz. 1979. The control of plasminogen activator secretion by Sertoli cells in culture, and its possible role in spermatogenesis. In *Hormones and Cell Culture, CSH Conferences on Cell Proliferation*. Vol. 6. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

- 239-248.
- Lacroix, M., M. Parvinen, and I. B. Fritz. 1981. Localization of testicular plasminogen activator in discrete portions (stage VII and VIII) of the seminiferous tubule. *Biol. Reprod.* 25:143-146.
- Larsson, L. I., L. Skriver, L. S. Nielsen, J. Grøndahl-Hansen, P. Kristensen, and K. Danø. 1984. Distribution of urokinase-type plasminogen activator immunoreactivity in the mouse. *J. Cell Biol.* 98:894-903.
- Lecander, I., and B. Astedt. 1986. Isolation of a new plasminogen activator inhibitor from pregnancy plasma. *Br. J. Haematol.* 62:221-228.
- Levin, E. G. 1983. Latent tissue plasminogen activator produced by human endothelial cells in culture: evidence for an enzyme-inhibitor complex. *Proc. Natl. Acad. Sci. USA.* 80:6804-6808.
- Loskutoff, D. J., J. A. van Mourik, L. A. Erickson, and D. Lawrence. 1983. Detection of an unusually stable fibrinolytic inhibitor produced by bovine endothelial cells. *Proc. Natl. Acad. Sci. USA.* 80:2956-2960.
- Marotti, K. R., D. Belin, and S. Strickland. 1982. The production of distinct forms of plasminogen activator by mouse embryonic cells. *Dev. Biol.* 90:154-159.
- Moller, C. C., and P. M. Wassarman. 1989. Characterization of a proteinase that cleaves zona pellucida glycoprotein ZP2 following activation of mouse eggs. *Dev. Biol.* 132:103-112.
- Ossowski, L., D. Biegel, and E. Reich. 1979. Mammary plasminogen activator: correlation with involution, hormonal modulation and comparison between normal and neoplastic tissues. *Cell.* 16:929-940.
- Ossowski, L., and Reich, E. 1983. Antibodies to plasminogen activator inhibit human tumor metastasis. *Cell.* 35:611-619.
- Reich, E. 1978. Activation of plasminogen: a general mechanism for producing localized extracellular proteolysis. In *Molecular Basis of Biological Degradative Processes*. R. D. Berlin, H. Hermann, I. H. Lepow, and J. M. Tanzer, editors. Academic Press, Inc., New York. 155-169.
- Rickles, R. J., A. L. Darrow, and S. Strickland. 1988. Molecular cloning of complementary DNA to mouse tissue plasminogen activator mRNA and its expression during F9 teratocarcinoma cell differentiation. *J. Biol. Chem.* 263:1563-1569.
- Rijken, D. C., G. Wijngaards, M. Zaal-de Jong, and J. Welbergen. 1979. Purification and partial characterization of plasminogen activator from uterine tissue. *Biochem. Biophys. Acta.* 580:140-153.
- Rijken, D. C., and D. Collen. 1981. Purification and characterization of the plasminogen activator secreted by human melanoma cells in culture. *J. Biol. Chem.* 256:7035-7041.
- Saling, P. M. 1981. Involvement of trypsin-like activity in binding of mouse spermatozoa to zonae pellucidae. *Proc. Natl. Acad. Sci. USA.* 78:6231-6235.
- Salonen, E.-M., A. Zitting, and A. Vaheri. 1984. Laminin interacts with plasminogen and its tissue-type activator. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 172:29-32.
- Sappino, A.-P., N. Busso, D. Belin, and J. D. Vassalli. 1987. Increase of urokinase-type plasminogen activator gene expression in human lung and breast carcinomas. *Cancer Res.* 47:4043-4046.
- Schlafke, S., and A. C. Enders. 1975. Cellular basis of interaction between trophoblast and uterus at implantation. *Biol. Reprod.* 12:41-65.
- Stambaugh, R. 1978. Enzymatic and morphologic events in mammalian fertilization. *Gamete Res.* 1:65-85.
- Stoppelli, M. P., A. Corti, A. Soffientini, G. Cassani, F. Blasi, and R. K. Assoian. 1985. Differentiation-enhanced binding of the amino-terminal fragment of human urokinase plasminogen activator to a specific receptor on U-937 monocytes. *Proc. Natl. Acad. Sci. USA.* 82:4939-4943.
- Strickland, S., E. Reich, and M. I. Sherman. 1976. Plasminogen activator in early embryogenesis: enzyme production by trophoblast and parietal endoderm. *Cell.* 15:393-403.
- Suenson, E., O. Lützen, and S. Thorsen. 1984. Initial plasmin-degradation of fibrin as the basis of a positive feed-back mechanism in fibrinolysis. *Eur. J. Biochem.* 140:513-522.
- Theiler, K. 1989. *The House Mouse. Development and Normal Stages from Fertilization to 4 Weeks of Age.* Springer-Verlag, Berlin/Heidelberg/New York. 15-72.
- Unkless, J. C., S. Gordon, and E. Reich. 1974. Secretion of plasminogen activator by stimulated macrophages. *J. Exp. Med.* 139:834-850.
- Vassalli, J. D., J. Hamilton, and E. Reich. 1976. Macrophage plasminogen activator: modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors, and cyclic nucleotides. *Cell.* 8:271-281.
- Vassalli, J. D., D. Baccino, and D. Belin. 1985. A cellular binding site for the M_r 55,000 form of the human plasminogen activator urokinase. *J. Cell Biol.* 100:86-92.
- Vihko, K. K., T.-L. Penttilä, M. Parvinen, and D. Belin. 1989. Regulation of urokinase- and tissue-type plasminogen activator gene expression in the rat seminiferous epithelium. *Mol. Endocrinol.* 3:52-59.
- Wohlwend, A., D. Belin, and J. D. Vassalli. 1987. Plasminogen activator-specific inhibitors produced by human monocytes/macrophages. *J. Exp. Med.* 165:320-339.
- Yagel, S., R. S. Parhar, J. Jeffrey, and P. Lala. 1988. Normal nonmetastatic human trophoblast cells share in vitro invasive properties of malignant cells. *J. Cell. Physiol.* 136:455-462.