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The Plasminogen Activator/Plasmin System

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Over the last 10 years, our knowledge of extracellular proteolysis has progressed dramatically. Different enzymatic cascades cooperate to achieve extracellular matrix (ECM)¹ degradation, and a number of participant proteins have been characterized and cloned. Physiological inhibitors have been identified for most of these enzymes. Also, the concept of focused proteolysis, through binding of enzymes and inhibitors to specific regions of the extracellular milieu, has received broad experimental support. Finally, the biosynthesis of many of the relevant proteases and inhibitors has been shown to be under the control of hormones and growth factors. Plasminogen activators (PAs) and their inhibitors (PAIs) are thought to be key participants in the balance of proteolytic and antiproteolytic activities that regulates matrix turnover. This article summarizes the evidence that supports this contention, discusses the role of PA-specific cell surface binding sites, and also draws attention to a number of instances in which the presence of PAs cannot be reconciled with an exclusive function in ECM degradation.

The plasminogen activator/plasmin system

ENZYMES

PAs are serine proteases of tryptic specificity. Two enzymes, differing mostly in the domain organization and function of their noncatalytic regions, have been identified in mammals: urokinase-type PA (uPA) and tissue-type PA (tPA) (1). They are the products of distinct genes, and are secreted as single-chain (sc) proteins; whereas sc tPA is active, sc uPA is essentially inactive (pro-uPA) (2). Cleavage of pro-uPA by plasmin, kallikrein, Factor XIIa or cathepsin B (3) yields the disulfide-linked two-chain active enzyme. The two PAs have distinct targeting determinants in their noncatalytic regions: the "growth factor domain" of uPA directs the binding of the enzyme (and that of pro-uPA) to a plasma membrane receptor (4, 5), whereas other structural domains in tPA (the "finger" region and the "kringles") allow its binding to fibrin and other

components of the ECM (6). These and perhaps additional interactions, for instance with heparinlike molecules, could have a dramatic effect on the focusing of PA-controlled proteolysis (7). The different extracellular addressing of the two PAs suggests that they play different biological roles.

Plasminogen is the preferred substrate for PAs, but other molecules may also be cleaved by one or the other PA; for instance, avian uPA exerts a plasmin-independent effect on the morphology of chick fibroblasts (8). Plasminogen is present in plasma and extracellular fluids at a 1–2 μ M concentration, in the range of the K_m of the activation reaction. It can associate with fibrin and other proteins via lysine-binding sites located in the kringles of its noncatalytic portion (9). The PA-catalyzed cleavage of an Arg-Val bond yields the active enzyme plasmin, consisting of two polypeptide chains linked by a disulfide bond; Arg-esterases other than PAs can activate plasminogen, but only tPA and uPA appear to do so under physiological conditions. Like PAs, plasmin belongs to the serine protease family, and has tryptic specificity; by contrast to PAs, however, it has a very broad spectrum of substrates. Whereas the "classical" plasmin substrate is fibrin, in the context of ECM proteolysis it is clear that most other matrix proteins can also be cleaved by this enzyme. In addition, plasmin is one of the activators of metalloproteases precursors (10), and could thus play a pivotal role in ECM degradation; whether the PA-plasmin system controls matrix turnover is, however, debatable, because the rate-limiting step in such enzymatic cascades may vary from situation to situation, and need not be the most proximal. Plasmin has also been found to activate latent forms of certain growth factors (11), and its role should hence not be considered as limited to degradative events. PA-catalyzed activation of plasminogen results in a dramatic amplification of the proteolytic capacity of PA-producing cells, by recruitment of the vast reservoir of potential, broad spectrum proteolysis represented by plasminogen in the pericellular environment.

INHIBITORS

Four arginine-specific serine-protease inhibitors (Arg-serpins) are of particular relevance to the PA/plasmin system (12). Their preferred target enzyme(s), as determined by the kinetic constants of complex formation, are different.

Plasminogen activator inhibitor 1 (PAI-1) is the major PAI in plasma. It has high affinity for tPA, both sc and two-chain (tc), as well as for uPA. PAI-1 is secreted as an active antiprotease, but it rapidly converts to an inactive latent form, which can be reactivated by exposure to chaotropic agents or phospholipids; this latency is not shared by other serpins, and its molecular basis remains unexplained. PAI-1 is often found associated with plasma and ECM vitronectin, which stabilizes it in the active conformation (13). In addition, vitronectin and heparin affect the specificity of PAI-1, by enhancing its reactivity toward thrombin (14).

It is impossible to select a small set of references that would provide appropriate coverage of the entire field discussed. We have thus included references to reviews and to recent papers that can be used to trace back earlier work. We apologize to all our colleagues whose contributions are not adequately referenced, as well as to our readers.

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1. *Abbreviations used in this paper:* ECM, extracellular matrix; PA, plasminogen activator; PAI, plasminogen activator inhibitor; sc, single chain; tc, two-chain; tPA, tissue-type PA; uPA, urokinase-type PA.

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Plasminogen activator inhibitor 2 (PAI-2) inhibits uPA and also, albeit less efficiently, tPA (tc but not sc). The rate constants of uPA and tPA inhibition are not as favorable as with PAI-1, and this raises the possibility that PAI-2 may control the activity of other, yet unidentified, enzymes. In this context it is intriguing that PAI-2, in addition to being a secreted protein, is also abundant in the cytosol of cells that synthesize it (15), whereas PAs are exclusively secretory proteins.

Protease-nexin I (PN-I) inhibits uPA, plasmin, and thrombin; its reactivity towards thrombin (but not towards the other two enzymes) is dramatically enhanced in the presence of heparin. The dual role of PN-I as an inhibitor of fibrin deposition and removal suggests that it may be designed to dampen multiple enzymatic pathways which, if active, would affect the metabolism of the extracellular milieu.

α 2-antiplasmin is the primary plasmin inhibitor in plasma. Interestingly, if the lysine-binding sites of plasmin are occupied, inhibition by α 2-antiplasmin is markedly reduced (16); this is thought to localize the activity of plasmin to specific regions of the extracellular environment, such as on fibrin or in the immediate vicinity of the cell surface.

CELL SURFACE BINDING SITES

A sharper image of the biological role of the PA/plasmin system has come from observations indicating that PAs and plasminogen can bind to cell surfaces.

uPA receptor is present on the plasma membrane of a number of cell types (4, 5). It binds with similar kinetics uPA and pro-uPA, formerly considered as secreted proteins acting in solution in extracellular fluids. This receptor comprises at least one polypeptide chain, which is inserted in the plasma membrane through a glycerophospholipid anchor (17). Binding involves the "growth factor-like" domain of uPA, and receptor-bound uPA remains functionally active at the cell surface with a half-life of 4–5 h (18). Recent studies (18, 19) demonstrate that receptor-bound uPA is not protected from inhibition by PAI-1 or PAI-2.

Different cellular binding sites for tPA have been described. Some of these are clearly involved in the clearance (in particular by the liver) of the enzyme, or of tPA-PAI complexes (20), whereas others localize tPA to the plasma membrane, for instance on endothelial cells and neurons (21, 22); they are different from the characterized uPA receptor. Because tPA also binds to components of the ECM, positive identification of these cellular binding site(s) will be required to ascertain whether they are plasma membrane constituents.

Plasminogen and plasmin bind to different cell types, including cells that also express uPA receptors (9). Binding appears to be to proteins with an exposed carboxy-terminal lysine residue, and recent results identify a plasma membrane form of enolase as one major plasminogen receptor site (23). This and other observations suggest that the lysine-binding domains of plasminogen are involved.

Cell biology of plasminogen activation

REGULATION OF SYNTHESIS

The range of cell types that can produce one (or more) of the constituents of the PA/plasmin system is extremely broad, and includes derivatives from the three germ layers, cells from the male and female germ lines, as well as cells from extraembryonic tissues. In fact, it may be that, given the circumstances, all cell types can produce PAs and/or PAIs. PAs and PAI-1 are

present in blood plasma, and so is PAI-2 during late pregnancy. PAI-1 is also a major constituent of certain ECM. However, these proteins are by no means constitutive components of the extracellular milieu: expression of the PA/plasmin system is a highly dynamic aspect of the cellular phenotype.

A large body of work has explored the regulation of expression of PAs (1) and PAIs (12), in many different cell lineages. Most often, transcriptional mechanisms have been shown to play a determining role (24–26), although in at least one instance regulation is clearly posttranscriptional (27). The important concept to emerge from these studies is that probably all signal transduction pathways can affect the PA/plasmin system. Some pathways have similar effects in many different cell types: for instance glucocorticoids seem to uniformly decrease uPA transcription and mRNA levels, and often increase expression of the PAI-1 gene. By contrast, a given pathway can have opposite effects depending on the cell type: cAMP and related agents can increase (26) or decrease (28) uPA gene transcription. In this context, the observation that the same nuclear factor can activate uPA and collagenase transcription provides a new example of cooperation between the plasmin and metalloprotease cascades (24). The intricate regulation of PA and PAI synthesis allows expression of the PA/plasmin system to be precisely controlled in time, in a manner that depends upon the endocrine or growth factor balance. One striking illustration of this is provided by the effect of bFGF and TGF β on endothelial cells: both agents enhance PAI-1 and uPA mRNA and protein synthesis, but the kinetics and amplitude of the changes in expression of the two genes are remarkably different, so that the overall balance is tilted towards enhanced proteolysis in response to bFGF, and towards antiproteolysis in response to TGF β (29). This shift in the proteolytic balance of endothelial cells probably plays an important part in the TGF β -mediated antiangiogenic properties of cartilage (30, 31).

SECRETION

It is in general considered that PAs and PAIs follow the constitutive secretory pathway, i.e., that secretion rapidly follows synthesis (with the exception of the cytosolic form of PAI-2 [15]). However, immunohistochemical analysis of uPA in the mouse *vas deferens* suggests that the antigen is present in apical granules (32), and can therefore follow the pathway for regulated secretion. Also, in human PMNs expression of uPA activity at the cell surface can be triggered by exposure to secretagogues (33). Thus, regulation of enzyme or inhibitor secretion may represent yet another control point for expression of the PA/plasmin system. One aspect that has not yet received appropriate attention is the possible polarization of PA and PAI secretion by epithelial cells (34). A systematic study of this issue may help elucidate the physiological role(s) of the PA/plasmin system in epithelial tissues. In addition, a perturbation of the polar release of PAs or PAIs could be detrimental to tissue architecture or function, and pathogenic; for instance the basolateral release of a PA normally destined for apical secretion may result in proteolytic damage to the underlying basement membrane and hence play a part in the acquisition of invasive properties by malignant epithelial cells.

CELL SURFACE EXPRESSION: uPA RECEPTOR

The biological consequences of plasminogen activation may differ as a function of the type of PA produced: for instance, uPA favors cell migration through the ECM, whereas catalytic-

cally equivalent amounts of tPA are ineffective (35). This could result from the binding of each PA to different cell surface or ECM sites. The uPA receptor thus probably plays an important part in the spatial control of PA-catalyzed proteolysis.

Cell types expressing uPA receptors include monocytes/macrophages, PMNs, fibroblasts, endothelial cells, keratinocytes. Expression of uPA receptors can be modulated, so that their number and affinity will vary under the influence of growth factors (5, 36). Immunochemical studies have demonstrated the presence of uPA, presumably receptor bound, at sites of attachment of fibroblasts to the substratum and its colocalization with the cytoskeletal component vinculin (37). Furthermore, there is a rapid redistribution of the uPA receptor to the leading edge of monocytes induced to migrate in response to a chemotactic gradient (18). Thus, expression of the uPA receptor can be a highly dynamic property of at least certain cell types.

The existence of plasma membrane binding sites for uPA, plasminogen, and plasmin points to the cell surface as the site of assemblage of a powerful proteolytic system. At least two steps in the PA/plasmin cascade are dramatically enhanced by this process: (a) activation of pro-uPA is markedly increased when it is receptor-bound and when plasminogen is simultaneously present on the cell surface, and this in turn accelerates plasmin formation (38); (b) plasmin on the cell surface is protected from α 2-antiplasmin in plasma and extracellular fluids (9). Taken together, these observations suggest how plasmin generation and activity can be focused to restricted domains of the cellular environment, such as the leading edge of migrating cells. Current views on the involvement of uPA in cell migration and invasion are illustrated in Fig. 1.

The source of uPA that binds to the receptor may differ for different cell types. In certain cases uPA is synthesized by receptor-bearing cells, and binds in an autocrine way after secretion (5). However, pro-uPA is present in blood plasma and could bind to receptor-bearing circulating cells. Similarly, mouse spermatozoa bind uPA secreted by epithelial cells of the

vas deferens (39). In human colon adenocarcinomas, increased levels of uPA are produced by stromal cells, whereas uPA receptor mRNA is present in the malignant cells (40). The possibility of paracrine loading of uPA on receptor-bearing cells increases the versatility of this system, and could provide an example of cooperation between different cells in tissues.

The uPA receptor may also serve other functions. Membrane-bound uPA/PAI-1 and uPA/PAI-2 complexes are rapidly cleared from the cell surface by endocytosis, suggesting that the receptor promotes their intracellular degradation (5, 18). Most interestingly, recent results indicate that uPA binding to its receptor can stimulate the differentiation or proliferation of myeloid and other cells (41). These observations suggest that the uPA receptor can also function in a signal transduction mode, and thus that uPA should perhaps be viewed as a bifunctional molecule with both growth factor and enzymatic activities.

Plasminogen activators in physiology and pathology

In addition to their established role in fibrinolysis, PAs might catalyze localized pericellular proteolysis in a very wide spectrum of physiological and pathological situations. A striking illustration of this is provided by a histological assay that reveals PA activity in sections of mouse embryos (Fig. 2). A number of tissues, in particular kidney, skin, intracranial structures, and lung, express PA activity, in a pattern which changes with the developmental stage. Whereas recent *in vivo* experiments have confirmed that PAs are synthesized and secreted during cell migration and tissue remodeling, other observations have demonstrated that PAs are also expressed by cells that are not involved in such processes. PAs may thus fulfill a larger spectrum of related functions than had previously been suspected.

PA_s AND ECM TURNOVER DURING CELL MIGRATION AND TISSUE REMODELING

Embryonic development. The expression of PAs by invasive embryonic cells *in vivo* has been best documented in tropho-

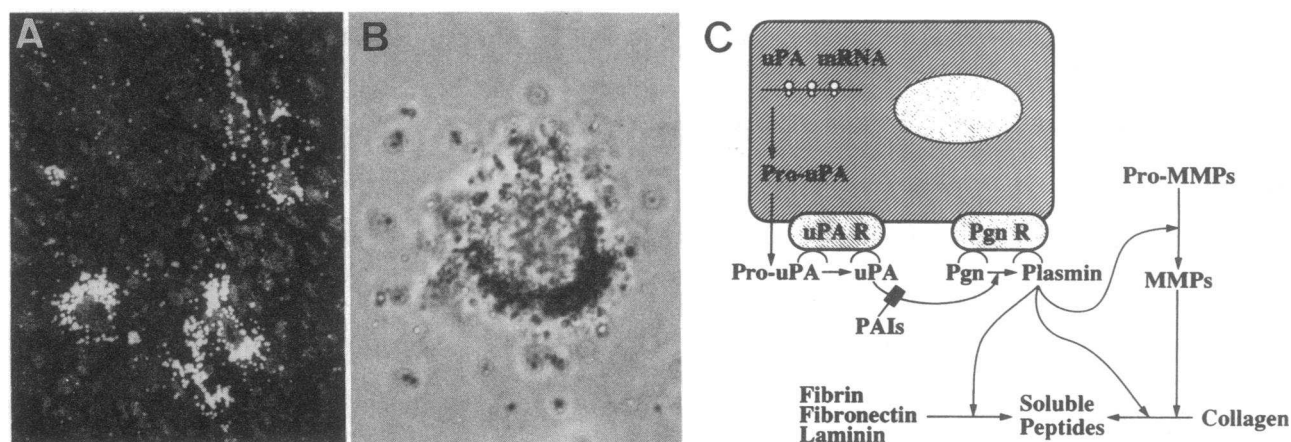


Figure 1. uPA in cell migration. (A) *In situ* hybridization with a ^3H -labeled cRNA probe reveals uPA mRNA in invasive trophoblasts migrating from a 6.5-d-old mouse embryo (top) into the surrounding uterine stroma. See reference 42 for experimental details. Silver grains appear as white spots in this dark-field micrograph (magnification, 800). (B) Autoradiographic detection of receptor-bound ^{125}I -uPA at the surface of a human monocyte in a chemotactic gradient. The source of the gradient is at the bottom of the photograph. See reference 18 for experimental details. Silver grains appear as black spots in this light-field micrograph, and reveal that the uPA receptor is concentrated at the leading edge of the cell (magnification, 1,400). (C) Schematic representation of the uPA-plasmin system at the surface of receptor-bearing cells. MMPs, matrix metalloproteases; Pgn, plasminogen; R, receptors.

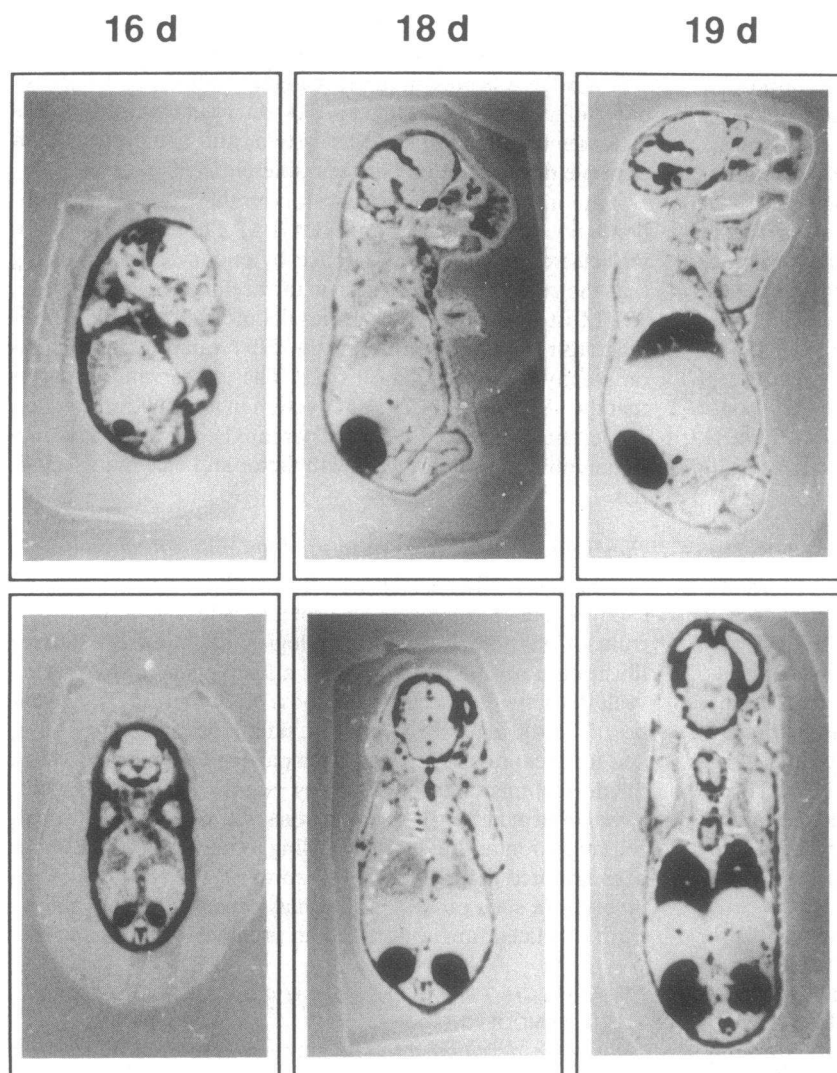


Figure 2. Visualization of PA enzymatic activities on tissue sections of murine embryos. Histological zymographies were performed as described (49). Sagittal (*top*) and frontal (*bottom*) cryostat tissue sections of 16-, 18-, and 19-d-old murine embryos were overlayed with a solution containing agar, plasminogen, and casein. Slides were incubated at 37°C for 3 h. PAs diffuse from the tissues into the overlay, where they activate plasminogen to plasmin, which in turn lyses the insoluble casein. No lysis is observed in the absence of plasminogen in the overlay (not shown). Photographs were taken using dark-ground illumination, and caseinolytic areas appear black. Note PA enzymatic activity in kidneys, skin, lungs, and intracranial structures (magnification, 2.2).

blast cells (42), which produce uPA in a temporal pattern compatible with its involvement in the endometrial disruption accompanying both implantation and early growth of the embryo. Similarly, other migrating embryonic cells, such as hemopoietic cells colonizing the bursa of *Fabricsius* (43), neural crest cells, and cerebellar granular neurons, have been found to produce PAs (44).

Ovulation. The production of PAs by rodent ovaries correlates with ovulation. The participation of the enzymes in follicular rupture has received strong experimental support: both anti-tPA antibodies and $\alpha 2$ -antiplasmin can block ovulation (45). The regulated expression of tPA in maturing rodent oocytes has led to the suggestion that it might participate in oocyte maturation and fertilization (46).

Inflammation, wound healing, and angiogenesis. Monocytes/macrophages and PMNs produce uPA, suggesting that it plays a role in the migration of inflammatory cells to the sites of inflammation and in the extensive extracellular remodeling that follows tissue injury. In addition, other cell types involved in inflammatory processes, such as keratinocytes in wound reepithelialization and endothelial cells in angiogenesis, also produce uPA. The angiogenic activity of endothelial cells reflects the balance of PAs and PAIs, as illustrated by the behavior of

hemangiomas, in which a shift of the balance towards proteolysis results in abnormal vessel formation (47).

Neoplasia. A large body of evidence implicates uPA in the invasive properties of malignant cells (1). The most direct comes from studies showing that inhibitory anti-uPA antibodies interfere with metastasis of tumor cells in chick embryos. In this model, cell surface receptor-bound uPA is required for one specific stage in metastasis, i.e., stromal invasion (35). In vivo, animal and human tumors often contain high levels of uPA and, in a number of cases, metastatic propensity correlates with the levels of enzyme production: for instance, uPA is abundant in squamous but not in basal cell carcinomas, the former having a higher invasive and metastatic potential (48).

PAs AND THE MAINTENANCE OF FLUIDITY IN TUBULAR STRUCTURES

Endothelial cells produce tPA; the enzyme, which may remain bound to the cells and/or be released into the blood, is thought to prevent inappropriate clot formation. Recently, uPA and tPA synthesis has been demonstrated in various epithelial cells lining tubular structures, a situation reminiscent of the function of tPA in the vascular bed. In the murine kidney, both PAs are secreted into urine by epithelial cells lining distinct seg-

ments of urinary tubules (49). Similarly, epithelial cells in the mammary gland, the vas deferens and the seminal vesicle produce and secrete uPA (32, 39). These cumulated observations support a role for PAs in the maintenance of patency and fluidity in tubular structures.

PAS AND THE BIOLOGY OF GROWTH FACTORS, HORMONES, AND NEUROPEPTIDES

PAs have been detected in the adult central nervous system (44), as well as in a majority of endocrine glands. Because ECM turnover is probably not a very active process in such tissues, other functions for PA-catalyzed extracellular proteolysis should be envisioned. This has led to the suggestion that PAs might participate in prohormone processing; recent data, which have tentatively identified intracellular processing proteases, do not support this hypothesis. However, the concept of proteolytic activation of hormones and growth factors should be revisited in the light of recent studies: plasmin can activate the latent form of TGF β and release bFGF-glycosaminoglycan complexes from the matrix and the cell surface (11), whereas PAs might cause posttranslational processing of hepatocyte growth factor, which has extensive homology with plasminogen. In addition, proteolytic inactivation of growth factors could involve the PA/plasmin system. Modulation of expression of receptors for growth factors, hormones, and neurotransmitters may also be achieved by PA-catalyzed proteolysis, as initially suggested for the EGF receptor (50). Finally, it is conceivable that PAs and PAIs could play a part in controlling the plasticity of synaptic structures.

In conclusion, the PA/plasmin system is part of a cohort of enzymatic pathways that catalyze extracellular proteolysis. We now know that it is expressed in a remarkably broad spectrum of biological situations, indicating the dynamic nature of the interaction of most cells with their environment. To elucidate the functions of PA-catalyzed proteolysis, we need to develop new tools: these may come from the identification of selective and pharmacologically useful inhibitors, or from the genetic approach of transgenic animals. Meeting this challenge will teach us much about the ecology of cells in the organism.

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