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Review

The plasminogen activator system: biology and regulation

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Abstract. The regulation of plasminogen activation involves genes for two plasminogen activators (tissue type and urokinase type), two specific inhibitors (type 1 and type 2), and a membrane-anchored urokinase-type plasminogen-activator-specific receptor. This system plays an important role in various biological processes involving extracellular proteolysis. Recent studies have revealed that the system, through interplay with integrins and the extracellular matrix protein vitronectin, is also involved in the regulation of cell migration and proliferation in a manner independent of proteolytic activity. The genes are expressed in many different cell types and their expression is under the control of diverse extracellular signals. Gene expression reflects the levels of the corresponding mRNA, which should be the net result of synthesis and degradation. Thus, modulation of mRNA stability is an important factor in overall regulation. This review summarizes current understanding of the biology and regulation of genes involved in plasminogen activation at different levels.

Key words. Cell invasion; cell motility; fibrinolysis; metastasis; mRNA stability; plasminogen activator; plasminogen activator inhibitor; signal transduction; transcriptional regulation; uPA receptor.

Introduction

As plasminogen is a ubiquitous zymogen and its activated form plasmin is a potent trypsin-like protease with wide substrate specificity, the unrestrained generation of plasmin from plasminogen by the action of plasminogen activator (PA) is potentially hazardous to cells. Thus, the process of plasminogen activation in a healthy organism is strictly controlled through the availability of PAs, localized activation, and interaction with specific inhibitors (PAIs). One of the two PAs, the urokinase type (uPA), is recruited to the cell membrane immediately after its secretion via a specific uPA receptor (uPAR) expressed on the cell surface, and plays a role in localized cell-associated proteolysis. The other PA, the tissue type (tPA), has a high affinity for fibrin, and its enzymatic activity is enhanced by fibrin binding, resulting in a stronger inclination towards the fibrinolytic process. Thus, despite their common enzymatic activities, the two PAs appear to play distinct roles in the organism. The difference is further highlighted by recent demonstration of a role for the uPA/uPAR system in the regulation of cell-cell/cell-matrix interactions. In this context, the system exhibits functions other than proteolysis, such as cell adhesion and migration.

Reflecting these diverse biological roles, the genes of fibrinolysis are expressed in many cell types, and their expression is controlled by a variety of extracellular signals. To study this regulation at the molecular level, the promoters of the relevant genes have been cloned from different species and used in functional analyses. The cytoplasmic levels of the corresponding mRNAs

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are the result of de novo synthesis and degradation. The importance of the regulation of mRNA stability for overall gene expression has received increasing attention, and numerous studies on the metabolism of mRNAs encoding fibrinolytic genes have recently emerged.

The plasminogen activation system

The zymogen of plasminogen is converted into the two-chain structure of plasmin by proteolytic cleavage of a single peptide bond. The cleavage is catalyzed by uPA or tPA, as well as by certain bacterial proteins [1]. Plasminogen is a ubiquitous protein produced mainly in the liver [2] but elsewhere also, for example in testis [3] and epidermal cells [4]. It binds to many molecules other than uPA [5], namely laminin [6], fibronectin [7], fibrin [8], thrombospondin [9], tetranectin [10], and cytokeratin 8 [11]. Plasminogen receptors are expressed on the cell surface of various cell types, including blood monocytes, granulocytes, and lymphocytes [12], [13] and endothelial cells [14]. The two main candidate plasminogen receptors are α-enolase [15] and annexin II [16]. The interaction of plasminogen with its cellular receptors accelerates its conversion to plasmin, which has enhanced enzymatic activity on the cell surface (compared to plasmin in solution) and is protected from inactivation by inhibitors [17].

Cell-surface-associated plasmin catalyzes the breakdown of many of the known extracellular matrix (ECM) and basement membrane molecules, such as fibronectin, laminin [18], vitronectin [19], proteoglycans [20], fibrin [21], and collagen [18, 22, 23]. These events occur directly by the action of plasmin or indirectly via the plasmin-dependent activation of other matrix-degrading proteases like pro-stromelysin [24] and pro-collagenase [25]. Latent forms of basic fibroblast growth factor (bFGF) and transforming growth factor-β (TGF-β) can also be activated by plasmin [26, 27]. Extracellularly, plasmin also acts on single-chain pro-uPA and uPA [28, 29].

Plasminogen-deficient mice are able to complete embryonic development, reach adulthood and reproduce, although they suffer multiple spontaneous thrombotic lesions, organ damage, and high early morbidity [30]. These mice also have impaired skin wound healing [31], which, however can be rescued by concomitant disruption of the fibrinogen gene [32]. The loss of plasmin-mediated proteolysis could be compensated by a functional overlap between plasmin and other extracellular proteases; however, in processes such as tissue remodeling and cell migration (wound healing) where fibrin is widespread, plasmin-mediated proteolysis is particularly important [31, 33].

The urokinase-type plasminogen activator

The human single-copy gene uPA codes for a 53-kDa serine protease produced as a single-chain protein (scuPA or pro-uPA) [34]. When secreted, pro-uPA is converted to the active two-chain form uPA (tcuPA or uPA) by cleavage of the peptide bond K158-I159 by plasmin [35]. The activation reaction can also be carried out, at least in vitro, by many other proteins including plasma kallikrein, blood coagulation factor XIIa, and cathepsins, but the physiological significance of these proteins as activators remains obscure [36]. Pro-uPA is a true zymogen with much lower PA activity than uPA [37].

Secreted pro-uPA binds to uPAR and is subsequently activated by plasmin to uPA, which then converts neighboring membrane-bound plasminogen to plasmin [38]. Pro-uPA bound to uPAR is activated by plasmin much faster than when free in the fluid phase [28, 39]. The two peptide chains of uPA are linked by disulfide bridges, and the molecule contains three functional domains. In the carboxyl-terminal region is a serine protease domain (SPD, residues 144–411; also called the B chain) making up most of the low-molecular-weight (LMW) uPA with full specific activity [40, 41]. The non-catalytic amino-terminal fragment (ATF), corresponding to the A chain, contains the kringle domain (triple-disulfide-containing structure that binds protein, residues 47–135) and the epidermal growth factor (EGF)-like domain (GF, residues 4–43). The two chains are linked by a connecting peptide [42]. Thus, the uPA molecule has at least two completely independent parts: the catalytic carboxyl-terminal chain and the non-catalytic amino-terminal chain, of which the initial GF domain of 32 residues is responsible for the specific interaction with uPAR [43].

uPA activity is controlled by binding of PAIs and endocytosis (see below). Besides converting pro-uPA to active uPA, plasmin can further cleave uPA to a 33-kDa form which lacks the GF and the kringle domains but still retains full activity [44].

Apart from plasminogen, uPA directly activates pro-hepatocyte growth factor/scatter factor (HGF/SF) [45, 46], and it can cleave fibronectin [47] as well as its own inhibitor PAI-1 [48, 49] in a plasminogen-independent manner. uPA, ATF and diisopropylfluorophosphate (DFP)-inactivated uPA, but not LMW uPA, compete with pro-uPA for binding to uPAR; thus pro-uPA may be the physiological ligand of uPAR [50, 51].

The tissue-type plasminogen activator

tPA is a 70-kDa protein which is secreted as a precursor in single-chain form [40]. Plasmin converts the precursor by cleaving the peptide bond Arg275-Ile276 to give

an active two-chain form held together by a single interchain disulfide bond as in uPA. Unlike uPA, however, single-chain tPA also has a significant activity [52]. The tPA molecule is composed of four functionally distinct domains: (1) an amino-terminal region of 47 residues (residues 4–50) known as the fibronectin-like domain or finger domain, (2) an EGF-like domain (GF, residues 50–87), (3) two kringle regions (residues 87–176, K1 domain, and 176–262, K2 domain), and (4) a serine protease region (residues 276–527) with the active-site residues His322, Asp371, and Ser478 [40]. The first three domains are located in the amino-terminal chain and have modulatory activities for the enzyme. K2 and to a lesser extent finger/GF domains are responsible for the high affinity of tPA for fibrin and its activation by fibrin [53, 54]. The finger and EGF-like domains are responsible for the rapid clearance of tPA in hepatocytes [55].

The presence of specific receptors for tPA has been reported in hepatocytes [56], brain [57], endothelial [58], and preimplantation embryonic cells [59]. Interestingly, a 20-kDa receptor purified from endothelial cells has been found to interact in vitro with tPA with a 1: 1 stoichiometry and to enhance plasminogen activation activity of tPA in soluble and immobilized form by 34- and 90-fold, respectively [58]. In liver, the tPA-binding molecule seems to be the cell surface α_2-macroglobulin receptor/low-density-lipoprotein-receptor-related protein (α_2-MR/LRP). In parenchymal liver cells, the tPA/PAI-1 complex is recognized by the receptor with high affinity in a PAI-1-dependent manner and cleared from the circulation [55]. LRP is a multifunctional cell surface receptor expressed in liver that binds with high affinity and endocytoses several structurally and functionally distinct ligands, including apolipoprotein-E-activated β-migrating very low density lipoprotein, tPA, and α_2-MR. This LRP apparently also interacts with tPA in neuronal cells [60].

Due to its high affinity for fibrin and activation by fibrin binding [61], the main biological role of tPA seems to be associated with fibrinolysis. Expression of tPA is observed in locations with close contact to fibrin clots, e.g., vascular endothelial cells [62], and is induced in physiological situations caused by or prone to thrombosis, such as ischemia [63], wounding [64], and ovulation [65]. However, in some activities, tPA and uPA have the same role. When either of the two genes is deleted from mice, skin wound healing proceeds quasi normally, while inactivation of both genes severely impedes this process [31]. Interchangeability of the two enzymes was suggested for the ovulation process as mouse and rat express different PAs [66].

The PA inhibitors

Unrestrained proteolytic activity by even low levels of uPA is potentially hazardous for cells. As one form of protection, cells secrete a surplus of inhibitor to guarantee restraint of pericellular proteolysis. Indeed, secreted uPA is often associated with PAI-1 and remains inactive [67, 68].

PAIs are members of the serine protease inhibitor superfamily (SERPIN) and belong to the arg-SERPIN subgroup possessing an arginine in the reactive center. The inhibitors include PAI-1, PAI-2, protease nexin 1, and protein C inactivator (PCI) [69–72]. They have a peptide bond for targeting proteases which resembles a pseudo-substrate, stably coupling them in an inactive conformation with a 1: 1 stoichiometry. Generally, SERPINs are specific (with distinct biological characteristics), fast-acting inactivators, present in most body fluids, tissues and cell lines [reviewed in ref. 73].

Plasminogen activator inhibitor type 1

PAI-1 is a single-chain 45- to 50-kDa glycoprotein secreted by many cell types. The secreted form is quickly transformed into a dormant conformation unable to form complexes with PAs. The physiological cofactors vitronectin and heparin, by interacting with PAI-1, can stabilize the active conformation and broaden the substrate specificity toward a further serine protease, thrombin [74]. Unlike the secreted form, the substratum-bound immobilized inhibitor remains active in the matrix for prolonged periods of time [75]. PAI-1 can also couple to uPAR-bound uPA, thereby inhibiting ECM degradation initiated by cell-associated uPA [76].

Regulation of PAI-1 activity can be modulated in several ways. It may be cleaved and inactivated by uPA [77] or form a quaternary complex of uPAR-uPA-PAI-1 with α_2-MR/LRP or with gp330 (very low density lipoprotein receptor endocytosis receptors) which is then internalized (see below). uPA-PAI-1 is then engulfed by lysosomes and digested, while uPAR is recycled to the cell surface [78, 79].

Besides the direct PA-inactivating role, PAI-1 binding to the ECM also modulates pericellular proteolysis carried out by receptor-bound uPA. PAI-1 is also involved in the regulation of cell adhesion and migration (see below).

Plasminogen activator inhibitor type 2

PAI-2 is a single-chain protein of 47 kDa [80]. Its inhibitory potency measured as an association constant towards receptor-bound uPA is 15 times less than that of PAI-1 [81]. PAI-2 is generally characterized as an

inhibitor of the extracellular serine protease uPA. However, most newly synthesized PAI-2 remains intracellular, with only a fraction of PAI-2 secreted as a glycosylated product [82]. Recently, an intracellular role for PAI-2 has been postulated from the observation that cytoplasmic expression of PAI-2 protects cells from tumor necrosis factor-α (TNF-α)-mediated apoptosis [83, 84] and induces a low level of autocrine interferon (IFN)-α/β [85]. PAI-2 is rapidly induced in monocyte macrophages in response to TNF-α and lipopolysaccharide (LPS), with the predominant proportion of newly synthesized PAI-2 remaining intracellular [84]. This suggests the interesting possibility that the physiological role of PAI-2 in inflammatory macrophages is to protect cells from the cytotoxic effect of their own TNF-α. A role for PAI-2 in the inhibition of apoptosis is further supported by the observation that PAI-2 can inhibit *Mycobacterium avium*-induced apoptosis of macrophages [86]. Thus, PAI-2 has the unique feature of existing in both secreted and cytosolic forms. In its secreted form, PAI-2 participates in the control of tissue remodeling and fibrinolysis. In its cytosolic form, it plays an important role in intracellular proteolysis involved in processes such as apoptosis and inflammation. In a recent report, HeLa cells expressing intracellular PAI-2 were protected from the cytopathic effects (CPEs) of alphavirus infection [85]. Infection in the absence of intracellular PAI-2 did not induce apoptosis in HeLa cells, indicating that protection against CPEs in PAI-2-expressing cells was not related to PAI-2-mediated inhibition of apoptosis. Instead, protection was associated with a PAI-2-mediated induction of constitutive low-level autocrine IFN-α/β production and IFN-stimulated gene factor (ISGF3), which primed the cell for rapid induction of antiviral genes. Thus, after virus infection, PAI-2-transfected cells expressed antiviral genes, which led to a rapid inhibition of viral replication. In contrast, there was no IFN-α/β or antiviral gene induction in control cells and consequently rapid viral replication and cell death.

The uPA receptor

uPAR, a 270-residue glycosyl-phosphatidyl-inositol (GPI)-anchored molecule, consists of three cysteine-rich CD59-like domains connected by short linker regions [87, 88]. Soluble uPAR variants (without the GPI anchor) may arise by differential splicing or by phospholipase C cleavage of the GPI anchor, but their function(s) is still unknown [89]. The amino-terminal domain (D1) has uPA-binding activity; the other two domains (D2 and D3) bind vitronectin. uPAR can also bind integrins at sites distinguishable from its uPA- and vitronectin-binding sites. The linker region connecting domains D1 and D2 is a protease-sensitive domain in vivo with chemotactic activity and binds an unknown surface adaptor (see below) [90, 91]. Pro-uPA, DFP-inactivated uPA, uPA, and ATF all bind with the same affinity to uPAR [92].

Regulation of uPAR can be achieved in two ways: uPAR can be inactivated by uPA-mediated cleavage between domains D1 and D2 [90]. Alternatively, PAI-1 can complex with uPAR-uPA-LRP and trigger internalization-recycling of uPAR, re-exposing free uPAR at a site where it can bind novel uPA [93]. Internalization of uPAR-bound u-PA/inhibitor complexes is much faster than that of fluid-phase uPA/inhibitor complexes [94]. This recycling process is controlled by the cellular concentrations of α_2-MR/LRP and uPAR, and it involves protein kinase C (PKC) [94–96] and cAMP-dependent protein kinase [79, 97] signal transduction pathways.

Biological role of the uPA/uPAR system

Cell invasion, adhesion, and migration

Identification of a uPA-specific receptor on the surface of various cell types was soon followed by the observation that both plasminogen receptor and uPAR are expressed on the same cell [98]. Furthermore, newly synthesized and secreted pro-uPA binds to the receptor, where it is efficiently converted to active uPA [29, 38, 92]. Receptor-bound uPA suggested that the uPA/uPAR system is mainly involved in site-directed proteolysis by localizing the plasminogen activation cascade to cell surfaces [99, 100]. Accordingly, the uPA/uPAR system was expected to contribute to cell-associated proteolysis in biological processes where cells are invasive. The most conspicuous example is tumor invasion. Several model systems indicated that the ability of tumor cells to invade and metastasize can be downregulated by uPA inhibitors, anti-uPA antibodies, antisense uPAR expression, and uPAR antagonists, or increased by uPA overexpression [101–105]. High levels of uPA and uPAR have also been observed in non-malignant cell-remodeling processes, such as normal embryogenesis and wound healing [106, 107] and post-lactational involution [108]. This raises the critical question as to what distinguishes the regulation of the uPA/uPAR system in controlled cell migration or tissue remodeling from that of tumor invasion.

Concomitant to uPA and uPAR expression, high levels of PAI-1 expression were documented in many of these processes [109, 110]. At first, this appeared to be paradoxical, since PAI-1 was understood to be an inhibitor of PAs. This observation suggested that the process of cell invasiveness was not simply a function of cell-associated uPA activity level. It could be argued that dynamic changes in cell-associated proteolytic activities

were required for cell movement, because cells would need at one time or another some foothold in order to move. In this context, it is worth noting that a deficiency of PAI-1 expression in host mice prevented local invasion and tumor vascularization of transplanted malignant keratinocytes. Invasion was restored by intravenous injection of a replication-defective adenoviral vector expressing human PAI-1. This is clear evidence for a role of PAI-1 in cancer cell invasion and angiogenesis [111]. It was reported that the uPA/uPAR complex is internalized when uPA is bound by PAI-1 [93, 112], indicating that PAI-1 controls cell-associated uPA activity by two mechanisms: the suppression of uPA enzymatic activity and reduction in the amount of uPA.

At this point, the extracellular protein vitronectin entered the picture. Vitronectin is a protein abundant in serum that promotes adhesion and spreading of tissue cells in culture [reviewed in ref. 113]. Vitronectin binds several integrins expressed on the cell membrane, including $\alpha_v\beta_3$ [reviewed in ref. 114]; it is also bound tightly by PAI-1 [115, 116]. In fact, smooth muscle cell movement on vitronectin was shown to be dependent on $\alpha_v\beta_3$ integrin, and was strongly reduced by active PAI-1 through its competition with integrin for a specific region on the vitronectin molecule [117]. uPA binding to PAI-1 suppressed this effect by masking the binding site for vitronectin. Important to this regulation is that it involves neither proteolytic activity of uPA nor enzyme inhibitory activity of PAI-1 [117], revealing new aspects of fibrinolytic gene involvement in biological processes.

Vitronectin interacts not only with integrins and PAI-1 but also with other molecules, including uPAR [118, 119] and, with a rather low affinity, uPA [120]. Interestingly, uPAR binds more readily to vitronectin when it is pre-bound to pro-uPA, uPA, ATF, or the uPA/PAI-1 complex [119, 121]; the pre-binding induces a conformational change in uPAR exposing its vitronectin-binding site [122]. uPAR and PAI-1 share binding regions on the vitronectin molecule [121, 123] and thus bind competitively. They have opposite effects on uPAR-dependent cell adhesion to vitronectin [124]: uPA enhances whereas PAI-1 inhibits the interaction of cells with vitronectin [125, 126]. Note that this regulation involves neither proteolytic activity of uPA nor enzyme inhibitory activity of PAI-1. In fact, uPA is replaceable here with pro-uPA or ATF, which have no proteolytic activity [121]. At the same time, PAI-1 regulates the proteolytic activity of cell-associated uPA by inducing internalization of the uPA/uPAR/PAI-1 complex [93], which is dependent on a further cell-associated receptor, α_2-MR/LRP [95]. The influence of PAI-1 on cell adhesion to vitronectin is not always negative. It has been shown that myogenic cells adhere and spread on PAI-1-coated plastic dishes by a mechanism dependent on uPAR, uPA, and $\alpha_v\beta_3$ integrins [78], indicating the involvement of both activated uPAR and $\alpha_v\beta_3$ in this process. Failure of receptor internalization due to the conditions applied in this work may have suppressed an otherwise inhibitory effect of PAI-1. Alternatively, as receptor internalization is dependent on LRP, the system may have lacked LRP.

A further level of regulation of uPAR-mediated cell adhesion and motility is the phosphorylation of pro-uPA on Ser138 and Ser303 residues. This prevents the catalytic-independent ability of pro-uPA to promote myelomonocytic cell adhesion and motility [127]. The phosphorylation is induced by PKC activation [128]. The effects on cell adhesion and motility, however, do not occur through modulation of the interaction between pro-uPA and uPAR or uPAR and vitronectin [128]. A step subsequent to pro-uPA binding to uPAR must be affected by pro-uPA phosphorylation.

Cell adhesion and spreading require cytoskeleton involvement [129]. As uPAR is a GPI-linked membrane protein lacking a cytoplasmic tail [87] with free lateral movement on the memberane in its uPA-unbound form [130], its role in these processes assumes the presence of partner membrane-associated molecules with cytoplasmic domains which can exert an influence on the cytoskeleton system. Various types of integrins have been reported to interact with uPAR and modulate uPAR-mediated cell interaction with the ECM. The mode of interaction varies with the integrin type. In embryonal kidney 293 cells, uPAR can be recovered as multimeric membrane complexes including uPAR, β_1 integrin and caveolin [131]. Caveolin is a protein associated with intracellular signaling pathways and cytoskeletal elements [132]. In 293 cells, β_1 integrin-dependent cell adhesion to vitronectin was markedly enhanced and that to fibronectin was repressed by uPAR expression or even by soluble uPAR [131]. In neutrophils, intact uPA, but not ATF, induces a reversible physical interaction between β_2 integrins (Mo1/Mac-1/CR3) and uPAR, regulating cell motility [133, 134]. In HT1080 fibrosarcoma cells, association of uPAR with β_3 integrins was observed when cells were attached to fibronectin, laminin, or vitronectin. In contrast, association of uPAR with β_1 integrins was observed only when cells were attached to vitronectin [135]. In pancreatic carcinoma FG cells, uPA induces the interaction of uPAR with $\alpha_V\beta_5$ integrin, leading to cell migration on vitronectin. PAI-2 did not affect cell adhesion but blocked migration, suggesting a direct role for uPA enzymatic activity in this process [136]. Thus, the functional regulation of different types of integrins is modulated by the uPA/uPAR complex. Indeed, uPAR can be considered a modulator of integrin function.

The uPA system is also involved in cell recruitment in normal physiological as well as pathological circum-

stances. uPA-deficient mice fail to recruit T cells and macrophages, thus succumbing to bacterial infection [137]. uPA and its precursors or derivatives lacking protease activity (i.e., pro-uPA or ATF) are able to stimulate chemotaxis in many cell types, such as activated blood leukocytes, neutrophils, and macrophages [138–140].

The uPAR is a true chemoattracting molecule. Upon ligand binding (such as to pro-uPA, uPA, or ATF), uPAR undergoes either a conformational change or a proteolytic cleavage (by uPA or a different protease), exposing a chemotactic epitope located in the linker region connecting domains D1 and D2. Soluble uPAR when cleaved by chymotrypsin at tyrosine 87 also undergoes a conformational change, similar to that induced by uPA binding, to generate a soluble chemokine [140]. It has been suggested that the conformational change is enough to expose the chemotactic epitope, implying that the proteolytic event is irrelevant [140]. The cell surface component that interacts with this chemotactic epitope and mediates chemokine activity has not yet been determined.

Intracellular signaling

On the cell, the uPAR-induced chemotactic effect requires the transient activation of a Src family member tyrosine kinase [139] and is pertussis toxin sensitive. This suggests a link between G proteins and intracellular protein tyrosine kinases [140]. Supporting this idea, it was shown that 3T3 fibroblasts from wild-type but not from $Src^{-/-}$ mice respond to uPAR-mediated chemoattraction [140]. Despite the fact that GPI-anchored molecules can generate signals, they do not possess a transmembrane domain. They must, therefore, associate with transmembrane 'adaptors' (i.e., with a true signaling molecule), like CD14, which can signal through a heterotrimeric G protein [141]. These candidate cell surface adapter proteins are molecules such as integrins [131, 142] and caveolin [143] [see review in ref. 124]. The transmembrane adaptor may bind uPAR via its extracellular domain and associate with a non-receptor protein tyrosine kinase (Hck, Fyn, Lyn, and Fgr) through its cytoplasmic tail [144, 145]. In HT1080 cells, uPA treatment induced the activation of Hck, p38 and extracellular-signal-regulated kinase (Erk)-2, and c-*fos* gene transcription [146]. In aortic endothelial cells, occupancy of the uPAR by uPA leads to phosphorylation of focal adhesion kinase (FAK). This is completely inhibited by genistein but not by a specific inhibitor of Src family kinases [147]. uPAR can also associate with PKCε in human epithelial cells, leading to serine phosphorylation of cytokeratins 8 and 18 [148]. As PKCε can activate Raf [149], uPAR occupancy would lead to the activation of Erk, a member of the mitogen-activated protein (MAP) kinase family. It has been reported recently that binding of uPA to its receptor in breast cancer MCF-7 cells activates Erk-1 and Erk-2, and that this signaling event is required for uPA-stimulated breast cancer migration [150]. Besides protein kinases, some transcription factors are upregulated or activated after uPA treatment. These include AP1 in HT1080 cells [151], as well as c-Jun and c-Myc in SAOS2 cells [152]. Thus, uPA induces uPAR-mediated activation of various kinases and transcription factors, and this may influence cytoskeletal structure, cell morphology, cell adhesion, and cell motility and migration.

Pathophysiology of the PA system

Various pathological situations are associated with imbalanced expression of components of the plasminogen activation system. The most intensively studied in this context are cardiovascular diseases and tumor metastasis. One of the well-characterized substrates of plasmin is fibrin, an essential component of blood clots. While fibrin aggregation is crucial to the suppression of hemorrhaging from wounded blood vessels, abnormal deposition of a fibrin clot leads to cardiovascular diseases such as thrombosis, arterial neointima formation, and atherosclerosis [see reviews in refs 153, 154]. Fibrin deposition may be a consequence of low plasminogen activation due to high expression of PAIs or low expression of PAs. For example, obesity may be accompanied by cardiovascular disease [reviewed in refs 155, 156], most likely as the result of high PAI-1 expression in adipocytes [157, 158].

Tumor growth may not be fatal if tumorigenic cells remain at their original sites. However, some tumorigenic cells acquire the capacity to leave the place of origin, penetrate blood vessels, travel to remote places and settle in different organs, a process collectively termed metastasis. Metastatic tumor cells would be expected to express high levels of cell-associated proteolytic activity. Epidemiological studies of tumor libraries have shown that uPA and PAI-1 are independent prognostic markers of disease recurrence and poor survival for patients with cancer of various organs such as breast, ovary, and colon [159, 160]. In addition, most cancer cells express uPAR, often at rather high levels [161]. The significance of high PAI-1 expression in malignant tumors was discussed above.

Studies in mice using targeted inactivation of uPA, tPA, PAI-1, uPAR, and plasminogen genes have confirmed that disruption of the plasminogen activation system contributes not only to various cardiovascular disorders but also to the perturbation of reproduction, embryonic development, wound healing, infection, cancer, and brain function. Phenotypes resulting from genetic inac-

Table 1. Phenotypes resulting from the targeted gene disruption of components of the plasminogen activation system in mice.

Deficiency	Phenotype
Tissue-type plasminogen activator (t-PA)	• increased thrombotic susceptibility • mild glomerulonephritis • teduced neurotoxicity in hippocampus • abnormal long-term potentiation • impaired neuronal migration
Urokinase-type plasminogen activator (u-PA)	• increased thrombotic susceptibility • impaired neointima formation • glomerulonephritis • impaired macrophage function • reduced decidual vascularization • reduced trophoblast invasion • reduced platelet activation • reduced tumor invasion (e.g. reduced progression of malignant melanomas)
t-PA:u-PA	• severe spontaneous thrombosis • impaired neointima formation • reduced ovulation and fertility • cachexia and shorter survival • severe glomerunonephritis • abnormal tissue remodeling
Urokinase receptor (u-PAR)	normal
Plasminogen	• severe spontaneous thrombosis • reduced ovulation and fertility • cachexia and shorter survival • severe glomerulonephritis • reduced neurotoxicity • impaired skin healing • reduced corneal wound healing • spontaneous ligneous conjunctivitis • reduced macrophage and keratinocyte migration • reduced metastasis (reduced Pym-T induced mammary cancer) • reduced morbility and mortality of Lewis lung carcinoma • accelerated atheroscleoris
Plasminogen activator inhibitor-1 (PAI-1)	• reduced thrombotic incidence • no bleeding • accelerated neointima formation • reduced lung inflammation • reduced atherosclerosis • reduced cancer invasion and vascularization
Plasminogen activator inhibitor-2 (PAI-2)	normal

tivation of different components of the plasminogen activation system in mice, compiled from reviews [162–164] and original reports [111, 165–169], are summarized in table 1.

Transcriptional regulation

Of numerous types of extracellular signal, only a limited number are recognized by the cell as active signals: the cell recognizes only signals to which it has corresponding receptors. A receptor is a molecule on the cell surface or within the cell that interacts stoichiometrically with a signal and triggers a relay of information through multiple molecules in the form of structural change and/or spatial relocation to various parts of the cell. There are many types of signal transduction pathways, but the number of extracellular signals and pathway end points easily outnumber them. The complexity of cellular responses is achieved through interaction between pathways and by multi-modal states of the targets depending on cell type and the phase in the cell cycle or developmental stage. There are a number of recent review articles on the types of extracellular signals, receptors and signal transduction, and it is not within the scope of this article to consider in detail each individual pathway. Rather, we will summarize recent information about the regulation of fibrinolytic genes and attempt to sort general from specific features. Relevant review articles on signaling pathways are cited in the discussion.

Although transcription regulation is a very complex process involving hundreds of proteins and factors, very important information about potential regulation can be obtained or is inferred from the primary sequence of the gene itself. Regulatory mechanisms essential for gene expression may be conserved during evolution, suggesting concurrent conservation of the primary regulatory sequences, i.e., cis-acting elements. Therefore, we will begin our discussion by brief sequence comparisons of promoter regions of homologous genes from different species, which may reveal important regulatory mechanisms. Then, we describe regulation for which specific regions in the promoter have not yet been identified.

The uPA gene

The uPA gene has been isolated from several mammalian species [170–172]. Sequence data from 5′-flanking regions for the pig (4.6 kb), human (5.7 kb), and mouse (8.2 kb) genes are available in the EMBL data bank with accession numbers Y11872, Y11873, and Y11874, respectively. The uPA gene promoter contains a TATA box (a characteristic of regulated genes) and a GC-rich region of about 200 bases immediately upstream of the cap site (a characteristic of housekeeping genes). Several copies of GGGCGG are present in this GC-rich region. The gene also contains one copy of the CAAT sequence several hundred bases upstream of the cap site. These sites are potentially functional because they are bound by nuclear proteins, as determined by footprinting analysis of the pig promoter [173]. The GGGCGG and CAAT sequences are recognized by the ubiquitous transcription factors SP1 and CTF, respectively. Reflecting this, the uPA gene is expressed at low levels in a variety of cells. uPA gene expression is also inducible by many different signals, such as growth factors, peptide hormones, steroid hormones, UV light, and cell morphology changes [reviewed in refs 174, 175]. The most characterized regulatory region of the promoter is an enhancer located about 2 kb upstream of the cap site [176, 177]. This region is composed of an Ets/$AP1_A$ composite site, the downstream $AP1_B$ site, and the connecting 74-bp COM (cooperation mediator) region [178]. Originally, the Ets/AP1 site was termed PEA3/AP1 [179] but the present name is preferable because the group of proteins binding to the consensus ETS element belongs to the Ets family, of which one member is PEA3 [180, 181]. Several reports indicate that the proteins which actually bind to this site are the Ets1 and Ets2 transcription factors [182–185]. So far, only Ets1 and Ets2 have been shown to activate the uPA promoter by ectopic overexpression in transient transfection assays. There is a further Ets/AP1 composite site several kilobases upstream of the enhancer. In three mammalian uPA genes, all these elements are highly conserved, suggesting their importance in regulation [183].

The -2-kb enhancer was first shown to mediate the action of EGF and the tumor promoter phorbol myristate acetate (PMA, also called TPA) in keratinocytes [179]. Ets/$AP1_A$ and $AP1_B$ cooperate in a COM-dependent way [178, 186]. In isolation, however, COM showed no effect on transcription. Several proteins bind to the COM region [187] and one (UEF-3) has been purified and cloned [188]. The function of these binding proteins in uPA gene regulation still remains to be determined. When the entire uPA promoter, i.e., including both Ets/AP1 sites, was analyzed in transient transfection assays in LLC-PK_1 and NIH3T3 cells, deletion of downstream $AP1_B$ had no influence on induction involving Erk MAP kinase (D. Besser, D. D'Orazio and Y. Nagamine, unpublished data). These observations suggest cell specificity for the role of $AP1_B$ and the COM. In keeping with this possibility, the DNase I footprints of the COM regions in LLC-PK_1 and HepG2 cells were different [174].

Ets sites contain the minimum consensus GGAA sequence and are potentially recognizable by many Ets family members. As mentioned above, only Ets1 and Ets2 have been shown to activate the uPA gene by transfection assays. Accordingly, a high correlation between Ets1 and uPA expression has been reported in several cell types [189–191], and expression of antisense Ets1 oligonucleotides was shown to suppress uPA expression in endothelial cells [192, 193]. The AP1 site was originally identified as a phorbol-ester-responsive element. It is recognized by a transcription factor complex AP1 [194–196], which is either a homodimer of Jun or a heterodimer of Jun and Fos family members [196, 197]. Additionally, Jun family members heterodimerize with ATF2 and ATF3, which belong to the CRE-binding protein (CREB)/activating transcription factor (ATF) family [198, 199]. All of these families make up a large basic-leucine zipper superfamily, and the number of possible combinatorial heterodimer formations is very large [200]. Ets1 and Ets2 are phosphorylated and activated by the Ras/Erk signaling pathway [201, 202]. c-Jun is phosphorylated and activated by c-Jun amino-terminal kinase (JNK) (also known as stress-activated kinase, SAPK) [203, 204 reviewed in ref. 205]. It has been proposed that JunD is phosphorylated and activated by JNK in a c-Jun/JunD dimerization-dependent manner [206]. However, it could still be a target of Erk MAP kinase, because PKC activation, which activates Erk but not other MAP kinases, still induces phosphorylation of JunD without inducing c-Jun phosphorylation [207]. JunB was also shown to be phosphorylated by JNK [208]. The transcription factor ATF2 is phosphorylated by both JNK [209] and p38 [210]. Thus, one way or another, transcription factors binding to Ets/AP1 sites are activated by members of a MAP kinase family. As these kinases are activated by various extracellular signals, such as growth factors, cytokines, osmotic stress, and UV irradiation [211–214], the uPA promoter is potentially sensitive to a variety of signals. In fact, it is very difficult to find cells which do not respond to one of these signals by inducing the uPA gene (see below, and unpublished observation). The responsiveness of the cell and the gene to a particular signal may be governed by the presence of different sets of signaling molecules and by chromatin structures, but this aspect is not discussed in this review [215].

Ets/AP1 sites in the uPA promoter mediate signaling pathways that utilize one of the MAP kinases. We and others have characterized several signals leading to the

activation of the uPA promoter via the Ets/AP1 sites. These are PMA [179, 183, 216], okadaic acid [217], cytoskeletal reorganization [207, 216], growth factors [182, 218, 219], oncogenes [220, 221], UV [222], and TNF-α [186]. In all cases, except for UV and TNF-α induction, Erk MAP kinases seem to play an important role; UV induction was shown to be via JNK MAP kinases [222]. As the expression of c-Jun, c-Fos, and JunB is also under the control of signals like cAMP, serum, and calcium [223], the uPA gene may also be regulated indirectly by these signals. It should be noted that sequences of all three AP1 sites deviate from the consensus AP1 recognition sequence TGACTCA. This surely affects the specificity for AP1 components and the type of regulation. Furthermore, reflecting this deviation, we have shown that c-Fos acts as a negative regulator of uPA gene expression [218]. This sequence divergence and the nature of the cooperation between Ets and AP1 sites suggest that regulation of the uPA promoter through the Ets/AP1 site is very different from conventional AP1 site-dependent regulation.

NFκB is a heterodimer of Rel family transcription factors [224]. It remains inactive in the cytoplasm complexed with IκB. Phosphorylation of IκB by a specific kinase IκK triggers its proteolysis, thereby releasing NFκB which then moves into the nucleus and activates genes harboring NFκB-binding sites [reviewed in ref. 225]. Activity of IκK is induced by signals such as the inflammatory cytokines TNF-α and interleukin (IL)-1 UV irradiation, PMA, and oxidative stress [224]. The uPA gene has a functional NFκB-like sequence, GGGAAAGATC, at -1583 in the human promoter. The sequence is conserved among the human, pig, and mouse genes, and was shown to mediate PMA induction in HeLa and HepG2 cells [226]. It is not clear how important this sequence is in mediating PMA action, because its activity is only observable in the absence of the upstream sequence [226], and deletion of Ets/AP1 sites is enough to completely suppress PMA induction [183].

In LLC-PK$_1$ pig epithelial cells, the uPA gene is transcriptionally induced by the peptide hormone calcitonin or cAMP analogs [227]. Dnase-I-hypersensitive-site analysis and stable transfection assays suggest dynamic changes of the uPA gene chromatin and the presence of multiple cAMP-regulatory sites [228, 229]. The prominent cAMP-responsive enhancer is located 3.4 kb upstream of the cap site and is composed of three protein-binding domains. Two of these contain a cAMP response element (CRE)-like sequence and are bound by CREB and ATF1, and the third is recognized by kidney enriched transcription factor LFB3 [230]. For this enhancer to mediate cAMP induction, cooperation between CREB/ATF1 and LFB3 involving physical interaction is necessary [231, 232], thus ensuring tissue-specific hormonal regulation of the uPA gene in kidney. This cAMP enhancer sequence, however, is not present in the corresponding regions of the uPA promoters in other mammalian species, suggesting that this particular type of hormonal regulation evolved much later.

The tPA gene

The tPA gene has been isolated from human [233, 234], mouse [235], and rat [236, 237], and sequence information for their 5′-flanking regions is available in the EMBL data bank with the accession numbers K03021, M26065, and S73569, respectively. They are TATA-box-independent genes, and the major transcription initiation sites are conserved [238 and references therein]. In the mouse and human promoters, there are additional minor initiation sites at -23 and -110, respectively. The minor initiation site in the human promoter is TATA box dependent and was originally considered to be the main transcription initiation site [233].

Two major regulatory regions have been characterized in the tPA promoters. One is located close to the cap site, is highly homologous in all three promoters, and constitutes the minimal promoter region required for basal and inducible expression. The other region is located far upstream in the human tPA promoter, making up a unique enhancer necessary for retinoid and steroid induction of human tPA gene expression. The lengths of the tPA promoter regions isolated so far are 9.5, 4.0, and 7.7 kb for the human, mouse, and rat genes, respectively [235, 239, 240].

Two copies of closely spaced GC-boxes, which are binding sites for transcription factor Sp1, exist in the vicinity of the cap sites of all three mammalian genes. It has been shown that TATA-less promoters depend on Sp1 for the recruitment of the transcription initiation complex [241]. In deletion analysis of the tPA promoter in various cell lines, these GC boxes were shown to play an important role both for basal and induced transcription [242–245]. Besides Sp1, a novel GC-box-binding protein has been suggested as responsible for brain-specific tPA mRNA expression [246].

Upstream of the GC boxes, all three mammalian tPA promoters contain a CCAAT-like element known to bind the NF1/CTF transcription factor. The significance of this NF1-binding site seems to be species specific. In the rat tPA promoter, the NF1-like sequence CTGGCGTCAAGCCAA at position -145 to -158 is bound in vitro by NF1, and its deletion impairs follicle-stimulating hormone (FSH)-induced transcription in rat granulosa cells and basal expression in neuroblastoma cells [244]. In the mouse promoter, the NF1-like sequence at -162 to -172 can bind NF1, but deletion experiments in various tissues revealed that the site is not functional [246]. Finally, in vivo genomic footprinting analysis using HUVE and HeLa cells also

revealed the occupancy of a CTF/NF1 binding site at position −202 to −187 with respect to the major initiation site in the human tPA promoter. Specific antibodies identified CTF/NF1 as the factor binding to this site in both cell types. These results suggest that the site is functional in the human promoter. However, mutational analysis and transfection experiments are needed to establish the relevance of this element in the human promoter [245].

Upstream of the NF1-binding sites, a CRE-like sequence is found in all three promoters. The CRE sequence in the rat tPA promoter is a perfect consensus CRE sequence TGACGTCA, while in both human and mouse promoters this element differs in one central nucleotide, TGACATCA, changing it to a sequence resembling an AP1-binding sequence TGACTCA [236]. Corresponding to this difference, rat tPA is inducible in granulosa cells by gonadotropic hormones such as FSH and luteinizing hormone, which augment intracellular cAMP levels via the consensus CRE site located at position −184 to −178 [244]. This cAMP-responsive element binds CRE-binding protein from both granulosa cells and neuroblastoma B103 cells in vitro. Both the CRE site and NF1 site contribute to the FSH responsiveness of the rat tPA gene in granulosa cells, whereas only the NF1 site is important for constitutive tPA expression in B103 cells. In contrast, the human and mouse tPA genes are unresponsive to these signals [247]. Instead, the human uPA gene is induced by FSH in mouse granulosa cells by FSH via an AP2-like site located at −72 and −29 [248]. These results indicate functional interchangeability between uPA and tPA in certain situations.

As expected, functional analysis of the human tPA promoter revealed that the AP1-like sequence (at −222 to −214) and the GC box (at −50 to −36) are important for both constitutive and PMA-induced transcription in HeLa [243, 249] and HUVEC cells [245]. In the mouse tPA promoter, the AP1-like sequence located at −175 to −168 and the two GC boxes mentioned above have been shown to be important for the retinoic acid (RA)/cAMP-mediated expression of tPA in F9 teratocarcinoma cells [235]. The GC-binding factor required for tPA induction during F9 cell differentiation was shown to be immunologically related to Sp1 [242].

Interestingly, differential binding of CREB1 and ATF2 to the human AP1-like element appears to correlate with the differential regulation of tPA by phorbol esters in HT1080 and HeLa cells. PMA-mediated suppression of tPA expression in HT1080 cells involves a decrease in tPA transcription, with CREB1 as the major nuclear protein interacting with the tPA-CRE. In HeLa cells, ATF2 was the most active tPA-CRE-binding protein detected in both uninduced and PMA-induced cells. Since CREB1 can repress PMA-induced transcription of other target genes (including c-*jun*) [250], it was suggested that the mechanism for the transcriptional downregulation of tPA by PMA in HT1080 cells requires CREB1 binding to the tPA-CRE. ATF2, by associating with the same site, plays a role in PMA-mediated induction of tPA in HeLa cells.

In addition to the functional importance of the transcription factors CREB, NF1, and Sp1 binding to the tPA proximal promoter for the multi-hormonal regulation of the tPA gene in rat granulosa cells, a novel TAAT-containing promoter element has been recently identified [240]. This element, located at position −172 between the CRE and NF1 sites, is important for both constitutive and cAMP-induced expression of the rat tPA gene and for binding of a novel nuclear factor termed tPA promoter factor-1 (tPF-1). An 11-nucleotide sequence containing the TAAT motif is 100% conserved between rat, mouse, and human promoters and is important for the binding of tPF-1, indicating that this factor plays a role in tPA gene expression in all three species. TAAT motifs are often found in DNA regions interacting with homeodomain-containing transcription factors [251–253]. Thus, it is tempting to suggest that tPF-1 belongs to the homeodomain family of transcription factors which might be involved in tPA regulation during development.

Steroid hormones such as glucocorticoids and androgens, and retinoids such as vitamin A and RA have been shown to increase tPA synthesis in vivo and in vitro [254–259]. Some of these effects could be reproduced in HT1080 human fibrosarcoma cells, where dexamethasone and RA were able to induce tPA gene expression. Moreover, both agents induced tPA mRNA transcription in a cooperative manner. Studies by Bulens et al. [260] have identified a multi-hormonal responsive region located between −7.1 and −8.0 kb. This 0.9-kb DNA fragment acts as an enhancer which is activated by glucocorticoids, progesterone, mineralcorticoids, and androgens (but not estrogens) and by RA [260].

A functional RA response element (RARE) consisting of a direct repeat of the GGGTCA motif spaced by five nucleotides (tPA/DR5) was localized inside the multi-hormone responsive enhancer at −7319 bp (with respect to the major initiation site). The tPA/DR5 element interacted with the heterodimer composed of RA receptor a and retinoic X receptor in vitro and mediated regulation of tPA by RA in human fibrosarcoma, endothelial, and neuroblastoma cells [239].

The multi-hormone responsive enhancer in the far-upstream tPA promoter also contains a glucocorticoid responsive unit (GRU) with four functional binding sites for the glucocorticoid receptor (GR), located between −7501 and −7974 [260]. Site-specific mutagenesis of the four glucocorticoid response elements (GREs) eliminated

dexamethasone-mediated induction of the tPA multi-hormone-responsive enhancer. Therefore, the human tPA gene is a direct target for glucocorticoids, albeit through an unusually complex GRU composed of multiple binding sites for GR.

The 0.9-kb multi-hormone responsive enhancer of the human tPA gene was found to confer a cooperative induction by dexamethasone and RA to both its homologous and a heterologous promoter, irrespective of orientation. Interestingly, deletion of the intervening sequence between the enhancer and the proximal promoter did not affect tPA transcriptional induction by dexamethasone or by RA. Moreover, the synergistic effect of dexamethasone and RA increased with decreasing distance between the enhancer and the tPA promoter elements. Interaction between distal regulatory loci and the basic transcription complex requires looping, which depends on protein/protein interaction between transcription factors bound to the enhancer and to the proximal promoter [261]. The fact that the intervening sequence between the tPA enhancer and promoter can be deleted without reducing response to dexamethasone and RA is suggestive of such a mechanism. Transcription factor Sp1 has been shown to mediate looping of DNA [262, 263]. Sp1-binding sites are present in the tPA proximal promoter and also in the tPA multi-hormone enhancer. Thus, it is tempting to speculate that interaction between distally and proximally bound Sp1 molecules is involved in regulation of human tPA gene expression by hormones. It is conceivable that binding of the GR to the tPA/GREs facilitates binding of RAR/RXR receptors to the tPA/DR5 element and/or vice versa. Alternatively, simultaneous binding of GR and RAR/RXR receptors might facilitate binding of coregulators involved in the hormonal response of the enhancer by opening the chromatin structure more efficiently than either the GR or the RAR/RXR would do separately. Although this hypothesis needs to be tested, it would explain the synergistic interaction between the two pathways on the tPA 0.9-kb enhancer.

The PAI-1 gene

PAI-1 expression has been observed in various cell types, and multiple regulatory factors have been identified that play a role in PAI-1 transcription. Many different growth factors (TGF-β, EGF, platelet-derived growth factor, bFGF), inflammatory cytokines (IL-1, TNF-α) and hormones (corticosteroids, insulin) induce synthesis of PAI-1. Multiple pathways are involved in this regulation but the mechanism by which these factors alter PAI-1 expression is not yet clearly understood.

The human [264, 265], rat [266], and mouse [267] PAI-1 genes have been isolated and their promoter sequences characterized. The mouse gene was initially identified as the c-Myc-regulated gene *mr1* [267]. Comparison of 5′-flanking regions revealed two highly conserved elements with $>80\%$ identity in the proximal promoter (at -90 to -25) and in a distal sequence (at -753 to -512). The common features detected in the 5′-flanking regions of the PAI-1 genes of these three species are a consensus TATA box and sequences closely related to PEA3, AP1, CTF/NF-1, and Sp1 recognition sites [268–270].

The 5′-flanking region of the human PAI-1 gene contains four putative AP1-binding sites: -58 to -50 (TGAGTTCA), -79 to -72 (TGAGTGG), -662 to -656 (TGTATCA) and -721 to -714 (TGACACA), although none is identical to the consensus AP1 site [TGA(G/C)TCA] to which c-Fos/c-Jun heterodimers preferentially bind. The second and third of the above sites are not conserved in other species [270]. The proximal two sites were shown to be essential for basal as well as PMA-mediated induction of PAI-1 gene expression in HT1080, HeLa and Hep3B [269] and HepG2 cells [271]. The main protein complex binding to the site -58 to -50 is the c-Jun homodimer, whose binding is enhanced dramatically by PMA treatment [271]. The c-Fos/c-Jun heterodimer interacts poorly with this site, and c-Jun preferentially forms a complex with c-Fos [196]. Thus, there arises the interesting possibility that c-Fos induction, e.g., by a cAMP signal affecting the ratio of intracellular concentrations of c-Jun homodimers over that of c-Fos/c-Jun heterodimers, leads to downregulation of PAI-1 expression [272]. PMA treatment also enhanced binding to the site -79 to -72 of unknown nuclear proteins not related to c-Jun or c-Fos [271]. Sp1 and AP2 proteins and a further 72-kDa unknown component have been found to bind to the site -79 to -62 (TGAGTGGGTGGGGCTGGA) [269]. Studies in HeLa cells revealed that a further transcription factor, helicase-like transcription factor, is involved in basal expression and binds to an AP1-like transcription site (-79 to -72) [273]. Its rat homologue P113 has also been identified as a PAI-1-binding transcription factor [274]. These two factors belong to the SWI/SNF family of proteins which have DNA-dependent helicase/ATPase activities and are involved in functions such as transcription regulation, DNA repair, and DNA recombination [275]. Enhancement of transcription by members of this family is connected with disruption of chromatin structure.

An extracellular signal of particular interest is TGF-β, which induces PAI-1 expression markedly [276, 277]. This growth factor is released from activated platelets and leukocytes at sites of inflammation and thrombosis [278] and influences ECM formation. This potent activation of the PAI-1 gene by TGF-β is the basis of a luciferase-expressing reporter assay for the sensitive and specific quantification of mature TGF-β [279].

It has been shown that the 5′-flanking region of the human PAI-1 gene contains a major TGF-β-responsive element between positions -804 and -546 and a minor element between positions -328 and -186 upstream of the cap site [280]. Activation through the major responsive element involves the two AP1-like binding sites [281]. However, transactivator molecules binding to these elements have not been identified. Riccio et al. [282] showed that the promoter region -598 to -532, which contains abutting sequences with high homology to consensus binding sites for the CCAAT-binding transcription factor nuclear factor I (CTF/NF-I) and the ubiquitous factor (USF), is responsive to TGF-β induction in the natural promoter context and in isolation in HepG3 and NIH3T3 cells. Although these sequences are important for TGF-β induction, it remains to be determined whether they actually mediate TGF-β induction. Nuclear proteins prepared from non-treated or TGF-β pretreated cells bound to this region. In the rat PAI-1 promoter, the corresponding CTF/NFI site is thought to be responsible for basal expression in HTC rat hepatoma cells [270]. In TGF-β-treated HepG2 cells, an unknown nuclear protein with a molecular mass of approximately 100 kDa has been shown to bind to the site -726 to -707 [283]. TGF-β was shown rcently to activate the PAI-1 gene in HepG2 and MvlLu cells via three TGF-β-responsive elements [AG(C/A)CAGACA], termed 'CAGA boxes,' located at -730, -580, and -280 [284]. The protein binding to these sites is the Smad3/Smad4 heterodimer whose complex formation is induced by TGF-β. A further report demonstrates that a 12-bp element (AGACAAGGTTGT) at -732 to -721, termed 'TGF-β-responsive sequence (TRS),' partially overlaps a CAGA box and is required for mediating strong transcriptional activation by TGF-β [285]. Mutations of the AGAC sequence within this element, which exists in all previously described CAGA boxes, abolished Smad3/Smad4 binding and TGF-β induction. Interestingly, TRS mutants in which the AGAC sequence remained intact also failed to mediate TGF-β induction and to compete with the wild-type TRS for Smad3/Smad4 binding. This suggests an important role for TRS sequences other than AGAC in TGF-β induction [285]. TGF-β is known to induce association of c-Jun with Smad3, which complements Smad3-Smad4 and c-Jun-c-Fos interactions and increases the rate of transcription [286]. The presence of an AP1 site close to the TRS (or the most distal CAGA box) in the human PAI-1 promoter suggests a potential transcription synergy between the two sites. This AP1-binding site has already been implicated in TGF-β-induced transcription of the PAI-1 gene [282]. Recently, a further transcription factor μE3 (TEF3) was identified and shown to activate TGF-β-induced PAI-1 gene transcription by binding to the E box sequence CACGTG at -523 to -528, 3′ to the middle CAGA box, and synergizing with the nearby Smad3/Smad4 complex [287]. Furthermore, interaction between the transcriptional adaptor p300 or CBP with Smad3 is induced by TGF-β treatment, suggesting that p300 (or CBP) acts as a coactivator in bridging the Smad3/Smad4 complex and the general transcriptional machinery [288].

An increased intracellular cAMP concentration decreases the levels of PAI-1 expression in a variety of cell lines [289–292]. cAMP inhibits PMA-induced transcription via the most proximal AP1 element of the PAI-1 promoter [293], to which c-Jun homodimers bind [271]. Although cAMP is known to induce AP1 activity, it is through the induction of c-Fos [294] and not c-Jun. In this respect, PAI-1 expression may be reduced by cAMP signals, since they decrease the formation of c-Jun homodimers in favor of c-Jun/c-Fos heterodimers.

Glucocorticoids are potent inducers of PAI-1 expression in a variety of cells and tissues [255, 268, 295, 296; reviewed in refs 297, 298]. Two regions of glucocorticoid induction have been reported in the human PAI-1 promoter between positions -100 and $+75$, and -800 and -549 [299]. However, these two sequences have little homology with the GRE consensus sequence, which suggests an indirect mechanism of activation. In the rat PAI-1 promoter, a consensus GRE has been identified which binds GR at position -1212 to -1196 [300].

Various studies have indicated a strong correlation between non-insulin-dependent diabetes mellitus and cardiovascular diseases for which high blood levels of PAI-1 may be responsible [157, 301, 302]. The increased production of PAI-1 seen in diabetic disease has been attributed directly to high glucose levels in the blood [303]. Glucose regulates PAI-1 gene expression through two Sp1 sites located between -85 and -42 of the PAI-1 promoter in vascular smooth muscle cells [304]. It is suggested that hyperglycemic conditions dissociate unidentified repressor molecules from Sp1 complexes on the promoter. The involvement of Sp1 in glucose-mediated induction has been reported for the acetyl-CoA carboxylase gene in adipocytes, but in this case induction is through enhancement of the DNA-binding activity of Sp1 by its dephosphorylation [305].

Malignant and invasive tumors often express high levels of uPA together with PAI-1 proteins [306, 307]. There is evidence that the p53 tumor suppressor, whose level is altered in many cancers, directly influences PAI-1 expression. Overexpression of p53 leads to the induction of the human PAI-1 gene through a p53-binding site situated at -159 to -134 on the PAI-1 promoter [308]. However, this region is not conserved in other species.

An allele-specific increase in basal transcription of the PAI-1 gene has also been characterized [309]. One polymorphism of the PAI-1 gene, a single nucleotide insertion/deletion (4G/5G), is situated in the promoter region [310]. The 4G allele of this polymorphism has been associated with higher plasma PAI-1 activity. Transcriptional studies of this promoter region revealed that both alleles bind a transcription activator (at position −683 to −676), whereas the 5G allele also binds a repressor protein to an overlapping binding site (at position −672 to −676). None of the known PAI-1-binding transcription factors were associated with this response.

It has been reported that the PAI-1 mRNA level in quiescent cells increases rapidly following serum stimulation and decreases prior to the DNA synthesis phase of the cell cycle [311]. The expression of several genes whose products are needed for the initiation and completion of DNA synthesis are regulated by E2F1 [312, 313]. Studies in our laboratory have shown that overexpression of E2F1 suppresses PAI-1 gene expression. DNA-binding and transactivation domains of E2F1 are important for this effect, but the retinoblastoma tumor suppressor gene Rb was not involved in this downregulation. The inhibitory effect is specific in the sense that E2F1 inhibits the PAI-1 promoter but not other promoters. However, cis-acting elements mediating this response have not yet been identified (M. Koziczak, unpublished data).

Endotoxin and LPS, a component of the cell wall of Gram-negative bacteria, also cause increased synthesis of PAI-1 in endothelial liver cells [314, 315]. This effect of LPS is mediated in part by TNF-α and IL-1, which are inflammatory response cytokines [316, 317]. However, TNF-α and IL-1-responsive cis-acting elements and their respective trans-acting factors required for the induction of PAI-1 have not yet been identified.

Other growth factors reported to stimulate PAI-1 biosynthesis include EGF [318], heparin-binding EGF-like growth factor (HB-EGF) [319], vascular endothelial growth factor (VEGF) [320, 321], and bFGF [322]. The molecular mechanisms by which these factors exert their effect on PAI-1 gene expression have not been described. c-Jun probably plays a key role, but cis-acting elements and transactivators required for promoter induction by these growth factors have not yet been characterized.

The PAI-2 gene

The involvement of PAI-2 in multiple physiological and pathological processes suggests the existence of multiple mechanisms controlling PAI-2 gene expression. PAI-2 gene expression can be modulated at both the transcriptional and post-transcriptional levels by agents such as growth factors (TGF-β, EGF, macrophage-colony-stimulating factor, and granulocyte-macrophage-colony-stimulating factor), hormones (RA, dexamethasone, and vitamin D3), cytokines (TNF-α, IL-1, and IL-2), vasoactive peptides (angiotensin II), toxins (dioxin and endotoxin), and tumor promoters (phorbol esters and okadaic acid) [82].

The complete PAI-2 gene has only been isolated from the human genome [323]. Sequence information of 2 kb of the 5′-flanking region has been reported [324] and is available in the EMBL data bank under the accession number M22469. The human PAI-2 gene utilizes a TATA-dependent start site to initiate transcription. Three major transcription regulatory domains have been defined in the 5′-flanking region. One domain in the proximity of the PAI-2 mRNA start site contains both a positive regulatory region and a repressor region. A second domain located upstream between −1100 to −1800 bp contains a negative regulatory region or silencer. The third domain located further upstream between −3300 to −5100 contains a second positive regulatory region, which appears to overcome inhibition of the silencer in a cell-specific manner.

There are several potential protein-binding sites in the region between −215 and −91, as revealed by DNase I footprinting analysis, including two AP1-like elements, AP1a (TGAATCA; position −103 to −97) and AP1b (TGAGTAA; position −114 to −108), and one CRE-like element (TGACCTCA; position −187 to −182) [241]. Site-directed mutagenesis demonstrated the requirement of the AP1a and CRE-like sites for both basal and PMA-induced PAI-2 transcription [241]. The CRE-like site might be functionally closer to an AP1 element. It seems that c-Jun and JunD are the major components binding to the AP1a element under both basal and PMA-treated conditions [151].

Basal and PMA-induced transcription of the PAI-2 gene promoter in HT1080 and U937 cells was significantly greater with a −219-bp than with a −1100-bp promoter construct, suggesting the presence of a repressor site between −219 and −1100 [151].

The PAI-2 gene is the most TNF-α responsive gene identified so far in several cell types. The first 219 bp of the proximal promoter region contains the positive elements required for TNF-α inducibility, as assessed by transfection of promoter-deletion mutants into HT1080 cells; however, the proximal PAI-2 promoter is TNF-α responsive only upon removal of repressor regions located upstream of −219 [325]. Two repressor regions have been identified: a distal one between positions −1859 and −1100, and a proximal one between positions −259 and −219. That removal of both repressor regions results in a selective increase in TNF-α responsiveness suggests that the induction of PAI-2 gene transcription by TNF-α is associated with derepression

[325], the mechanism for which is currently unknown. Different signaling pathways can be activated by TNF-α to induce gene expression, including MAP kinase pathways such as Erk, JNK, and p38, and the NFκB pathway [326–328]. The proximal promoter region is devoid of conventional NFκB-binding sites but contains two functional AP1-like elements. Therefore, it is most likely that one of the MAP kinases is involved in TNF-α induction (see the section on the uPA gene). It would be very interesting to know how AP1 activity is regulated by a protein binding to the repressor region.

A RARE was identified at position -1659. This element is responsible for RA potentiation of the PMA-mediated induction of PAI-2 in human myeloid leukemia cells [329]. The PAI-2 RARE is composed of a GRE half-site separated by seven bases from a RARE half-site. Nuclear receptors binding to this site and mediating the RA effect have not been defined.

A distal repressor region located between -1859 and -1100 was suggested to be essential for basal and PMA- and okadaic-acid-induced PAI-2 transcription in HT1080 and U937 cells [325]. Deletion analysis further showed that the PAI-2 silencer activity was associated with a 303-bp region from -1977 to -1675. Addition of this DNA region to a transcriptionally active heterologous promoter suppressed transcription in a position- and orientation-independent fashion (properties characteristic of a transcriptional silencer) but not in a cell-specific manner. The motif responsible for silencer activity was located in a 28-bp DNA sequence containing a 12-bp palindrome centered at an *Xba*I restriction site at position -1832, CTCTCTAGAGAG. This motif was termed the PAI-2 upstream silencer element (PAUSE-1) and was shown to bind a specific PAUSE-1 binding factor, as determined by mobility shift analysis [330].

The uPAR gene

The uPAR genes have been cloned from human [331] and mouse [332], and sequence information is in the EMBL data bank under the accession numbers s78532 and u12235, respectively. The determined sequences in the 5′-flanking regions were 1.5 and 0.9 kb, respectively. Only the first 140 bp from the transcription start sites exhibit homology. One of the two genes may have a repetitive sequence insert. Within this first 140 bp, a common feature of the uPAR genes is the absence of TATA and CAAT boxes and the presence of a short GC-rich region, which is recognized by either SP1 or one member of the EGR family [333, 334]. Both genes contain a canonical AP1 site but not at equivalent positions: mouse at -72, downstream of the GC-rich region, and human at -184, upstream of the GC-rich region. The human promoter has a further imperfect AP1 sequence, TGACTCG, at -69.

The uPAR gene is induced by various signals [335–339]. Lengyel et al. [340] characterized the first 180 bp of the human uPAR gene promoter in colon cancer cells and showed that the AP1 site at -184 plays an important role in both basal expression in a high-uPAR-expressing cell line and PMA induction in a low-expressing cell line. As partial deletion of the GC-rich region downstream of the AP1 site significantly reduced basal template activity in a high-expressing cell line, there may be cooperation between AP1 and SP1 [341]. It has been reported recently that a cis-acting element between -152 and -135 is important for high basal and PMA-mediated uPAR induction in colon cancer cells. The region contains overlapping binding motifs for AP2 and SP1/3, and the protein which binds to this region and is important for activity is an AP2α-related factor [342]. Both Erk and JNK MAP kinases seem to be involved in the full induction of uPAR by PMA [343].

The uPAR gene is also induced by TGF-β [335], TNF-α [337], cAMP [338] and HIV-1 infection [339]. However, in none of these cases have cis-acting elements and trans-acting factors been determined.

Post-transcriptional regulation

In yeast, a major mRNA decay pathway is initiated by deadenylation at the 3′ end that triggers decapping at the 5′ end and 5′ to 3′ exonucleolysis of the transcript [344, 345]. Shortening of the poly(A) tail to 10–15 residues abolishes binding of the poly(A)-binding protein (PABP), whose function is to inhibit decapping [346]. The decapping enzyme, Dcp1, which recognizes the mRNA substrate by interaction with both the cap and the RNA moiety [347], is required for normal decay of both unstable and stable yeast mRNAs [348]. The product of decapping is degraded by the cytoplasmic 5′ to 3′ exoribonuclease Xrn 1p [344]. A second pathway brings about 3′ to 5′ degradation of the mRNA. An exosome is likely to be responsible for the exonucleolytic activity, with Ski6p/Rrp41p and Rrp4p as components, while Ski2p, Ski3p, and Ski8p modulate exosome activity. Interestingly, both 3′ to 5′ and 5′ to 3′ decay pathways are required for cell viability [349, 350].

Even though poly(A) shortening seems to be the initial step in the degradation of most polyadenylated mRNAs in mammalian cells [351], the degradation pathway has not been elucidated [342, 352]. This process is probably catalyzed in vivo by a mammalian poly(A)-specific 3′-exoribonuclease, identified by Korner and Wahle [353]. Recently Couttet et al. [354] provided evidence that decapping is also preceded by poly(A) shortening in mammalian cells. mRNAs lacking the cap are less stable than their capped counterparts in cell extracts, and enzymes that catalyze the decapping and 5′ to 3′ exonu-

cleolysis have been identified [345, 352]. Recently, a mouse homologue (mXrn 1p) of yeast Xrn 1p has been cloned, which exhibited a novel preference for G4-RNA-tetraplex-containing substrates [355]. In addition, Somoskeoy et al. [356] described a 5′ to 3′ exoribonuclease from rabbit reticulocytes that degraded both capped and uncapped RNAs. An interaction between the 3′ poly(A) tail and the 5′ untranslated region (5′UTR) of the transcript, involving the translation initiation factor eIF4 A, has been reported in yeast and mammalian cells [357–359]. It was suggested that such an interaction selects transcripts with an intact poly(A) tail and cap as templates for translation and may protect them from degradation.

The variation in the decay rates of different mRNAs is determined by cis elements in the transcript. These include sequences in the 3′ untranslated region (3′UTR) like AU-rich elements (AREs) or the iron-responsive element (IRE), those within the coding region of fos, myc, and tubulin mRNA, and a region in the 5′UTR [360]. The ARE is usually present as multiple repeats in many mRNAs with short half-lives encoding cytokines, lymphokines, and proto-oncogenes [361, 362]. The minimum consensus sequence in an ARE is UUAUUUAUU [363] or UUAUUU(A/U)(A/U) [364]. The cytoplasmic proteins reported to bind to AREs include AU-A [365], AU-B [366, 367], the ELAV family of RNA-binding proteins [368], hnRNP A1 [369], hnRNP C [369], AUF1 [370, 371], and tristetraprolin [372]. The binding of these proteins to the ARE is correlated with mRNA decay. Only for HuR, an ELAV protein, was it shown that overexpression suppresses ARE-mediated degradation of c-fos mRNA in vivo [373]. In these reports, it is assumed that extracellular signals regulate the mRNA half-life by modulating the binding of these proteins to the ARE. For example, HuR was shown to be involved in stabilization of VEGF mRNA under hypoxic conditions [374]. Another example is the destabilization of IL-3 mRNA through the ARE by the immunosuppressors cyclosporin A, FK-506 [375], and rapamycin [376] in the mast tumor cell line in which the IL-3 mRNA has a longer half-life than normal. The IREs present in transferrin receptor mRNA confer a longer half-life on the transcript binding to an iron-regulatory protein when the intracellular iron concentration is low. This dynamic regulation reflects the physiological requirement for the end product [377, 378]. One of the two coding-region determinants of the c-fos mRNA half-life is 320 nucleotides near the center of the transcript; this RNA structure confers instability to the c-fos mRNA [379]. In myc mRNA, the region coding for the last 60 amino acids influences mRNA half-life when translation is inhibited [380]. In the case of β-tubulin, the stability of its mRNA is inversely correlated with the intracellular concentration of β-tubulin monomers and regulated through an autoregulatory mechanism involving the amino-terminal β-tubulin tetrapeptide [381, 382].

uPA mRNA

In LLC-PK_1 pig kidney epithelial cells, uPA mRNA is inducible by cAMP or PMA treatment and has a short half-life of 70 min, but becomes very stable upon inhibition of protein synthesis by cycloheximide, puromycin, or pactamycin [383]. This phenomenon was further analyzed in a cell-free decay reaction system based on post-mitochondrial supernatant [384]. In this system, we found that the effect of cycloheximide on uPA mRNA stability was delayed compared with the effect on protein synthesis, suggesting that a labile protein is involved in uPA mRNA metabolism. Extracts from control cells did not stabilize uPA mRNA from cAMP/cycloheximide-treated cells, arguing that the labile factor is not a soluble protein but rather is associated with polysomes [384]. The stabilization of uPA mRNA by cycloheximide or anisomycin was also reported in a metastatic rat adenocarcinoma, BCI [385]. mRNA stabilization upon protein synthesis inhibition has been observed for almost all unstable mRNAs, including PAI-1 and PAI-2 mRNAs (see below). However, in no case has the putative labile protein been identified. In LLC-PK_1 cells, the uPA mRNA half-life is also prolonged by PKC downregulation and calcium ions [386, 387].

Studying the molecular mechanism underlying rapid turnover of uPA mRNA, we first focused on the 3′UTR, because this relatively long region (900 bases) is highly conserved between rat, mouse, cow, pig, and human [174]. Insertion of the entire 3′UTR of uPA mRNA immediately upstream of the poly(A) addition signal sequence rendered otherwise stable globin mRNA as unstable as uPA mRNA, suggesting that almost all information governing rapid uPA mRNA turnover resides in the 3′UTR [388]. Three regions in the 3′UTR were found to contribute independently to the rapid turnover of mRNA. These regions include a sequence with a stem structure, a region that requires ongoing transcription to destabilize the transcript, and an ARE that is responsible for the PKC-downregulation-induced uPA mRNA stabilization [388].

In a metastatic human breast cancer cell line, MDA-MB-231, the uPA mRNA is stable with a half-life of 17 h. Interestingly, the ARE-mediated degradation of the uPA transcript is impaired. We identified a cytoplasmic protein of 40 kDa (p40) which specifically interacts with the ARE detected by UV cross-linking experiments. p40 binding activity was immunodepleted with a specific antibody against heterogeneous nuclear ribonucleoprotein C (hnRNP C). We observed that the levels of p40 binding were higher in MDA-MB-231 and PKC-

downregulated LLC-PK$_1$ extracts than in untreated LLC-PK$_1$ cells, and that binding of p40 to the ARE was drastically reduced by treatment of the cytoplasmic extract with protein alkaline phosphatase [389]. A positive correlation between ARE-dependent mRNA stabilization in vivo and binding of hnRNP C (or closely related protein) to the ARE in vitro suggested that hnRNP C protects mRNA from ARE-mediated degradation and that uPA mRNA stability is regulated by a phosphorylation/dephosphorylation event [389].

In colon cancer HCT116 cells, Wang et al. [390] showed that PMA and cycloheximide increase uPA mRNA accumulation by acting at the post-transciptional level, while amiloride strongly reduces accumulation by acting both transcriptionally and post-transcriptionally. Together with its inhibitory activity against uPA catalytic activity [391], amiloride may be a powerful tool to modulate overall uPA expression. It would be interesting to see whether its action on mRNA stability is specific for uPA mRNA.

Henderson et al. [392] observed enhanced stability of uPA mRNA in metastatic rat mammary tumor cell lines compared with non-metastatic lines. The high stability of uPA mRNA in one of these cell lines, MAT 13762, diminished upon dexamethasone treatment [393]. uPA expression was also modulated at the decay level in the transformed human keratinocyte cell line SCC-12F, where the mRNA was stabilized by TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) [394].

tPA mRNA

Levels of tPA protein in developing oocytes are determined by translational silencing or activation of tPA mRNA, which is controlled by the poly(A) tail length, at least in rat [395]. In primary growing rat oocytes, tPA mRNA is synthesized with a long poly(A) tail characteristic of nuclear transcripts, undergoes deadenylation to $\sim 40-60$ As and is stored stable and translationally silent until oocyte maturation [396]. Upon meiotic maturation, the tPA mRNA poly(A) tail is elongated and the transcript becomes translationally active. After being translated, tPA mRNA is degraded and is not detected in fertilized eggs [397]. The deadenylation and the silencing are regulated by an UA-rich region in the transcript 3′UTR, termed the adenylation control element (ACE); readenylation and translation require, in addition, the canonical AAUAAA adenylation signal [396]. By injection of oligonucleotides antisense to different 3′UTR sequences, Stutz et al. [398] observed that both AAUAAA and ACE are protected from hybridization by being in a masked configuration until the resumption of meiosis. Recently, Stutz et al. [399] demonstrated that the mRNA silencing is due to the binding of an 80-kDa protein to ACEs in primary oocytes. The masking of ACEs by this protein decreases when meiosis reassumes, before translation starts. Sequences similar to ACEs are present both in mouse and human messages at the corresponding sites, suggesting that the observed regulation occurs in other species.

PAI-1 mRNA

All the PAI 1 mRNAs cloned in mammals have long 3′UTRs (~ 2.7 kb) containing AREs. The human, mouse, and pig transcripts contain four repeats of the AUUUA pentamer, while the rat, mink, and bovine mRNAs contain three, two, and six copies, respectively. In humans and higher primates, two species of PAI-1 mRNA are produced as a result of alternative cleavage and polyadenylation of a single pre-mRNA and, therefore, their 3′UTRs differ in the length [264, 400, 401]. The longer mature species is 3.2 kb and contains an ARE followed by a single copy of the AUUUA pentamer, which is absent in the 2.2- to 2.4-kb form. The difference in the 3′UTR sequences causes a distinct post-transcriptional modulation of the two species. The 3.2-kb form has a shorter half-life than the 2.4-kb form (51–56 min vs 2.5–2.8 h) [280, 402].

Several growth factors have been reported to increase the stability of PAI-1 mRNA. In the human hepatoma cell line HepG2, TGF-β increases the half-life of the 3.2-kb form, but not the 2.4-kb form, twofold [280]. Elevated plasma levels of PAI-1 were seen in patients with hyperinsulinemia [403], and both PAI-1 mRNA and protein increased after stimulation with insulin or insulin-like growth factor-1 (IGF-1) at concentrations found in obese and diabetic patients [404]. Interestingly, insulin and IGF-1 do not affect the rate of transcription but increase the levels of PAI-1 by stabilizing its mRNAs. While the 2.4-kb form is stabilized only by IGF-1, the 3.2-kb form is stabilized by both insulin and IGF-1 and also by cycloheximide [405].

In rat hepatoma cells, 8-bromo-cAMP reduces the levels of PAI-1 mRNA, mainly through post-transcriptional regulation [406]. Analysis using chimeric β-globin constructs showed that the 3′UTR sequence is sufficient to confer cyclic nucleotide responsiveness to the otherwise stable globin mRNA. At least two regions in the 3′UTR of PAI-1 mRNA are involved in the modulation of mRNA decay; one of these is located in the most 3′ 134 nucleotides and contains both U-rich and A-rich elements [406]. A 38-kDa cytoplasmic protein interacts with the U-rich element in the cyclic nucleotide responsive region, while cytoplasmic 50-, 61-, and 76-kDa proteins and a multi-protein complex interact with the A-rich element. Mutation of the A-rich element prevents both its interaction with the cytoplasmic proteins and the cyclic-nucleotide-regulated destabilization of chimeric globin-PAI-1 transcripts in HTC cells. This suggests that the identified proteins play a role in the cyclic nucleotide regulation of PAI-1 mRNA stability [407].

Interestingly, decay of PAI-1 mRNA seems to require ongoing RNA synthesis, as shown for PMA-induced messages in HepG2 cells [402], cAMP-induced messages in rat hepatoma cells [406], and PMA-induced messages in mink lung CCl64 cells [408].

PAI-2 mRNA

Maurer et al. [409] showed that PAI-2 mRNA in HT1080 human fibrosarcoma cells is induced by cotreatment with PMA and TNF-α and involves both transcriptional and post-transcriptional regulation. An insertion experiment showed that the 368-nucleotide sequence preceding the poly(A) tail and containing the consensus nanomeric motif UUAUUUAUU makes otherwise stable β-globin mRNA unstable and that cotreatment with PMA and TNF-α stabilizes this unstable chimeric mRNA. In a chimeric globin mRNA containing the entire 3′UTR of PAI-2 mRNA, mutagenesis of the nanomeric motif abolished the destabilizing effect of PAI-2 3′UTR [409]. These results suggest that the nanomeric motif plays an essential role in regulating PAI-2 mRNA stability, but it has not been determined whether this regulation is important for PAI-2 regulation in other species, since the PAI-2 mRNA sequence is known only for humans.

uPAR mRNA

uPAR mRNA levels are increased by cycloheximide and proinflammatory agents, like PMA, LPS and TGF-β, in human mesothelioma cells, and this induction is, at least partly, due to increased stability of the message. A 50-kDa cytoplasmic protein has been identified that specifically interacts with a region of 51 bases in the protein-coding region. Binding of the protein to this sequence, as revealed by UV cross-linking followed by gel shift assays, was markedly reduced when the cytoplasmic fractions were prepared from cells pretreated with these uPAR mRNA inducers. Interestingly, insertion of the 51 bases rendered otherwise stable β-globin mRNA very unstable and, furthermore, treatment of cells with PMA stabilized this chimeric mRNA. These results suggest that the 50-kDa protein is positively engaged in uPAR mRNA degradation by interacting with the 51-base region, and that uPAR inducers inactivate this protein by an unknown mechanism [410]. Stabilization of uPAR mRNA by PMA and TGF-β has also been reported in human lung carcinoma A549 cells, but the underlying molecular mechanism has not been reported [411].

In Jurkat human T cells, uPAR mRNA is induced by activation of β_2 integrin lymphocyte-function-associated antigen-1 (LFA-1). The induction is post-transcriptional and involves the ARE in the 3′UTR, which contains the classical nanomeric UUAUUUAUU motif [412]. LFA-1 activation in these cells rendered the unstable chimeric β-globin/uPAR ARE mRNA stable. Protein binding to the ARE and the signaling pathway involved in this regulation remain to be studied. The ARE sequence in uPAR mRNA is highly conserved among human, bovine, rat, and mouse. Thus, modulation of the stability of the uPAR mRNA through the ARE could be a conserved mechanism.

Perspectives

We have discussed the functions of fibrinolytic genes and shown that they are not limited to proteolytic processes but also include control of cell-ECM interactions without the involvement of proteolysis. The discovery of various interactions of uPAR with other membrane-located molecules makes it important to study the role of the fibrinolytic genes in a still wider context. Reflecting the wide range of biological roles of this system, the regulation mechanisms of the genes are multitudinous—from signal processing through gene transcription to mRNA metabolism. There are many molecular descriptions of the regulation at different steps for each gene. We have also shown that activities formerly thought to be in opposition are coordinately engaged in some biological processes, such as that observed in the high expression of both PAI-1 and uPA in metastatic breast cancer. However, our understanding of the mechanisms underlying coordinated regulation between genes and between different regulatory steps is still limited. Furthermore, descriptions of gene regulation are still rather static and often lack reference to time-dependent changes, which are intrinsic to biological systems. With its involvement in various biological processes and the availability of molecular tools to examine each component, the fibrinolytic gene system is an excellent model for studying a multi-component biological system.

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