

Urokinase-Type Plasminogen Activator: Proenzyme, Receptor, and Inhibitors

Francesco Blasi,* Jean-Dominique Vassalli,† and Keld Danø‡

*International Institute of Genetics and Biophysics, Consiglio Nazionale delle Ricerche, Naples, Italy; †Institute of Histology and Embryology, University of Geneva, Switzerland; ‡Finsen Laboratory, Rigshospitalet, 2100 Copenhagen, Denmark

NEW information has recently become available that we believe may be of value for a more precise focusing of research on the role of plasminogen activators (PAs) in cellular physiology and pathology. PAs convert the abundant extracellular zymogen plasminogen into plasmin, an active protease that, directly or indirectly, can promote degradation of all components of the extracellular matrix. The urokinase-type PA (u-PA) is one of two activators with similar catalytic specificities that have been described in each mammalian species so far studied. Many cell types synthesize and secrete u-PA at different stages in their life cycle; it has been a consistent finding that the expression of u-PA-catalyzed proteolysis is under the control of hormonal-type mediators, the nature of which depends on the cell population considered. u-PA is thought to play a central role in regulating extracellular proteolysis in a variety of normal and pathological processes involving tissue destruction and cell migration, e.g., organ involution, inflammatory reactions, and invasive growth of trophoblasts and cancer cells (for reviews, see 8, 18, 44, 47, 49, 50). Recent findings show that u-PA is synthesized and released from mammalian cells as a proenzyme with little or no activity (pro-u-PA), that cells possess a specific binding site for u-PA and pro-u-PA, and that they can also synthesize different rapidly acting, high affinity inhibitors of PAs. Thus, activation of pro-u-PA, u-PA receptor occupancy, and PA-inhibitors may finely regulate the location and extent of plasminogen activation.

The Proenzyme

The human u-PA gene, located on chromosome 10, is 6.4-kb long. It is organized in 11 exons and gives rise to a 2.5-kb-long mRNA, which in turn yields a single-chain glycosylated polypeptide of ~50,000 D (25, 45, 48, 52, 63, 67). Pig and mouse u-PA show strong homologies with the human enzyme; mouse u-PA, however, contains no N-glycosylation site (7, 37). It is the single-chain form of u-PA that is released from a variety of cultured normal and neoplastic cell types (20, 29, 39, 55, 59, 64, 70), and that appears to be the predominant extracellular form of u-PA in the intact organism (30, 54). Single-chain pro-u-PA is converted by limited proteolysis into an active M_r 50,000 enzyme consisting of two polypeptide chains held together by one disulfide bond; this conversion can be catalyzed by plasmin. Results from several laboratories have demonstrated that single-chain u-PA is a zymogen that in contrast to the two-chain active enzyme has little or no proteolytic activity against the natural substrate

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

plasminogen, does not react with macromolecular antiproteases, lacks amidolytic activity against synthetic plasminogen activator substrates, and is unable to react with the active site titrant diisopropylfluorophosphate (2, 18, 26, 29, 39, 55, 64, 70). It has recently been reported (33, 60), however, that certain purified preparations of single-chain u-PA may also have catalytic activity; recombinant single-chain u-PA produced in *Escherichia coli* had a catalytic efficiency superior to that of the two-chain enzyme, and single-chain u-PA purified from different mammalian sources had variable and low, but detectable, catalytic activity. The basis for these differences in the activity of single-chain u-PA preparations is not known. It may be related to differences in the extent of glycosylation, in the three-dimensional folding, or in a partial degradation of the protein, depending on the cell of origin or the procedure of purification.

The u-PA Receptor

Several types of cells possess specific high affinity ($K_d \sim 10^{-10}$ M) plasma membrane binding sites for u-PA (9, 57, 66). Human peripheral blood monocytes, the monocyte-like U937 cell, fibroblasts, and HT1080 fibrosarcoma cells all have $\sim 10^5$ such specific receptors per cell. Receptor binding does not require u-PA catalytic activity, and single-chain u-PA binds with about the same affinity as does the $M_r \sim 50,000$ two-chain enzyme. The region of u-PA that binds to the receptor is located on the 135-residue-long amino terminal fragment of the A-chain; this amino terminal fragment peptide, which comprises two structural domains, a "growth factor" region with homology to EGF and a kringle (43), binds to the receptor with the same affinity as does intact u-PA. Recent data show that the binding region is located within the second loop of the growth factor domain (4). In contrast, the $M_r \sim 33,000$ form of u-PA, a partial degradation product of the $M_r \sim 50,000$ enzyme that lacks the amino-terminal fragment but maintains catalytic activity, does not interact with the receptor. Bound u-PA does not dissociate rapidly from the cell surface ($t_{1/2} > 5$ h), nor is it appreciably endocytosed and/or degraded. The bound enzyme maintains its plasminogen activating ability, and binding of both one-chain and two-chain u-PA to the u-PA receptor on cultured human monocytes-macrophages results in the generation of plasmin-mediated pericellular proteolysis. Thus the presence of a u-PA receptor allows cells to acquire surface-bound plasminogen-activating ability. It will be of interest to determine whether the receptor-bound one-chain protein itself catalyzes plasmin formation, or whether it is first cleaved to the two-chain enzyme. The receptor-bound single

chain pro-u-PA can be converted to two-chain u-PA by plasmin with high efficiency (16).

Cultured human monocytes and monocyte-like U937 cells do produce u-PA, although at a rather low rate. In contrast, many malignant cells produce high levels of u-PA. Do they express a u-PA receptor? Human epidermoid carcinoma-derived A431 and fibrosarcoma HT1080 cells bind little or no ¹²⁵I-labeled u-PA. However, uncovering of u-PA receptors can be achieved by dissociation of single-chain pro-u-PA from the cell surface by short exposure to low pH conditions (6, 58). These cells, therefore, possess u-PA receptors that are very similar to, and possibly identical with, those of U937 cells, except that they are fully occupied by cellular pro-u-PA. Immunochemical and kinetic studies have shown that pro-u-PA is first secreted from A431 cells and then binds to the plasma membrane (58). Thus, cells that produce little or no u-PA may have free u-PA receptors, while those that produce larger amounts of the proenzyme have receptors constantly saturated through an autocrine process, and may now release excess u-PA into the extracellular milieu.

PA Inhibitors (PAI)

Two distinct protease inhibitors specific for PAs have recently been identified and purified. One of these, PAI type-1, is released by endothelial cells and some neoplastic cell lines (2, 21, 23, 32, 34, 62, 65); it is also present in thrombocytes and blood plasma (22, 68). The other, PAI type-2, first purified from placenta extracts (5), is also released by cultured monocytes-macrophages (12, 31, 51, 56, 64, 69). These two inhibitors are proteins of $M_r \sim 50,000$; they differ in immunological reactivity and in some physiological characteristics, such as activation by denaturing agents (27). Recent protein and c-DNA sequence data (3, 24, 31, 41, 42) have shown that both inhibitors belong to the serine protease inhibitor family (serpins, see reference 11), PAI type-1 being an Argserpin with arginine at its reactive center (3). A third PA inhibitor is the fibroblast-produced protease-nexin that, in contrast to the PA-specific inhibitors, also inhibits plasmin, thrombin, and other trypsin-like serine proteases (53); complexes of protease-nexin with proteases bind to fibroblasts in a manner that is independent of the u-PA receptor, and are endocytosed and degraded (35). These three PA inhibitors react with u-PA, but not with pro-u-PA (2, 20, 64). Several cultured cell types produce both pro-u-PA and one of the PA inhibitors; the proenzyme and the inhibitors have been found to coexist in the extracellular milieu. The reaction rate of the three inhibitors for u-PA is several orders of magnitude higher than that of the major plasma protease inhibitors; it is thus likely that they play an important part in controlling u-PA-initiated extracellular proteolysis *in vivo*. It is noteworthy that in endotoxin-treated macrophage cultures a PA inhibitor is induced, which inhibits the secreted enzyme but does not affect the cell-associated (presumably receptor-bound) activity (13). Furthermore, the addition of protease nexin or of anti-u-PA antibodies blocks the degradation of fibroblast-produced extracellular matrix by human HT 1080 cells (10). The role of the u-PA receptor in this reaction is certainly one of the first questions that should be posed.

The endothelial cell inhibitor is proteolytically converted to an inactive form by u-PA (38) and it may be speculated that, apart from inhibiting PA, this inhibitor, its conversion

product, or a putative released peptide may have other functions. Another exciting subject for further study is the regulation of inhibitor production; hormones and growth factors have been found to affect inhibitor levels in different cell types (1, 14, 15, 17, 19, 36, 40).

Modulation of u-PA Activity on Cell Surfaces and in Body Fluids

To summarize our current understanding of the cell biology of u-PA, it is now established that: (a) the product of the u-PA gene is a single-chain proenzyme with little or no activity, which is released as such from at least some cell types; (b) released pro-u-PA can be bound to cell surfaces via a high affinity binding site; (c) pro-u-PA does not react with PA inhibitors, unlike active two-chain u-PA.

These data suggest a model, probably oversimplified, in which receptor-associated u-PA is the active form in cell migration and tissue destruction. Extracellular pro-u-PA interacts with its plasma membrane receptor, and, after activation, triggers localized proteolysis. It is possible that the binding to the receptor may either render the proenzyme more susceptible to activation by other factors (plasmin, u-PA itself, or other still unidentified components of the system), or directly induce activity in single-chain u-PA, e.g., by an allosteric effect. Furthermore, receptor-bound two-chain u-PA may be protected from certain inhibitors, in contrast to free two-chain u-PA that is rapidly inactivated by PA inhibitors.

In any event, it is clear that u-PA binding to its plasma membrane receptor results in focalization of plasminogen activation to the close environment of the cell surface; this appears as an optimal configuration if u-PA is to participate in the proteolytic events that are required for cell migration. In this respect, and although a discussion of the biology of the other mammalian plasminogen activator tissue-type PA is beyond the scope of the present review, it should be pointed out that tissue-type PA binds to fibrin (28, 46, 61); this interaction, which increases the enzyme's catalytic activity, suggests a preferred role for tissue-type PA in the maintenance of fluidity of the extracellular milieu.

Implications and Perspectives

Conflicting data have been obtained relating production of u-PA to malignancy. In light of the most recent information, this question should be reinvestigated; studies that include determination of two-chain versus single-chain forms of u-PA, of receptor distribution and occupancy, and of PA inhibitors are likely to give a clearer picture of the role of u-PA in tumor biology. Similarly, the multiple steps at which u-PA-catalyzed proteolysis can be modulated in response to growth factors and hormones can now be dissected at the molecular level. Finally, the discovery of a u-PA receptor and of PA-specific inhibitors suggests possible approaches for interfering with the cascade reactions leading to plasminogen activation, and may thus help in designing a therapeutic regimen for the control of extracellular proteolysis.

The evolving picture of the increasing number of factors involved in plasminogen activation is reminiscent of the unraveling of other proteolytic cascades, e.g., the coagulation and complement systems. It is likely that additional factors will be discovered that will complete our, at present, frag-

mentary understanding of the biochemistry of this system. For example, the activation of pro-u-PA under physiological conditions is still poorly understood. The possible role of u-PA receptors in this process appears an attractive target for further studies.

References

- Andreasen, P. A., T. H. Christensen, J.-Y. Huang, L. S. Nielsen, E. L. Wilson, and K. Danø. 1986. Hormonal regulation of extracellular plasminogen activators and $M_r \approx 54,000$ plasminogen activator inhibitor in human neoplastic cell lines, studied with monoclonal antibodies. *Mol. Cell. Endocr.* 45:137-147.
- Andreasen, P. A., L. S. Nielsen, P. Kristensen, J. Grøndahl-Hansen, L. Skriver, and K. Danø. 1986. Plasminogen activator inhibitor from human fibrosarcoma cells binds urokinase-type plasminogen activator, but not its proenzyme. *J. Biol. Chem.* 261:7644-7651.
- Andreasen, P. A., A. Riccio, K. G. Welinder, R. Douglas, R. Sartorio, L. S. Nielsen, C. Oppenheimer, F. Blasi, and K. Danø. 1986. Plasminogen activator inhibitor type-1: reactive center and amino-terminal heterogeneity determined by protein and cDNA sequencing. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 209:213-217.
- Appella, E., E. A. Robinson, S.-J. Ullrich, M. P. Stoppelli, A. Corti, G. Cassani, and F. Blasi. 1987. Identification of the receptor-binding sequence of urokinase: a biological function for the growth factor domain of several proteins. *J. Biol. Chem.* In press.
- Åstedt, B., I. Lecander, T. Brodin, A. Lundblad, and K. Löw. 1985. Purification of specific placental plasminogen activator inhibitor by monoclonal antibody and its complex formation with plasminogen activator. *Thromb. Haemostasis.* 53:122-125.
- Bajpai, A., and J. B. Baker. 1985. Cryptic urokinase binding sites on human foreskin fibroblasts. *Biochem. Biophys. Res. Commun.* 133:475-482.
- Belin, D., J.-D. Vassalli, C. Combépine, F. Godeau, Y. Nagamine, E. Reich, H. P. Kocher, and R. M. Duvoisin. Cloning, nucleotide sequencing and expression of cDNAs encoding mouse urokinase-type plasminogen activator. *Eur. J. Biochem.* 148:225-232.
- Blasi, F., A. Riccio, and G. Sebastio. 1986. Human plasminogen activator. Genes and protein structure. In *Human Genes and Diseases*. F. Blasi, editor. John Wiley and Sons, Ltd., London. 337-413.
- Blasi, F., M. P. Stoppelli, and M. V. Cubellis. 1986. The receptor for urokinase plasminogen activator. *J. Cell Biochem.* 32:179-186.
- Bergmann, B. L., R. W. Scott, A. Bajpai, S. Watts, and J. B. Baker. 1986. Inhibition of tumor-cell-mediated extracellular matrix destruction by a fibroblast proteinase inhibitor, protease nexin 1. *Proc. Natl. Acad. Sci. USA.* 83:996-1000.
- Carrell, R., and J. Travis. 1985. α_1 -antitrypsin and the serpins: variation and countervariation. *Trends Biochem. Sci.* 10:20-24.
- Chapman, H. A., and O. L. Stone. 1985. Characterization of a macrophage-derived plasminogen activator inhibitor. *Biochem. J.* 230:109-115.
- Chapman, H. A., Z. Vavrin, and J. B. Hibbs. 1982. Macrophage fibrinolytic activity: identification of two pathways of plasmin formation by intact cells and of a plasminogen activator inhibitor. *Cell.* 28:653-662.
- Coleman, P. S., P. D. Patel, B. J. Cwikel, U. M. Rafferty, R. Szyner-Laszuk, and T. D. Gelehrter. 1986. Characterization of the dexamethasone-induced inhibitor of plasminogen activator in HTC hepatoma cells. *J. Biol. Chem.* 261:4352-4357.
- Crutchley, D. J., L. B. Conanan, and J. R. Maynard. 1981. Human fibroblasts produce inhibitor directed against plasminogen activator when treated with glucocorticoids. *Ann. N.Y. Acad. Sci.* 370:609-616.
- Cubellis, M. V., M. L. Nolli, G. Cassani, and F. Blasi. 1986. Binding of single-chain pro-urokinase to the urokinase receptor of human U937 cells. *J. Biol. Chem.* 261:15819-15822.
- Cwikel, B. J., P. A. Barouski-Miller, P. L. Coleman, and T. D. Gelehrter. 1984. Dexamethasone induction of an inhibitor of plasminogen activator in HTC hepatoma cells. *J. Biol. Chem.* 259:6847-6851.
- 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.
- Eaton, D. L., and J. B. Baker. 1983. Phorbol ester and mitogens stimulate human fibroblast secretions of plasmin-activatable plasminogen activator and protease nexin, an antiactivator/antiplasmin. *J. Biol. Chem.* 97:323-328.
- Eaton, D. L., R. W. Scott, and J. B. Baker. 1984. Purification of human fibroblast urokinase proenzyme and analysis of its regulation by proteases and protease nexin. *J. Biol. Chem.* 259:6241-6247.
- Emeis, J. J., V. W. M. van Hindsberg, J. H. Verheijen, and G. Wijngaards. 1983. Inhibition of tissue-type plasminogen activator by conditioned medium from cultured human and porcine vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 110:392-398.
- Erickson, L. A., C. M. Hekman, and D. J. Loskutoff. 1985. The primary plasminogen activator inhibitors in endothelial cells, platelets, serum and plasma are immunologically related. *Proc. Natl. Acad. Sci. USA.* 82:8710-8714.
- Gelehrter, T. D., P. A. Barouski-Miller, P. L. Coleman, and B. J. Cwikel. 1983. Hormonal regulation of plasminogen activator in rat hepatoma cells. *Mol. Cell. Biochem.* 53/54:11-21.
- Ginsburg, D., R. Zeheb, A. Y. Yang, U. M. Rafferty, P. A. Andreasen, L. Nielsen, K. Danø, R. V. Lebo, and T. D. Gelehrter. 1986. cDNA cloning of human plasminogen activator-inhibitor from endothelial cells. *J. Clin. Invest.* 78:1673-1680.
- Günzler, W. A., G. J. Steffens, F. Ötting, S.-M. A. Kim, E. Frankus, and L. Flohé. 1982. Structural relationship between human high and low molecular mass urokinase. *Hoppe-Seyler's Z. Physiol. Chem.* 363:1155-1165.
- Gurewich, V., R. Pannell, S. Louis, P. Kelley, R. L. Suddith, and R. Greenlee. 1984. Effective and fibrin-specific clot lysis by a zymogen precursor form of urokinase (pro-urokinase). A study in vitro and two animal species. *J. Clin. Invest.* 73:1731-1739.
- Hekman, C. M., and D. J. Loskutoff. 1985. Endothelial cells produce a latent inhibitor of plasminogen activators that can be activated by denaturants. *J. Biol. Chem.* 260:11581-11587.
- 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.
- Kasai, S., H. Arimura, M. Nishida, and T. Suyama. 1985. Proteolytic cleavage of single-chain pro-urokinase induces conformational change which follows activation of the zymogen and reduction of its high affinity for fibrin. *J. Biol. Chem.* 260:12377-12381.
- Kielberg, V., P. A. Andreasen, J. Grøndahl-Hansen, L. S. Nielsen, L. Skriver, and K. Danø. 1985. Proenzyme to urokinase-type plasminogen activator in the mouse in vivo. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 182:441-445.
- Kruithof, E. K. O., J.-D. Vassalli, W.-D. Schleuning, R. J. Mattaliano, and F. Bachmann. 1986. Purification and characterization of a plasminogen activator inhibitor from the histiocytic lymphoma cell line U-937. *J. Biol. Chem.* 261:11207-11213.
- 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.
- Lijnen, H. R., C. Zammaron, M. Blaber, M. E. Winkler, and D. Collen. 1986. Activation of plasminogen by pro-urokinase. I. Mechanism. *J. Biol. Chem.* 261:1253-1258.
- 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.
- Low, D. A., J. B. Baker, W. C. Koonce, and D. D. Cunningham. 1981. Released protease nexin regulates cellular binding, internalization, and degradation of serine proteases. *Proc. Natl. Acad. Sci. USA.* 78:2340-2344.
- Lund, L. R., A. Riccio, P. A. Andreasen, L. S. Nielsen, P. Kristensen, M. Laiho, O. Saksela, F. Blasi, and K. Danø. 1987. Transforming growth factor- β is a strong and fast acting positive regulator of the level of type-1 plasminogen activator inhibitor mRNA in WI-38 human lung fibroblasts. *EMBO (Eur. Mol. Biol. Organ.) J.* In press.
- Nagamine, Y., D. Pearson, M. S. Altus, and E. Reich. 1984. cDNA and gene nucleotide sequence of porcine plasminogen activator. *Nucleic Acids Res.* 12:9525-9541.
- Nielsen, L. S., P. A. Andreasen, J. Grøndahl-Hansen, L. Skriver, and K. Danø. 1986. Plasminogen activators catalyze conversion of inhibitor from fibrosarcoma cells to an inactive form with a lower apparent molecular mass. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 196:269-273.
- Nielsen, L. S., J. G. Hansen, L. Skriver, E. L. Wilson, K. Kaltoft, J. Zeuthen, and K. Danø. 1982. Purification of zymogen to plasminogen activator from human glioblastoma cells by affinity chromatography with monoclonal antibody. *Biochemistry.* 24:6410-6415.
- Ny, T., L. Bjersing, A. J. W. Hsieh, and D. J. Loskutoff. 1985. Cultured granulosa cells produce two plasminogen activators and an antiactivator, each regulated differently by gonadotropins. *Endocrinology.* 116:1666-1668.
- Ny, T., M. Sawdey, D. Lawrence, J. L. Millan, and D. J. Loskutoff. 1986. Cloning and sequence of a cDNA coding for the human β -migrating endothelial-cell-type plasminogen activator inhibitor. *Proc. Natl. Acad. Sci. USA.* 83:6776-6780.
- Pannekoek, H., H. Veerman, H. Lambers, P. Diergaarde, C. L. Verweij, A. J. van Zonneveld, and J. A. van Mourik. 1986. Endothelial plasminogen activator inhibitor (PAI): a new member of the serpin gene family. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2539-2544.
- Pathy, L. 1985. Evolution of the proteases of blood coagulation and fibrinolysis by assembly from modules. *Cell.* 41:657-663.
- Quigley, J. P., R. H. Goldfarb, C. Scheiner, J. O'Donnell-Tormey, and T. K. Yeo. 1980. Plasminogen activator and the membrane of transformed cells. In *Tumor Cell Surfaces and Malignancy*. R. O. Hynes and C. F. Fox, editors. Alan R. Liss, Inc. New York. 773-796.
- Rajput, B., S. F. Degen, E. Reich, E. K. Waller, J. Axelrod, R. L. Eppy, and T. B. Shows. 1985. Chromosomal locations of human tissue plas-

- minogen activator and urokinase genes. *Science (Wash. DC)*. 230:672-674.
46. Rånby, M. 1982. Studies on the kinetics of plasminogen activation by tissue plasminogen activator. *Biochim. Biophys. Acta*. 704:461-469.
 47. 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.
 48. Riccio, A., G. Grimaldi, P. Verde, G. Sebastio, S. Boast, and F. Blasi. 1986. The human urokinase-plasminogen activator gene and its promoter. *Nucleic Acids Res.* 13:2759-2771.
 49. Rohrich, S. T., and D. B. Rifkin. 1979. Proteases and cell invasion. *Annu. Rep. Med. Chem.* 14:229-239.
 50. Saksela, O. 1985. Plasminogen activation and regulation of pericellular proteolysis. *Biochim. Biophys. Acta*. 823:35-65.
 51. Saksela, O., T. Hovi, and A. Vaheri. 1985. Urokinase-type plasminogen activator and its inhibitor secreted by cultured human monocyte-macrophages. *J. Cell. Physiol.* 122:125-132.
 52. Salerno, G., P. Verde, M. L. Nolli, A. Corti, H. Szöts, T. Meo, J. Johnson, S. Bullock, G. Cassani, and F. Blasi. 1984. Monoclonal antibodies to human urokinase identify the single-chain pro-urokinase precursor. *Proc. Natl. Acad. Sci. USA*. 81:110-114.
 53. Scott, R. W., B. L. Bergmann, A. Bajpai, R. T. Hersh, H. Rodrigues, B. N. Jones, C. Barreda, S. Watts, and J. B. Baker. 1985. Protease nexin. Properties and a modified purification procedure. *J. Biol. Chem.* 260:7029-7034.
 54. Skriver, L., L.-I. Larsson, V. Kielberg, L. S. Nielsen, P. B. Andresen, P. Kristensen, and K. Danø. 1984. Immunocytochemical localization of urokinase-type plasminogen activator in Lewis lung carcinoma. *J. Cell Biol.* 99:753-757.
 55. Skriver, L., L. S. Nielsen, R. Stephens, and K. Danø. 1982. Plasminogen activator released as inactive proenzyme from murine cells transformed by sarcoma virus. *Eur. J. Biochem.* 124:409-414.
 56. Stephens, R. W., J. P. Golder, D. R. H. Faile, D. A. Hume, A. J. Hapel, W. Allan, C. J. Fordham, and W. F. Doe. 1985. Minactivin expression in human monocyte and macrophage populations. *Blood*. 66:333-337.
 57. Stoppelli, M. P., A. Corti, A. Soffientini, G. Cassani, F. Blasi, and R. K. Associan. 1985. Differentiation-enhanced binding of the amino-terminal fragment of human urokinase plasminogen activator to a specific receptor on U937 monocytes. *Proc. Natl. Acad. Sci. USA*. 82:4939-4943.
 58. Stoppelli, M. P., C. Tacchetti, M. V. Cubellis, A. Corti, V. J. Hearing, G. Cassani, E. Appella, and F. Blasi. 1986. Autocrine saturation of pro-urokinase receptors on human A431 cells. *Cell*. 45:675-684.
 59. Stoppelli, M. P., P. Verde, G. Grimaldi, E. K. Locatelli, and F. Blasi. 1986. Increase in urokinase plasminogen activator mRNA synthesis in human carcinoma cells is a primary effect of the potent tumor promoter, phorbol myristate acetate. *J. Cell Biol.* 102:1235-1241.
 60. Stump, D. C., M. Thienpont, and D. Collen. 1986. Urokinase-related proteins in human urine. Isolation and characterization of single-chain urokinase (pro-urokinase) and urokinase-inhibitor complex. *J. Biol. Chem.* 261:1274-1278.
 61. 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.
 62. Thorsen, S., and M. Philips. 1984. Isolation of tissue-type plasminogen activator-inhibitor complexes from human plasma. Evidence for a rapid plasminogen activator inhibitor. *Biochim. Biophys. Acta*. 802:111-118.
 63. Tripputi, P., F. Blasi, P. Verde, L. A. Cannizzaro, B. S. Emanuel, and C. M. Croce. 1985. Human urokinase gene is located on the long arm of chromosome 10. *Proc. Natl. Acad. Sci. USA*. 82:4448-4452.
 64. Vassalli, J.-D., J.-M. Dayer, A. Wohlwend, and D. Belin. 1984. Concomitant secretion of prourokinase and of a plasminogen activator-specific inhibitor by cultured human monocytes-macrophages. *J. Exp. Med.* 159:1653-1668.
 65. van Mourik, J. A., D. A. Lawrence, and D. J. Loskutoff. 1984. Purification of an inhibitor of plasminogen activator (antiactivator) synthesized by endothelial cells. *J. Biol. Chem.* 259:14914-14921.
 66. 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.
 67. Verde, P., M. P. Stoppelli, P. Galeffi, P. Di Nocera, and F. Blasi. 1984. Identification and primary sequence of an unspliced human urokinase poly(A)⁺ RNA. *Proc. Natl. Acad. Sci. USA*. 81:4727-4731.
 68. Wiman, B., J. Chmielewska, and M. Rånby. 1984. Inactivation of tissue plasminogen activator in plasma. Demonstration of a complex with a new rapid inhibitor. *J. Biol. Chem.* 259:3644-3647.
 69. Wohlwend, A., D. Belin, and J.-D. Vassalli. 1987. Plasminogen activator-specific inhibitors produced by human monocyte-macrophages. *J. Exp. Med.* 165:320-339.
 70. Wun, T.-C., L. Ossowski, and E. Reich. 1982. A proenzyme form of human urokinase. *J. Biol. Chem.* 257:7262-7268.