Abstract
A variety of proteases have the potential to degrade the extracellular matrix (ECM), thereby influencing the behaviour of cells by removing physical barriers to cell migration, altering cell–ECM interactions or releasing ECM-associated growth factors. The plasminogen activation system of serine proteases is particularly implicated in this pericellular proteolysis and is involved in pathologies ranging from cancer invasion and metastasis to fibroproliferative vascular disorders and neurodegeneration. A central mechanism for regulating plasmin generation is through the binding of the two plasminogen activators to specific cellular receptors: urokinase-type plasminogen activator to the glycolipid-anchored membrane protein uPAR, and tissue plasminogen activator to a type-II transmembrane protein recently identified on vascular smooth muscle cells. These binary complexes interact with membrane-associated plasminogen to form higher order activation complexes that greatly reduce the $K_m$ for plasminogen activation and, in some cases, protect the proteases from their cognate serpin inhibitors. Various other proteins that are involved in cell adhesion and migration also interact with these complexes, modulating the activity of this efficient and spatially restricted proteolytic system. Recent observations demonstrate that certain forms of the prion protein can stimulate tissue plasminogen activator–catalysed plasminogen activation, which raises the possibility that these proteases may also have a role in the pathogenesis of the transmissible spongiform encephalopathies.

Introduction
The serine proteases of the plasminogen activation system have traditionally been considered as part of the haemostatic mechanism owing to the dissolution of fibrin clots by plasmin (fibrinolysis). However, the plasminogen activation system has also been implicated in cellular migration and invasion. To some extent the existence of two distinct plasminogen activators accounts for these apparently distinct functions, with tissue plasminogen activator (tPA) being the primary fibrinolytic activator and urokinase-type plasminogen activator (uPA) the primary cellular activator. However recent evidence from knockout mice has demonstrated overlap in the functions of the two plasminogen activators, as well as highlighting novel functions for each of them.

It is now widely accepted that the generation of proteolytic activity is a key event in mediating cell migration and invasion, which dynamically modulates interactions between the cell and its surrounding extracellular matrix [1]. Owing to its broad substrate specificity, as well as the abundance and widespread distribution of plasminogen, plasmin is a central molecule in these processes. The diverse targets of this proteolytic system have led to it being implicated in a range of pathologies, including tumour invasion and metastasis, arthritis, cardiovascular disease and neurodegeneration; all linked by their dependence on cell migration and tissue degradation or remodelling.

In addition to acting in the pericellular environment, the activity of this proteolytic system...
is also regulated at the functional level by various interactions with cell surface molecules [2]. The most well characterized of these regulatory mechanisms involves the specific cellular uPA receptor (uPAR). Here we review briefly a number of recent developments both in the regulation of plasminogen activator function at the cell surface and also in the biological processes with which they may be involved.

Regulation of plasminogen activation by uPAR: general principles
Plasminogen activation is inherently efficient owing to the amplification of proteolytic activity resulting from a protease cascade, i.e. small amounts of plasminogen activator can generate large amounts of plasmin. The uPA-catalysed reaction has an additional level of amplification as plasmin can efficiently activate zymogen pro-uPA, a process of reciprocal zymogen activation. The presence of uPAR and the assembly of the cell-surface plasminogen activation system increases the catalytic efficiency of these two reactions, leading to a very large overall increase in plasmin generation. The principle interactions and reactions of the cell-surface plasminogen activation system are shown in Figure 1.

In kinetic terms, uPAR increases uPA activity by decreasing the $K_m$ for plasminogen activation by up to 200-fold [3], which is dependent on the cellular binding of plasminogen and cannot be replicated with recombinant soluble forms of uPAR [4]. Despite the importance of plasminogen binding and the variety of proteins and other molecules that contribute to the high plasminogen-binding capacity of the cell surface [5], the precise role of these various molecules in plasminogen activation is not yet clear. For example, they could contribute equally to plasminogen activation, or a discrete subset could be functionally involved. Mechanistic models have been proposed based on the high surface-density of plasminogen [6], but our own experimental results support the involvement of specific stoichiometric interactions between plasminogen and the uPA–uPAR complex. This evidence includes the following: (i) the effect of uPAR on plasminogen activation can be quantitatively mimicked in 1:1 complexes of uPA and plasminogen assembled on a monoclonal antibody [7]. (ii) Cell-surface plasminogen activation can be specifically blocked by certain low-molecular-mass anionic compounds in the absence of any effect on the cellular binding of uPA or plasminogen or on the catalytic activity of uPA or plasmin [8]. (iii) Plasminogen can bind to uPA via an 'exosite' interaction, i.e. independent of the active-site, with an affinity ($K_d = 50\, \text{nM}$ as determined by surface plasmon resonance) that is close to the $K_m$ for plasminogen activation ($100\, \text{nM}$), and much higher than the overall affinity for the cellular binding of plasminogen ($K_d = 1–2\, \text{µM}$) [9].

Non-active-site interactions have previously been implicated as part of the mechanism of pro-uPA activation by plasmin [10,11]. Together, these results suggest that the exosite interaction between uPA and plasminogen is necessary for the assembly of plasminogen activation complexes on the cell surface, with the exosite interaction possibly being responsible for orientating the membrane immobilized protease/zymogen components. We can therefore speculate that uPAR-bound uPA itself constitutes the functional cellular binding site for plasminogen. Interestingly, and in support of this hypothesis, it has been observed that a proportion of plasminogen co-localizes with uPA on a breast cancer cell line [12], although there are alternative interpretations of this observation.

Although it has been difficult to demonstrate a function for uPAR in mice with an ablated uPAR gene, evidence continues to accumulate that uPAR is an important regulator of uPA function in vivo. For instance, it has recently been shown that expression of a uPA transgene in the skin of mice is only pathogenic in the presence of a uPAR transgene [13]; this is consistent with a pathological role for this proteolytic system. It has also been shown that the toxicity of an engineered uPA-activatable anthrax toxin requires the presence of uPAR, and the assembly a functional cell-surface plasminogen activation system both in cell culture [14] and in vivo (T. Bugge, personal communication), suggesting that the generation of pericellular uPA activity does require uPAR.

Modulation of uPAR function by tetraspanin proteins
If the function of the uPA/uPAR system is to generate proteolytic activity to modify the extracellular matrix (ECM), it would seem advantageous for it to communicate with the systems involved in cell adhesion, and there has been much interest in potential interactions between uPAR and integrins, the major class of cell adhesion receptors. In various cell-types uPAR has been
Figure I

Receptor-mediated plasminogen activation

Two independent pathways for pericellular plasmin generation are shown in which all the principal reactions occur with membrane-bound components, making these pathways completely cell-associated. The binding of secreted pro-uPA to its cellular receptor, uPAR, leads to a reciprocal zymogen activation system in which uPA preferentially activates cell-bound plasminogen, and cell-bound plasmin can efficiently activate receptor-bound pro-uPA. Similarly, the binding of secreted tPA to its putative receptor(s) leads to the efficient activation of cell-associated plasminogen. Both pathways thus act to greatly enhance plasmin generation. Once generated, plasmin can degrade many non-fibrillar proteins of the ECM, can activate certain matrix metalloproteases (MMPs) (interstitial collagenase, stromelysin-1, gelatinase-B) and activate or release matrix-bound growth factors such as transforming growth factor β and basic fibroblast growth factor. The plasminogen activation pathways are also modulated by physiological inhibitors. α1-Antiplasmin (present in plasma) acts to further focus plasmin activity at the cell surface, as any plasmin that is generated is fully protected from inhibition while it remains bound, but is rapidly inhibited upon dissociation from the cell surface. PAl-1, in particular PAl-1, appears to have contrasting functions in the regulation of uPA and tPA activities. Pro-uPA is not inhibited by PAl-1 but once it is converted into active uPA the receptor-bound enzyme is fully available for inhibition. In contrast, nascent tPA is susceptible to inhibition by PAl-1 owing to its 'active zymogen' nature, but it becomes protected to some degree when bound to its cellular binding site. tPAR, tPA receptor.
despite unaltered levels of uPA and uPAR expression. Therefore, expression of CD82 results in uPAR being largely unable to bind its ligand and may provide a novel mechanism for downregulating the activity of this system. The mechanism underlying this effect is not yet fully elucidated, but the change in ligand binding behaviour of uPAR correlates with its redistribution on the cell surface, possibly as a consequence of CD82-mediated rearrangements of integrin distribution. This raises the possibility that CD82 downregulation of uPA–uPAR proteolytic function is a dynamic event, in a similar manner to many other events that regulate cell motility and migration.

**Regulation of tPA activity by vascular smooth muscle cells (VSMCs)**

Since the elucidation of the role of uPAR in mediating the generation of pericellular proteolytic activity there has been significant interest in identifying similar mechanisms for other proteases, including tPA. The expression of tPA in vivo is restricted, compared with that of uPA. A cell type that does expresses significant amounts of tPA in vivo is the VSMC. tPA expression by these cells is upregulated in response to arterial injury, which suggests that tPA plays a role in the migration and/or phenotypic changes of these cells which lead to intimal hyperplasia and thickening of the arterial wall. We have shown previously that VSMCs increase the activity of tPA by greater than 100-fold, and that this is owing to a specific and saturable binding of tPA, with a $K_d$ of approx. 25 nM [16]. This involves a novel binding protein, as the interaction could not be competed using a range of ligands for known tPA-binding molecules, including annexin 11, cytokeratin 8 and LRP (low-density lipoprotein receptor-related protein). Further characterization of the binding interaction revealed that it has a dual functional role in addition to increasing plasminogen activation as it also decreases the inhibition of tPA by plasminogen activator inhibitor 1 (PAI-1) [17], an effect not observed with uPA–uPAR (these and other comparisons are summarized in Table 1). The effect on tPA inhibition was also observed with certain low-molecular-mass inhibitors, and was found to correlate with a conformational change in the catalytic domain of the protease. tPA complexed with these inhibitors is unable to bind to the cellular binding site on VSMCs, which suggests an allosteric linkage between the cellular binding of tPA and its catalytic activity. This has also been observed for other serine proteases such as factor VIIa [18], although in the case of tPA it is not yet known whether the effects on plasminogen activation are due solely these to conformational changes or whether they also involve direct plasminogen binding as observed with the uPA–uPAR complex. The elucidation of this mechanism will be aided by our recent isolation and partial characterization of a 63 kDa type II transmembrane protein that appears to be responsible for tPA binding (T. M. Razzaq, R. Bass and V. Ellis, unpublished work). This protein, which is normally resident in the endoplasmic reticulum, is found on the plasma membrane of VSMCs, indicating that differences in trafficking of this protein may be involved in regulating tPA function on VSMCs.

**Plasminogen activation in neurodegenerative diseases**

Experiments in mice with ablated tPA genes have demonstrated that this protease has important functions in the central nervous system, being involved in both normal brain functions, such as synaptic plasticity and learning [19], and brain pathology, such as excitotoxin-induced seizure (reviewed in [20] and on pages 222–225 in this

<table>
<thead>
<tr>
<th>Comparison of functional regulation of plasminogen activators by pericellular binding sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>tPAR, tPA receptor</td>
</tr>
<tr>
<td>Stimulation of plasminogen activation</td>
</tr>
<tr>
<td>Dependence on cellular binding of plasminogen</td>
</tr>
<tr>
<td>Dependent on specific interaction with plasminogen</td>
</tr>
<tr>
<td>Direct effect on catalytic activity</td>
</tr>
<tr>
<td>Protection from inhibition</td>
</tr>
</tbody>
</table>

+ yes; - no; n.d., not determined
issue [21]). Most of these processes have been demonstrated to be dependent on the generation of plasmin activity, and ECM targets for plasmin have been identified. These observations suggest that there must be mechanisms to regulate tPA activity in the brain. tPA is known to be released from storage granules in neurons [22] and its activity is controlled by inhibitors such as PAI-1 and neuroserpin [23]. However, it is not known whether regulatory mechanisms, similar to those that stimulate the activity of tPA in other tissues, also exist in the brain. We have recently identified such a mechanism that could potentially fulfill this function in the group of neurodegenerative diseases known as the transmissible spongiform encephalopathies, or prion diseases, which include scrapie, bovine spongiform encephalopathy and variant Creutzfeldt–Jakob disease.

It has recently been found that plasminogen can bind to the prion protein (PrP), i.e. the infectious isomorph of the protein PrPSc in brain homogenates from scrapie-infected mice, but not from brains of uninfected mice (PrPSc) [24]. We have further investigated this phenomenon to determine whether the putative interaction between plasminogen and PrP has functional consequences, and found that PrP specifically stimulates tPA-catalysed plasminogen activation (V. Ellis and D. R. Brown, unpublished work). PrPSc, but not PrPSc, contains bound copper at the N-terminal 'octapeptide repeat' region [25], a characteristic that can be exploited to generate recombinant forms of PrP resembling the natural proteins. Holo-PrP (containing bound Cu2+) had no effect on plasminogen activation by either uPA or tPA; this is consistent with the inability of PrPSc to bind plasminogen. Apo-PrP (lacking bound Cu2+) also had no effect on plasminogen activation by uPA. However, apo-PrP was found to increase tPA-catalysed plasminogen activation by up to 300-fold. Competition experiments demonstrated that this was owing not only to the binding of plasminogen to apo-PrP, consistent with the previously observed binding of plasminogen to PrPSc, but also to the binding of tPA. Additional kinetic experiments have demonstrated that plasminogen and tPA bind to independent sites on apo-PrP, and that the binding of tPA is specific and of higher affinity than its binding to fibrin or cell surfaces. Together these characteristics suggest that the binding of tPA to PrP may be of significance in vivo and that the stimulated generation of plasmin may contribute to neurodegeneration in the prion diseases by mechanisms similar to those observed in excitotoxin-induced seizure, e.g. ECM degradation. As PrP is a glycosylphosphatidylinositol-anchored membrane protein in vivo these events are likely to occur at the neuronal cell surface, in common with the other mechanisms discussed here that occur on other cell types.

**Conclusions**

It now seems clear that a diversity of mechanisms exist to regulate the activation of plasminogen in the pericellular environment where plasmin has a non-haemostatic role. These mechanisms may be involved in distinct pathological functions, although operating through similar biological pathways, e.g. ECM degradation. Experiments using fibrinogen-knockout mice have confirmed fibrin as a major pericellular substrate in some pathological conditions [26], but not in others, such as liver regeneration [27] and pulmonary fibrosis [28]. Therefore, although many substrates for plasmin have been identified in vitro, the non-haemostatic substrates in vivo in various pathological situations remain to be fully elucidated.

V. E. is a Senior Research Fellow of the British Heart Foundation. We would like to thank various collaborators who have contributed to the work described here, in particular Fedor Berditchevski (University of Birmingham, U.K.) and David Brown (University of Bath, U.K.).

**References**

Abstract

Fibrotic disorders of the liver, kidney and lung are associated with excessive deposition of extracellular matrix proteins and ongoing coagulationcascade activity. In addition to their critical roles in blood coagulation, thrombin and the immediate upstream coagulation proteases, Factors Xa and VIIa, influence numerous cellular responses that may play critical roles in subsequent inflammatory and tissue repair processes in vascular and extravascular compartments. The cellular effects of these proteases are mediated via proteolytic activation of a novel family of cell-surface receptors, the protease-activated receptors (PAR-1, -2, -3 and -4). Although thrombin is capable of activating PAR-1, -3 and -4, there is accumulating in vivo evidence that the profibrotic effects of thrombin are predominantly mediated via PAR-1. Factor Xa is capable of activating PAR-1 and PAR-2, but its mitogenic effects for fibroblasts are similarly mediated via PAR-1. These proteases do not exert their profibrotic effects directly, but act via the induction of potent fibrogenic mediators, such as platelet-derived growth factor and connective tissue growth factor. In vivo studies using proteolytic inhibitors, PAR-1 antagonists and PAR-1-deficient mice have provided evidence that coagulation proteases play a key role in tissue inflammation and in a number of vascular pathologies associated with hyperproliferation of smooth muscle cells. More recently, coagulation proteases have also been shown to play a role in the pathogenesis of fibrosis but the relative contribution of their cellular versus their procoagulant effects awaits urgent evaluation in vivo. These studies will be informative in determining the potential application of PAR-1 antagonists as antifibrotic agents.

Introduction

The main function of the coagulation cascade is to ensure the formation of stable haemostatic clots, consisting of aggregated platelets enmeshed in fibrin, that plug injured vessels and prevent blood loss. It has been known for a long time that fibrin deposition plays a pivotal role in influencing subsequent tissue repair processes by acting as a provisional structural matrix for fibroblasts and inflammatory cells migrating into the area of injury and by acting as a reservoir of growth factors and fibrogenic cytokines [1]. The last decade has also seen a major re-evaluation of our perception that

Key words: connective tissue growth factor, Factor Xa, protease-activated receptor, thrombin. Abbreviations used: CTGF, connective tissue growth factor; EPR-I, effector-cell protease receptor-I; IL, interleukin; PAR, protease-activated receptor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β.

To whom correspondence should be addressed (e-mail r.chambers@ucl.ac.uk).

Received 14 December 2001