

# Glucocorticoid-modulated Gene Expression of Tissue- and Urinary-type Plasminogen Activator and Plasminogen Activator Inhibitor 1 and 2

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**Abstract.** Constitutive gene expression of four components of the plasminogen activating enzyme system, urinary and tissue-type plasminogen activator (u-PA and t-PA), plasminogen activator inhibitor 1 (PAI-1) and PAI-2 in HT-1080 human fibrosarcoma cells, was modulated by the synthetic glucocorticoid dexamethasone (Dex,  $10^{-7}$  M). More than 90% of u-PA, t-PA and PAI-1 antigen was found in conditioned medium, whereas PAI-2 was mainly cell associated. In 48-h culture supernatants (expressed per  $10^6$  cells) PAI-1 antigen increased from 350 to 3,300 ng and t-PA from 19 to 38 ng. u-PA and PAI-2 in the same samples decreased from 380 to 46 ng and from 3.5 to 1.8 ng, respectively. Northern blot hybridization and nuclear

“Run-on” transcription assays demonstrated that the increase of t-PA and PAI-1 and the decrease of u-PA were associated with equivalent changes of gene template activity. Modulation of u-PA, t-PA and PAI-1 gene expression by Dex was completely blocked by the glucocorticoid antagonist RU 38486, suggesting that all effects were mediated through the glucocorticoid receptor. Cycloheximide, an inhibitor of protein biosynthesis induced a rapid transient increase of t-PA, u-PA and PAI-1 mRNA and a sustained increase of PAI-2 mRNA, but blocked the more long term effects of Dex, suggesting that both constitutive and hormonally regulated maintenance of mRNA steady state levels required protein biosynthesis.

**P**LASMINOGEN activators (PAs)<sup>1</sup> are highly specific serine proteases of two genetically distinct types: t-PA and u-PA. They convert the abundant (200 mg/l) blood zymogen plasminogen into plasmin, a trypsin-like protease. Natural substrates of plasmin are fibrin, the solid protein matrix of the blood clot and a number of extracellular matrix proteins (Collen, 1980; Saksela, 1985; Danø et al., 1985). High PA activity is often associated with cells and tissues involved in extracellular matrix degradation in the course of morphogenetic events in ontogeny and malignant growth (Reich, 1978; Valinsky et al., 1981; Markus, 1984; Danø et al., 1985; Saksela, 1985). There is considerable literature on developmental and hormonal control of PA biosynthesis (Danø et al., 1985; Blasi et al., 1987), whereas in this respect much less is known about the recently discovered PAIs, PAI-1 (Loskutoff et al., 1983; Van Mourik et al., 1984; Pannekoek et al., 1986) and PAI-2 (Åstedt et al., 1985; Kopitar et al., 1985; Chapman and Stone, 1985; Kruithof et al., 1986; Schleuning et al., 1987). It has previously been observed that the expression of PA activity by various established cell lines, primary cultures and cultured tissues and organs was suppressed by glucocorticoids. This phenomenon was attributed to either a suppression of PA biosynthesis (Vassalli et al., 1976; Werb, 1978; Hamilton et al., 1981; Coleman et al., 1982; Mira-y-Lopez et al., 1985; Littlefield

et al., 1985; Mayer et al., 1986), to an increase of PAI biosynthesis (Seifert and Gelehrter, 1978; Crutchley et al., 1981; Coleman et al., 1982; Gelehrter et al., 1983; Cwikel et al., 1984; Andreasen et al., 1986) or to a combination of both effects (Busso et al., 1987a; Pearson et al., 1987). PA activity or antigen changes have been shown to be associated with respective changes in u-PA mRNA (Medcalf et al., 1986; Busso et al., 1986; Pearson et al., 1987), t-PA (Busso et al., 1987a) or PAI-1 (Andreasen et al., 1987) or gene template activities of u-PA (Collart et al., 1987) and t-PA (Busso et al., 1987b). A synoptic study however, addressing the coordinated, time-dependent changes of secreted and cell associated antigen of all four factors, the associated changes in cellular mRNA levels and respective gene template activities and their dependence on protein biosynthesis has not previously been performed.

## Materials and Methods

### Cell Culture and Harvesting of Media

HT-1080 cells were cultured to confluency using standard techniques in 150 cm<sup>2</sup> culture flasks (Corning Glass Works, Corning, NY) in 40 ml DME containing 2 mM glutamine and 10% heat-inactivated FCS (Gibco, Paisley, UK). Before the start of an experiment, cells were maintained overnight at 37°C in serum free DME. To start an experiment, medium was replaced with fresh serum free DME with or without  $10^{-7}$  M Dexamethasone (Dex, Sigma Chemical Co., St. Louis, MO) prepared from a stock solution of  $10^{-3}$  M in ethanol. Cells were subsequently maintained for various times

1. Abbreviations used in this paper: Dex, Dexamethasone; PA, plasminogen activator.

up to 48 h. Where indicated, cycloheximide (5 µg/ml) or the glucocorticoid antagonist RU 38484 ( $10^{-6}$  M) (Jung-Testas and Baulieu, 1983) was added to cell cultures 30 min or 14 h before the addition of Dex, respectively. (This concentration of RU 38486 was previously determined to be optimal at blocking induction of PAI-1 mRNA by  $10^{-7}$  M Dex). To quantitate PAI-2 antigen, conditioned medium collected at 24 and 48 h from nontreated or Dex treated HT-1080 cells, was dialyzed overnight against deionized water at 4°C, and concentrated 20-fold by vacuum centrifugation (Speed-vac, Savant, Farmingdale, NY) then stored at -30°C until further use.

### Activity Assay

Plasminogen-dependent fibrinolytic activity in conditioned medium was determined by the Astrup plate assay (Astrup and Müllertz, 1952).

### Preparation of Cellular Extracts

To quantify cell associated u-PA, t-PA, PAI-1 and PAI-2, cells were harvested after various incubation times by trypsinization, washed in 20 ml of PBS, centrifuged, and then disrupted by adding 5 ml of 0.5% ice cold NP-40 lysis buffer (10 mM Tris-HCl, pH 7.4; 10 mM NaCl; 3 mM MgCl<sub>2</sub>; 1 mM EDTA; 0.1 mM PMSF; 1 mM DTT;  $10^{-6}$  M antipain (Sigma Chemical Co. and 0.5% NP-40), vortexed, and kept on ice for 5 min. Nuclei were sedimented and the supernatant containing the cellular extract was removed and stored at -30°C until further use.

### Electrophoretic Procedures and Zymography

HT-1080 cell-conditioned medium obtained at various times from control and Dex-treated cultures was fractionated by SDS-PAGE (Laemmli, 1970), using 10% separation gels. After electrophoresis, plasminogen activator activity in the gels was visualized by fibrin zymography (Graneli-Piperno and Reich, 1978).

### Antigen Determinations

Conditioned medium was centrifuged to remove cellular debris and stored in aliquots at -30°C until further use. u-PA, PAI-1, and PAI-2 antigen were then determined by radioimmunoassay as previously described (Genton et al., 1987; Kruithof et al., 1987). t-PA antigen was determined using the Biopool IMULYSE 5 t-PA ELISA kit (Biopool, Umea, Sweden), according to manufacturers instructions.

### DNA Probes

The Bgl II fragment of the pPA II 4B cDNA clone, harboring a 1948 base pair (bp) insert of the human t-PA cDNA has been described (Fisher et al., 1985). The t-PA cosmid clone, pPA cos 3 harboring the complete t-PA gene including 10 kb of flanking sequence was isolated by Dr. R. Fisher (Biogen Res. Corp.) from a cosmid library (Grosveld et al., 1982) and kindly provided.

pUK 0321 (Schleuning, W.-D., L. Liebermann, and H. Mead, unpublished data) harboring a 1,023-bp fragment of the human u-PA cDNA was isolated from a cDNA library derived from human HT-1080 fibrosarcoma cells using mixed oligonucleotide probes synthesized according to a published sequence (Günzler et al., 1982).

PAI-1 cDNA (1,500 base pair) and cosmid clones were isolated by Van den Berg et al., (1987) and Bosma et al., (1987), respectively, and kindly provided.

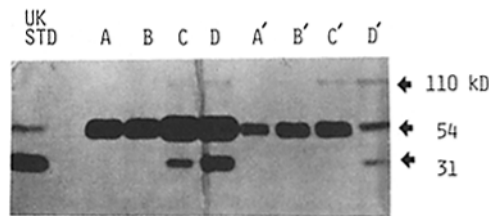
pPAI J7 harboring a 1,900 bp cDNA insert for PAI-2 was as described by Schleuning et al., 1987.

**Metallothionein IIa:** Plasmid pH MT-II3 (Karin and Richards, 1982), was a kind gift from Dr. Rob Richards, Howard Florey Institute (Melbourne, Australia).

Labeling of all probes with  $\alpha$ -<sup>32</sup>P dATP was performed by the random priming technique (Maniatis, et al., 1982). For the Run-on transcription assay, each probe was linearized and fixed to nitrocellulose as previously described (Greenberg and Ziff, 1984). 5 µg of DNA was applied per slot (1 µg for PAI-1).

### Northern Blot Analysis

The isolation of mRNA from cultured cells was performed as previously described (Metcalf et al., 1986). Agarose gel (0.9%) electrophoresis of mRNA in the presence of 20% formaldehyde followed by Northern blot transfer was performed as described by Thomas, (1980), but using Gene



**Figure 1.** Zymographic analysis of HT-1080 cell-conditioned medium. Regions of plasminogen-dependent fibrinolytic activity in HT-1080 cell-conditioned medium were determined by zymographic analysis. Conditioned medium was collected at the indicated times (below) from control (A, B, C and D) or Dex treated (A', B', C', and D') HT-1080 cell cultures. A: 4 h; B: 8 h; C: 24 h; D: 48 h. Low molecular mass urokinase (31 kD) was used as a standard (far left lane). 110 kD t-PA/PAI-1 complexes, 54 kD u-PA and 31 kD low molecular mass urokinase positions are indicated to the right of the figure. 25 µl of medium, mixed with an equal volume of 2X sample buffer were charged per well. Incubation time of SDS-PAGE gel with fibrin-agar underlay gel was 5 h.

Screen Plus membrane (New England Nuclear, Boston, MA) in place of nitrocellulose filter paper. Hybridization conditions and processing of filters were performed as previously described (Metcalf et al., 1986).

### Run-on Transcription Assay

The methods used were essentially those described by Greenberg and Ziff, (1984). Cells were lysed in 10 ml of ice-cold NP-40 lysis buffer (10 mM Tris-HCl, pH 7.4; 10 mM NaCl; 3 mM MgCl<sub>2</sub>; 1 mM EDTA; 0.1 mM PMSF; 1 mM DTT;  $10^{-6}$  M Antipain [Sigma Chemical Co., and 0.5% NP-40], the nuclei sedimented by centrifugation, resuspended in glycerol storage buffer (Greenberg and Ziff, 1984), distributed into 100-µl aliquots ( $10^7$  nuclei/aliquot), then snap frozen in liquid nitrogen and stored at -70°C. As a negative control 1 µl of 1 mg/ml  $\alpha$ -amanitin was added to replicate nuclear preparations (see figure legend) for 10 min before the addition of the reaction buffer (Greenberg and Ziff, 1984) while still on ice. Nuclear RNA was finally resuspended in 300 µl hybridization buffer (50 mM Hepes; pH 7.4, 0.3 M NaCl; 10 mM EDTA; 0.2% SDS; 100 µg/ml yeast tRNA and 1× Denhardt's solution [Maniatis et al., 1982]) in sterile Eppendorf tubes. To quantitate in vitro elongated u-PA, PAI-1, PAI-2, t-PA, and metallothionein IIa transcripts, regions of nitrocellulose filter strips containing "slot-blotted" plasmid or genomic DNA (above), were cut out and prehybridized for 30 min at 65°C in prehybridization buffer (50 mM Hepes, pH 7.4; 0.3 M NaCl; 10 mM EDTA; 0.2% SDS; 1 mg/ml yeast tRNA and 5× Denhardt's solution), and then transferred to the RNA samples in hybridization buffer prepared above. All filter strips containing cDNA slots were hybridized simultaneously for 36 h at 65°C, then washed, RNase treated (Greenberg and Ziff, 1984), air dried, and exposed to x-ray film for up to 14 d.

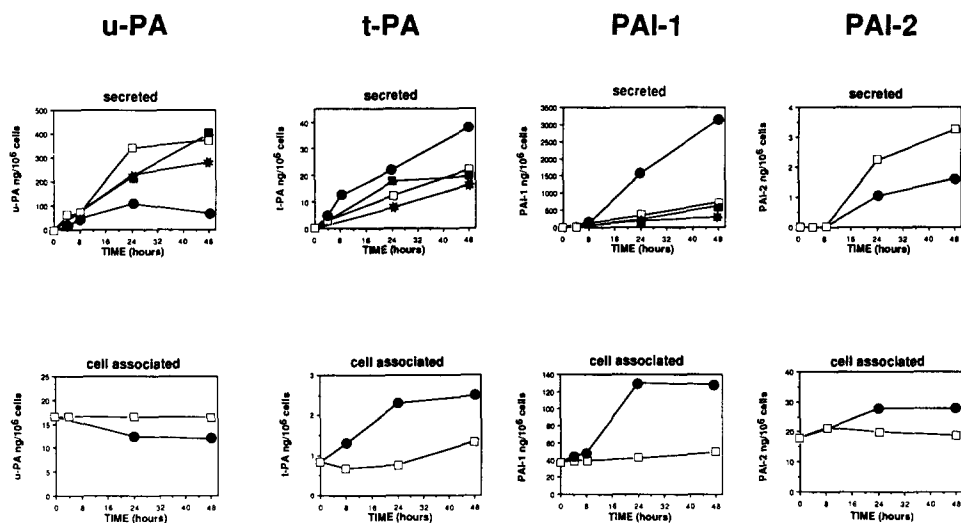
### Densitometry

Densitometric analysis of transcription and mRNA signals on autoradiograms was performed using an LKB Ultrosan XL model 2222 densitometer (LKB-Pharmacia, Switzerland). All calculations were expressed relative to an arbitrary value of 1 assigned to the intensity of the signal obtained for each sample at time point 0 unless otherwise stated (see figure legends).

### Results

#### Dex-Mediated Modulation of Fibrinolytic Activity and Antigen

The fibrinolytic activity of HT-1080 cell-conditioned medium was 30 U/ml and 50 U/ml after 24 and 48 h culture, respectively (data not shown). Treatment of the cells with Dex suppressed the fibrinolytic activity below detectable limits (0.1 U/ml). Analysis of PA activity by SDS-PAGE followed by zymographic analysis showed mainly one band at the position of u-PA (54 kD; Fig. 1), which was markedly



**Figure 2.** Quantitation of secreted and cell associated u-PA, t-PA, PAI-1, and PAI-2 antigen in HT-1080 cells. Conditioned medium was collected after 4, 8, 24, or 48 h from nontreated ( $\square$ ), Dex treated ( $\bullet$ ), Dex + RU 38486 treated ( $\blacksquare$ ) or RU 38486 alone treated ( $\star$ ) HT-1080 cells. The effect of RU 38486 was not performed on secreted PAI-2 nor on antigen levels in cell lysates. RIA's for u-PA, PAI-1, and PAI-2 and ELISA assay for t-PA antigen were performed as described in the text. Results presented represent the average of at least 2 separate determinations. Inter and intra assay variations were less than 5% in all cases. All results have been expressed as ng antigen/ $10^6$  cells.

reduced in conditioned medium of Dex treated cells. Minor bands at 31 kD (co-migrating with the low  $M_r$  form of u-PA), and at 110 kD were also visible, the latter representing a complex of t-PA with PAI-1 as demonstrated by immunoprecipitation (data not shown).

Accumulation of secreted and cell associated u-PA, t-PA, PAI-1, and PAI-2 antigen in HT-1080 cells was determined after 0, 2, 4, 8, 24, and 48 h exposure to Dex and is shown in Fig. 2. Expressed per  $10^6$  cells, secreted PAI-1 increased from 350 to 3,300 ng and t-PA from 19 to 38 ng. u-PA and PAI-2 in the same samples decreased from 380 to 46 ng and from 3.5 to 1.8 ng, respectively. Modulation of u-PA, t-PA, and PAI-1 by Dex was completely blocked if cells were pretreated overnight with the glucocorticoid antagonist RU 38486. Cell associated u-PA antigen decreased 0.3-fold, whereas t-PA and PAI-2 increased 2-fold and 1.5-fold respectively.

### Time Dependent Effects on mRNA

The association of Dex-mediated modulation of antigen with corresponding changes of mRNA was explored by Northern blot hybridization (Fig. 3, panels *a, d, g, and j*). Quantitation of changes in mRNA was assessed by densitometric analysis of the autoradiograms (Fig. 3: adjacent to the respective mRNA panels). Exposure times of all filters to autoradiograms were chosen to visually optimize the signals present. u-PA mRNA was suppressed 95% after 8 h (Fig. 3, *A*) as previously described (Medcalf et al., 1986) and is included here as a control. Dex caused an 11.9-fold and a 7.8-fold increase in the 3.4 and 2.4 kb PAI-1 mRNA species respectively, after 24 h (Fig. 3, panel *G*). Within a further 48 h, the 3.4 kb species decreased nearly 2-fold, whereas the 2.4 kb mRNA species increased 16-fold over basal levels. t-PA mRNA was constitutively expressed and increased 3.4-fold (Fig. 3, panel *D*). PAI-2 mRNA was present only at low concentration in control cells (exposure to the X-ray film: 15 d) yet suppressed 50% after 2 h and virtually absent after 8 h (Fig. 3, panel *J*). Constitutive levels of all 4 mRNAs remained unchanged under serum-free conditions during a 48-h time course (see

Fig. 4 and data not shown). Modulation of u-PA and PAI-1 mRNA was prevented by pretreatment of the cells overnight with RU 38486 before addition of Dex (Fig. 4).

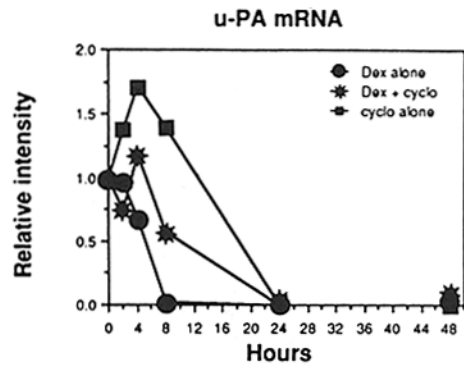
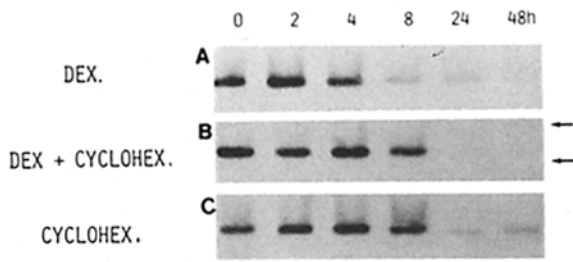
### Effects of Cycloheximide

To determine whether on-going protein synthesis was required for constitutive or regulated gene expression, cells were treated with cycloheximide alone or before exposure to Dex. Fig. 3 (panels *C, F, and I*) demonstrate that cycloheximide caused a transient augmentation of u-PA, t-PA and PAI-1 mRNA within a 4–8-h period. The 3.4 kb species of PAI-1 mRNA was most conspicuously increased (7.8-fold). After 8 h, however, the three mRNAs were reduced. The combination of both Dex and cycloheximide caused a transient 9-fold and 4.5-fold increase in the 3.4 and 2.4 kb PAI-1 mRNA species, respectively (Fig. 3, panel *H*). Hence protein biosynthesis was required for the glucocorticoid-mediated induction of PAI-1 and t-PA mRNA and the downregulation of u-PA mRNA. The effect of cycloheximide on PAI-2 gene expression was peculiar: by itself it caused a sustained 7.8-fold increase in mRNA, in combination with Dex a transient fivefold increase of mRNA (Fig. 3, panels *K and L*).

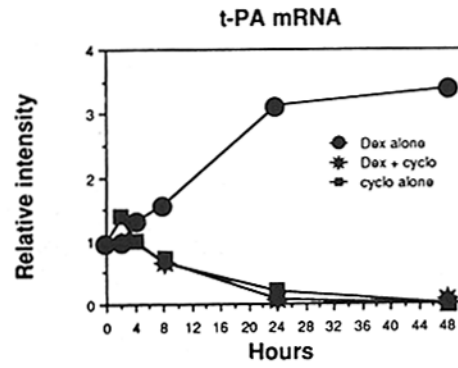
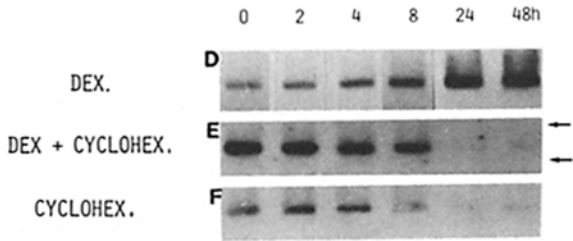
### Effect on Gene Transcription Rates

Fig. 5 (*A*) demonstrates the signals obtained from the Run-on transcription assays indicating the relative changes in transcriptional activity of u-PA, t-PA, PAI-1, PAI-2, and metallothionein after treatment with Dex. Quantitation of these signals by densitometric analysis is shown in Fig. 5 (*B*). It is evident that urokinase gene template activity was suppressed 50% after 2 h and was virtually absent after 4 h. t-PA and PAI-1 gene transcription rates were increased 3.4 and 10-fold, respectively. Metallothionein IIa (Mt IIa) gene transcription, known to be activated by Dex (Karin et al., 1980; Karin et al., 1984) was used as a positive control and is shown here to be transiently induced 50-fold.  $\alpha$ -Amanitin, an inhibitor of RNA polymerase II was added to replicate nuclear preparations (0 and 24-h time points) and was shown to block the transcriptional activity of PAI-1.  $\alpha$ -Amanitin also

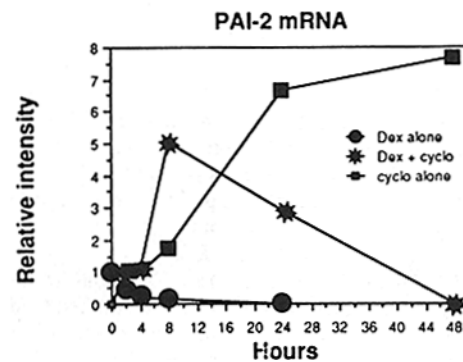
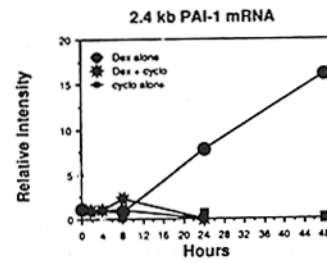
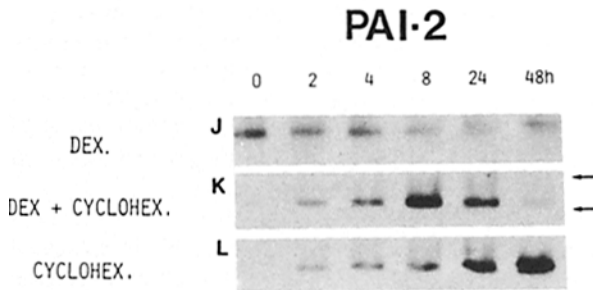
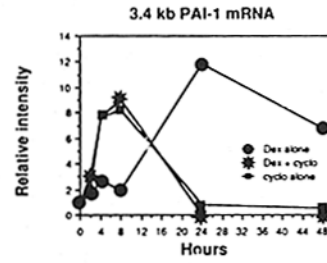
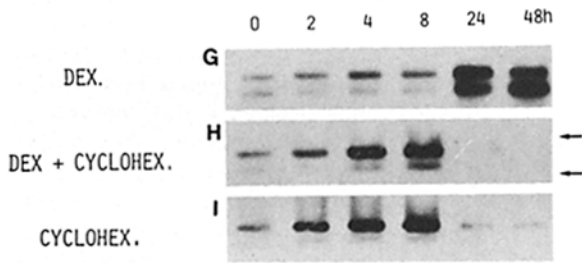
### u-PA

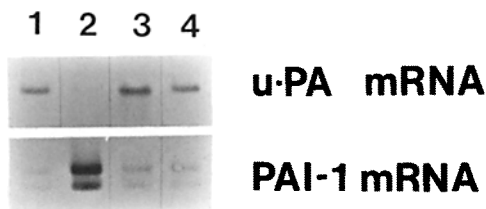


### t-PA



### PAI-1





**Figure 4.** Antagonism of RU 38486 on Dexamethasone induced modulation of u-PA and PAI-1 mRNA in HT-1080 cells. mRNA prepared from 24 hour nontreated (lane 1), Dex treated (lane 2), Dex plus RU 38 486 treated (lane 3) or RU 38486 alone treated (lane 4) HT-1080 cell cultures was prepared and assessed by Northern blot hybridization for u-PA and PAI-1 mRNA as indicated. 4  $\mu$ g mRNA was applied to each lane.

blocked constitutive expression of u-PA and the induction of t-PA and metallothionein (data not shown). Changes of PAI-2 gene transcription could not be determined accurately because of the sensitivity limits of the assay. Modulation of u-PA, t-PA and PAI-1 and MT-IIa gene transcription by Dex was blocked if cells were pretreated overnight with the glucocorticoid antagonist RU 38486 (data not shown). All changes in gene transcription corresponded to the respective changes in mRNA and antigen (cf. Figures 2, 3, and 5).

### Discussion

Exposure of HT-1080 cells to Dex entailed a disproportionate increase of PAI-1 and t-PA antigen, a decrease of u-PA and PAI-2 antigen and consequentially a net decrease of PA activity in culture supernatants. Like in the human histiocytic cell line U-937 (Genton et al., 1987; Wohlwend et al., 1987) and in contrast to the other factors, PAI-2 was mainly cell associated. The peculiar pathway of PAI-2 biosynthesis and secretion, characterized by the coexistence of a nonglycosylated intracellular and a glycosylated extracellular form (Genton et al., 1987) is therefore not an exclusive property of U-937 cells.

The relative amounts of u-PA, PAI-1, PAI-2, and t-PA mRNA changed concomitantly with antigen quantity. Initially both the 3.4 and 2.4 kb PAI-1 mRNA species, (differing in the length of their 3'-untranslated region because of two poly-A addition sites [Ny et al., 1986; Pannekoek et al., 1986]) were induced. Depending on time after hormone treatment the smaller species became more abundant. The Dex effect was transcriptional (see below) however the 2.4 kb PAI-1 mRNA (lacking one of the TATTTAT consensus se-

quences conferring mRNA instability [Shaw and Kamen, 1986]) was perhaps more stable.

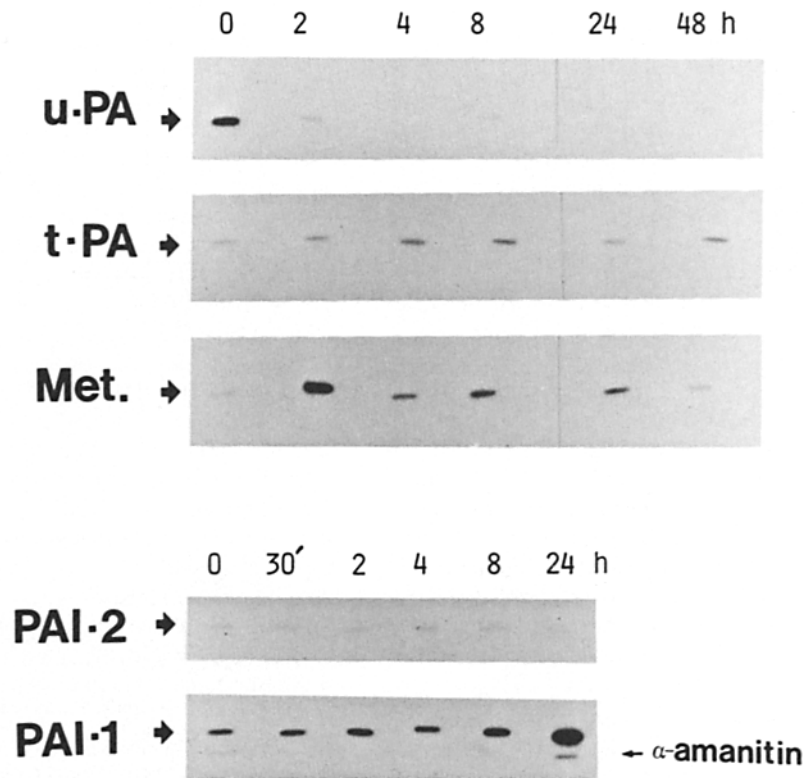
Cycloheximide caused a transient increase of u-PA, t-PA, and PAI-1(3.4 kb) mRNA and a sustained increase of PAI-2 mRNA. This finding suggests that unstable protein intermediate(s) which cause under constitutive conditions the suppression of transcription or the degradation of mRNA must play a pivotal role in the regulation of mRNA steady state levels. A similar effect of protein synthesis inhibitors on modulating calcitonin-induced u-PA gene transcription and mRNA stability has been described in LLC-PK<sub>1</sub> cells (Altus et al., 1987). Furthermore, Klein et al., (1987), have demonstrated that ongoing protein synthesis is necessary for glucocorticoid regulation of the rat  $\alpha_1$ -acid glycoprotein gene. Andreasen et al., (1987) recently reported that cycloheximide did not influence the induction of PAI-1 mRNA by Dex in HT-1080 cells. As only a single time point (16 h) was used in that study, none of the regulatory phenomena reported here were observed. The peculiar effect of cycloheximide alone or in combination with DEX on PAI-2 mRNA is consistent with the interpretation that under constitutive conditions PAI-2 mRNA is rapidly degraded by protein factor(s) with a limited half-life. The alternative interpretation would imply that constitutively expressed PAI-2 is regulated by a labile transcriptional suppressor and/or that Dex had a negative effect on PAI-2 mRNA stability. This second hypothesis appears less attractive in the light of what is now known about glucocorticoid hormone action (Yamamoto, 1985).

The constitutive template activity of the u-PA gene decreased after exposure to Dex. As the Run-on transcription experiment was performed in the absence of cycloheximide, there is the possibility that Dex may be having an indirect effect on the u-PA gene. It has previously been shown that the suppressive action of Dex on u-PA mRNA in the human mammary cell line HBL-100 required on-going protein biosynthesis (Busso, et al., 1986). From the Northern blot data presented in this study, it is evident that on-going protein biosynthesis is indeed required to maintain steady state levels of both u-PA, t-PA and PAI-1 mRNA. Although Dex suppressed u-PA gene transcription within a 2-h period, it is nevertheless a possibility that on-going protein biosynthesis is required for this effect. Furthermore, cycloheximide when added alone also suppressed constitutive u-PA and t-PA gene transcription over a 48-h period, and transiently induced PAI-1 gene transcription (data not shown).

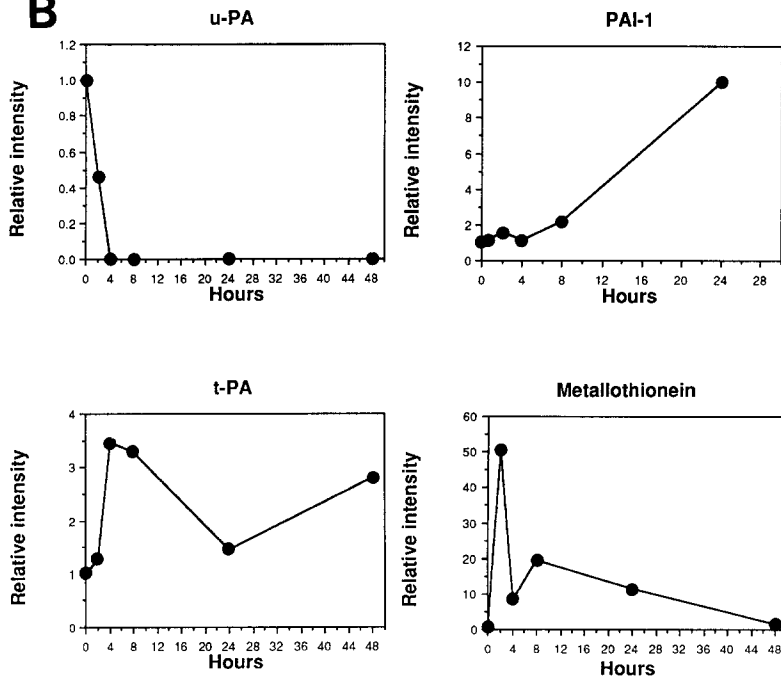
The effect of Dex was inhibited by the glucocorticoid antagonist RU 38486 and therefore most likely mediated through the glucocorticoid receptor. Hence the u-PA gene

**Figure 3.** Time course of u-PA, t-PA, PAI-1, and PAI-2 mRNA accumulation in HT-1080 cells. mRNA prepared from nontreated (0 h) cells, or from cells treated with Dex alone, Dex plus cycloheximide (*cyclohex*), or with cycloheximide alone for 2, 4, 8, 24, and 48 h was assessed by Northern blot analysis. Relative changes of u-PA, t-PA, PAI-1, and PAI-2 mRNA were determined by hybridization to random primer labeled u-PA, t-PA, PAI-1, or PAI-2 cDNA probes of similar specific activity. 4  $\mu$ g mRNA was applied in each lane. Arrows next to panels b, e, h, and k represent 28 S and 18 S ribosomal RNA markers. Exposure times of the filters to X-ray film were chosen to visually optimize the signals present. Panels A-D and F-I and K and L were exposed to X-ray film overnight, E for 2 d, and J for 15 d. Quantitation of changes of u-PA, t-PA, PAI-1 (3.4 and 2.4 kb species) and PAI-2 mRNA are indicated in the graphs adjacent to the respective mRNA panels. Changes in relative intensity of the signals presented were quantified by densitometric analysis by assigning an arbitrary score of 1 to the intensity of the mRNA signal obtained at the zero point time for each component. Quantitation of PAI-2 mRNA after treatment with Dex + cycloheximide (*cyclo*) and with cycloheximide alone was calculated from the arbitrary score being assigned to the 2-h time point.

**A**



**B**



**Figure 5.** (A) Time course of Dex-induced modulation of u-PA, t-PA, PAI-1, PAI-2, and metallothionein gene transcription. HT-1080 cell nuclei were prepared from nontreated (0 h) cells, and from cells treated for 30 min 2, 4, 8, 24, or 48 h with Dex. Elongation and labeling of initiated RNA transcripts in isolated nuclei and hybridization to immobilized cDNA (u-PA, metallothionein [Met], and PAI-2) or genomic (t-PA and PAI-1) slot blots was performed as described in the materials and methods section.  $\alpha$ -Amanitin was added to replicate nuclei aliquots at time 0 and 24 h and assessed simultaneously during hybridization for PAI-1 transcripts. Exposure times were chosen to visually optimize the signals present: u-PA and metallothionein, 14 d; t-PA and PAI-1, overnight; PAI-2, 10 d. (B) Quantification of changes in u-PA, t-PA, PAI-1 and metallothionein gene transcription. Changes in relative intensity of the signals presented in A were quantified by densitometric analysis of the autoradiograms described in the legend to Fig. 3.

probably contains a cis-acting, glucocorticoid responsive, negative regulatory element. Such an element has been identified in the regulatory region of the proopiomelanocortin gene (Charron and Drouin, 1986). The respective findings on Dex modulation of t-PA and PAI-1 gene transcription on the other hand, suggest in contrast the presence of positive regulatory elements, possibly similar to those identified and

studied in considerable detail in other genes (Yamamoto, 1985). We note here that the Dex stimulation of PAI-1 gene template activity, although clearly observed in five consecutive experiments, was in terms of intensity and time dependence more variable than the other effects. Time dependence of PAI-1 mRNA induction by Dex was also slightly variable although the magnitude of the induction was always consis-

tent. PAI-1 biosynthesis is modulated by factors present in serum like TGF- $\beta$  (Laiho et al., 1986; Lund et al., 1987) and it is possible that variations in the quality of serum accounted for some of the differences observed. Furthermore, we cannot exclude that Dex also affects PAI-1 mRNA stability as in the case of growth hormone (Pael and Axel, 1987), in addition to the effect on transcription as the appropriate experiments to rigorously rule out such a possibility were not performed.

Our results demonstrate that the PA enzyme system is modulated by glucocorticoids via negative and positive regulation of transcription. Both, constitutive and regulated transcription are dependent on unstable protein factors which may include the glucocorticoid receptor. Although the net effect of glucocorticoid in our experimental system was a decrease of PA activity, the surprising finding of positive regulation of t-PA and negative regulation of PAI-2, precluded any generalizations. The glucocorticoid receptor is widely distributed and the observed mechanisms are therefore likely to operate in many cells and tissues. With the data in mind however that other workers have obtained by studying different cell lines or tissues, it appears likely that other hormonal, tissue specific or developmentally programmed pathways must cooperate with the mechanisms described in this study.

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