Expression of urokinase-type plasminogen activator, its receptor and type-1 plasminogen activator inhibitor is differently regulated by inhibitors of protein synthesis in human cancer cell lines

Leif R. Lund[a,b,*]

[a]*Finsen Laboratory, Rigshospitalet, Strandboulevarden 49, 2100 Copenhagen Ø., Denmark*
[b]*Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143, USA*

Received 23 January 1996; revised version received 18 February 1996

Abstract Expression of the various components of the plasminogen activation system is under tight regulation by hormones, cytokines, and growth factors under physiologic conditions. Like early-response genes, these components are modulated by inhibitors of protein synthesis in some cell lines. To clarify the specific expression and regulation of mRNAs for urokinase (uPA), its receptor (uPAR), and type-1 plasminogen activator inhibitor (PAI-1), I analyzed RNA from four human cancer cell lines by RNA blotting after treatment with cycloheximide, anisomycin, emetine or puromycin. These inhibitors, all of which induced translational arrest, induced a very diverse response in the various transcripts, suggesting that the inhibitors mediate their effects through different molecular mechanisms. Dose-response analysis showed that, in A549 cells, anisomycin strongly induced uPAR and PAI-1 mRNA at concentrations that did not cause complete inhibition of protein synthesis, whereas cycloheximide induced these transcripts in a dose-dependent manner only at concentrations sufficient to inhibit total protein synthesis by >90%. Puromycin induced the 3.4-kb transcript of PAI-1 mRNA in A549 and RD cells, whereas it decreased the expression of both the 3.4-kb and 2.4-kb PAI-1 transcripts in HT-1080 cells. Different time patterns of induction for uPA, uPAR and PAI-1 mRNA suggest that even in the same cell type, inhibitors of protein synthesis mediate their effects on various genes through different mechanisms. Thus, induction of uPA, uPAR and PAI-1 transcripts by inhibitors of protein synthesis was dependent on the gene, the cell line, and the type of inhibitor, and inhibition of protein synthesis per se was not sufficient for induction of these transcripts.

Key words: Urokinase-type plasminogen activator; Tissue-type plasminogen activator; Plasminogen activator inhibitor

1. Introduction

Activation of the zymogen plasminogen to the active proteinase plasmin by urokinase-type plasminogen activator (uPA) [1] plays an important role during processes involved in the degradation of the extracellular matrix, e.g., during wound healing, cell migration during trophoblast implantation, mammary gland involution, and invasion of cancer cells [1,2]. Activation of plasminogen by tissue-type plasminogen activator (tPA) is involved in lysis of fibrin clots [3]. The plasminogen activation process is regulated by two distinct and fast-acting specific inhibitors, PAI-1 and PAI-2 [4]. A specific and high-affinity receptor for uPA (uPAR) has also been identified in all examined cell lines and tissues, although the level of expression varies [5]. Binding of uPA to its receptor strongly enhances the activation of plasminogen, making the cell surface an important site for focused proteolysis [6].

In vitro the synthesis of the plasminogen activators, the inhibitors and the receptor is regulated by a variety of cytokines, hormones and tumor promoters [1,2,4,7]. In some cell lines, treatment with the inhibitor of protein synthesis cycloheximide transiently induces or increases the level of mRNA for uPA [8], tPA [9], PAI-1 [10], PAI-2 [8], and uPAR [11], as it does for the normally labile early-response genes [12]. Investigators have sought to explain this phenomenon by two different molecular mechanisms: the presence of specific, short-lived mRNA-degrading enzymes, or the existence of labile repressors that control specific gene transcription [12]. According to the second hypothesis, treatment of cells with inhibitors of protein synthesis will rapidly decrease the level of the repressor molecule within the cells, thereby allowing transcription of the repressed genes. Although the existence of such factors has been postulated for years, labile repressors have not yet been biochemically characterized in eukaryotic cells. Recent results obtained in C3H 10T1/2 cells, in which anisomycin loses its ability to block protein synthesis at concentrations <70 ng/ml, show that at even lower concentrations anisomycin is still able to induce c-*fos* and c-*jun* mRNA [13,14]. These results are not consistent with a role for labile repressor molecules or short-lived specific mRNA-degrading enzymes; instead, they suggest direct effects of protein synthesis inhibitors on signal transduction pathways for c-*fos* and c-*jun* [14].

The data obtained with protein synthesis inhibitors on the various components of the plasminogen activation system have all centered on the effects of cycloheximide at concentrations that induce complete inhibition of protein synthesis. To determine if modulation of uPAR, uPA and PAI-1 mRNA expression is a general feature of protein synthesis inhibitors, I examined the effects of cycloheximide, anisomycin, emetine and puromycin, which mediate their blocking effects on protein synthesis through various molecular mechanisms [15], in four human cancer cell lines.

2. Materials and methods

2.1. Materials

Cycloheximide, anisomycin, emetine and puromycin were obtained from Sigma Chemical Co. (St. Louis, MO). All other materials were

*Corresponding author. Fax: (45) 31385450.

Abbreviations: uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; PAI, plasminogen activator inhibitor; uPAR, urokinase-type plasminogen activator receptor; TGF-β, transforming growth factor-β; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; TCA, trichloroacetic acid.

PII S0014-5793(96)00223-2

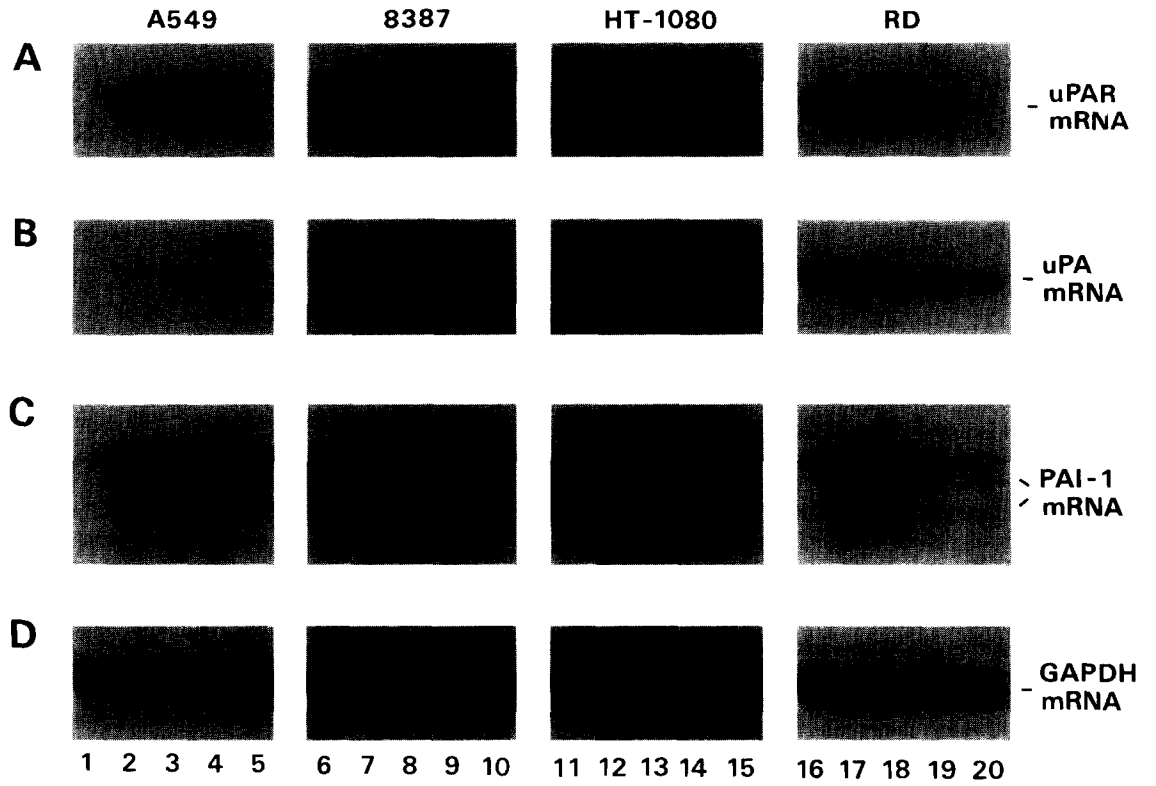

Fig. 1. Effects of inhibitors of protein synthesis on uPAR, uPA and PAI-1 mRNA levels in human cell lines (RNA blot analysis). Confluent cells were incubated for 6 h under serum-free conditions with buffer alone (lanes 1, 6, 11 and 16), 10 μg/ml cycloheximide (lanes 2, 7, 12 and 17), 250 ng/ml anisomycin (lanes 3, 8, 13 and 18), 10 μg/ml emetine (lanes 4, 9, 14 and 19), or 50 μg/ml puromycin (lanes 5, 10, 15 and 20). Cells were harvested, and total RNA was purified as described in section 2. For the RNA blot analysis, 30 μg of the total RNA was electrophoresed in 1.5% agarose gels under denaturing conditions and blotted onto a nitrocellulose filter. The membrane was hybridized with a randomly primed ^{32}P-labeled uPAR cDNA probe (A), uPA cDNA probe (B), PAI-1 cDNA probe (C) or GAPDH cDNA probe (D). Cell lines are indicated at the top, and positions of the specific mRNA bands are indicated on the right.

those described previously [11,16] or of the best grade commercially available.

2.2. Cell culture

The human lung carcinoma cell line A549 (American Type Culture Collection CCL 185) and the human rhabdomyosarcoma cell line RD (CCL 136) were obtained from Flow Laboratories (Irvine, UK). The fibrosarcoma cell lines HT-1080 (CCL 121) and 8387 were obtained from A. Vaheri, University of Helsinki, Finland. Approximately 10^6 trypsinized cells were seeded into 15-cm petri dishes and grown to confluence in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, as described previously [17]. Before being used for experiments, cells were kept under serum-free conditions for 48–72 h. The inhibitors were added to the medium for various times

Table 1
Summary of effects of treatment with protein synthesis inhibitors on uPAR, uPA and PAI-1 mRNA levels in human cancer cell lines

mRNA	Inhibitor	A549	8387	HT-1080	RD
uPAR	Cycloheximide	11.3 ± 2.4	1.9 ± 1.2	1.0 ± 0.2	6.3 ± 1.4
	Anisomycin	18.4 ± 3.6	4.2 ± 1.9	4.0 ± 1.0	0.9 ± 0.3
	Emetine	8.3 ± 1.8	2.4 ± 1.3	1.3 ± 0.4	1.2 ± 0.7
	Puromycin	ND	ND	0.4 ± 0.3	0.4 ± 0.3
uPA	Cycloheximide	2.7 ± 1.1	2.4 ± 1.5	0.6 ± 0.4	7.4 ± 1.4
	Anisomycin	3.1 ± 0.8	3.7 ± 1.6	2.1 ± 0.6	1.1 ± 0.4
	Emetine	2.4 ± 1.3	2.6 ± 0.4	1.0 ± 0.2	2.1 ± 0.7
	Puromycin	ND	1.1 ± 0.3	0.3 ± 0.3	0.6 ± 0.5
PAI-1 (3.4 kb)	Cycloheximide	12.4 ± 2.3	4.8 ± 1.1	0.9 ± 0.3	18.4 ± 2.3
	Anisomycin	15.6 ± 3.4	7.7 ± 0.9	4.6 ± 0.8	7.3 ± 1.3
	Emetine	17.8 ± 1.7	5.1 ± 1.4	1.2 ± 0.4	2.1 ± 1.1
	Puromycin	4.6 ± 0.6	0.8 ± 0.3	0.3 ± 0.2	1.8 ± 0.7
PAI-1 (2.4 kb)	Cycloheximide	3.4 ± 1.7	4.1 ± 0.5	1.1 ± 0.4	5.7 ± 0.9
	Anisomycin	3.8 ± 2.3	5.4 ± 0.9	2.1 ± 0.6	2.7 ± 0.4
	Emetine	4.3 ± 1.4	4.7 ± 0.4	1.0 ± 0.3	1.4 ± 0.3
	Puromycin	1.3 ± 0.3	1.0 ± 0.2	0.2 ± 0.3	1.1 ± 0.2

Numbers represent fold induction (mean ± S.D.) after 6 h treatment, in three independent experiments. The densitometric value obtained for samples treated with solvent alone, normalized to the value for GAPDH, has been set to 1. ND, not detectable.

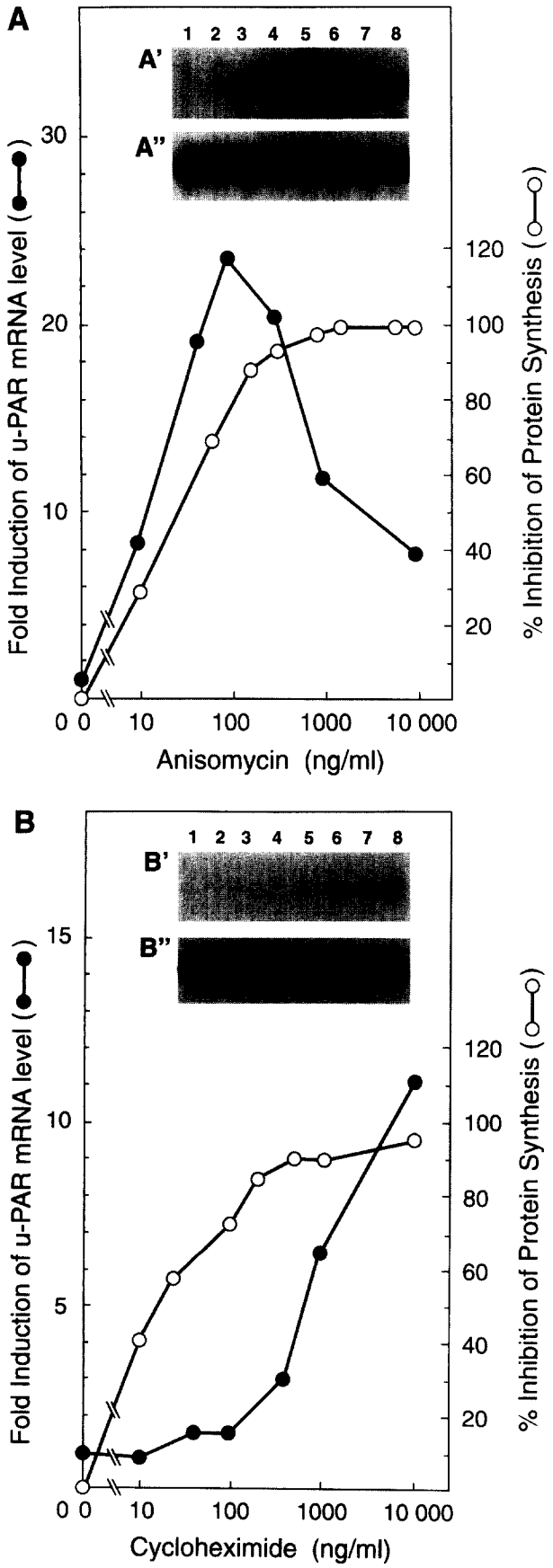

Fig. 2. Dose-dependent effects of anisomycin and cycloheximide on the induction of uPAR mRNA and inhibition of total protein synthesis in A549 cells. A549 cells were treated with increasing concentrations of anisomycin or cycloheximide for 6 h, and RNA blot analysis was performed as described in the legend to Fig. 1. Hybridization with the uPAR cDNA probe is shown in insets A′ and B′ and rehybridization with the GAPDH probe in insets A″ and B″. The normalized relative amounts of uPAR mRNA are indicated on the graph. The uPAR mRNA level in untreated cells was set equal to 1, and subsequent points indicate fold induction in treated cells. The numbers in the insets indicate untreated cells (lanes 1 and 2) and cells treated with anisomycin (A) or cycloheximide (B) at a concentration of 10 ng/ml (lane 3), 50 ng/ml (lane 4), 100 ng/ml (lane 5), 500 ng/ml (lane 6), 1000 ng/ml (lane 7), or 10000 ng/ml (lane 8). Inhibition of total protein synthesis was determined by measuring the incorporation of [^{35}S]methionine into TCA-precipitable proteins. Incorporation in untreated cells was set to 0, and subsequent points indicate inhibition in treated cells as a percentage of that in untreated cells.
←

and in various concentrations, as indicated for individual experiments. Each experiment was performed at least three times, with virtually identical results.

2.3. RNA analysis

Total RNA was isolated from cells and analyzed by hybridizing RNA blots as described previously [16]. The plasmid used as a probe for uPAR mRNA (puPAR-1) carries cDNA covering the entire coding region and part of the 3′- and part of the 5′-untranslated regions [18]. pHuK8 carries a 1.6-kb *Pst*I fragment of the human uPA cDNA [19]. A full-length cDNA-probe for PAI-1 was used for detection of PAI-1 mRNA [20], and a cDNA for rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [21] was used as a control for equal loading and transfer of the RNA samples. Specific signals were quantified by scanning of the autoradiographic films with a Shimadzu dual-wavelength TLC scanner CS-930. Various exposure times were used to ensure that the density of the bands on the autoradiographic films was within the linear range. The relative amounts of uPAR, uPA or PAI-1 mRNA were normalized against the corresponding relative amounts of GAPDH mRNA, which were found to be unaffected by treatment of the various inhibitors in A549 cells, as shown in the figures for the individual experiments.

2.4. Metabolic labeling of cells and quantitation of the labeled proteins

The effects of the various inhibitors on total protein synthesis were determined by measuring the incorporation of [^{35}S]methionine into cellular proteins. The cells were grown to confluence in 35-mm culture dishes, washed twice with phosphate-buffered saline, and kept in serum-free medium for 48 h. At the onset of the experiment, the medium was changed, and the inhibitors were added to fresh serum-free medium 1 h before the addition of [^{35}S]methionine (50 μCi/ml). After incubation for another 5 h the cells were quickly washed twice in ice-cold phosphate-buffered saline. The cells were then lysed in 1 ml 0.1 M Tris, pH 8.1, 10 mM EDTA, 0.5% Triton X-100 and 10 μg/ml of aprotinin for 15 min and scraped off with a rubber spatula. The proteins were precipitated by 7% trichloroacetic acid (TCA), and aliquots were analyzed either by SDS-polyacrylamide gel electrophoresis, followed by autoradiography, or by direct counting in a liquid scintillation counter.

3. Results

3.1. Cell-specific expression and regulation of uPAR, uPA and PAI-1 mRNA by various inhibitors of protein synthesis in human cancer cell lines

Cell-specific expression of uPAR, uPA and PAI-1 mRNA and their inducibility by various inhibitors of protein synthesis were assessed by RNA blot analysis (Fig. 1). A549 and 8387 are cell lines with very low or no detectable basal levels of uPAR mRNA, whereas a stronger signal was obtained in HT-1080 and RD cells (Fig. 1A). After treatment of the cells with various inhibitors of protein synthesis, a diversified picture

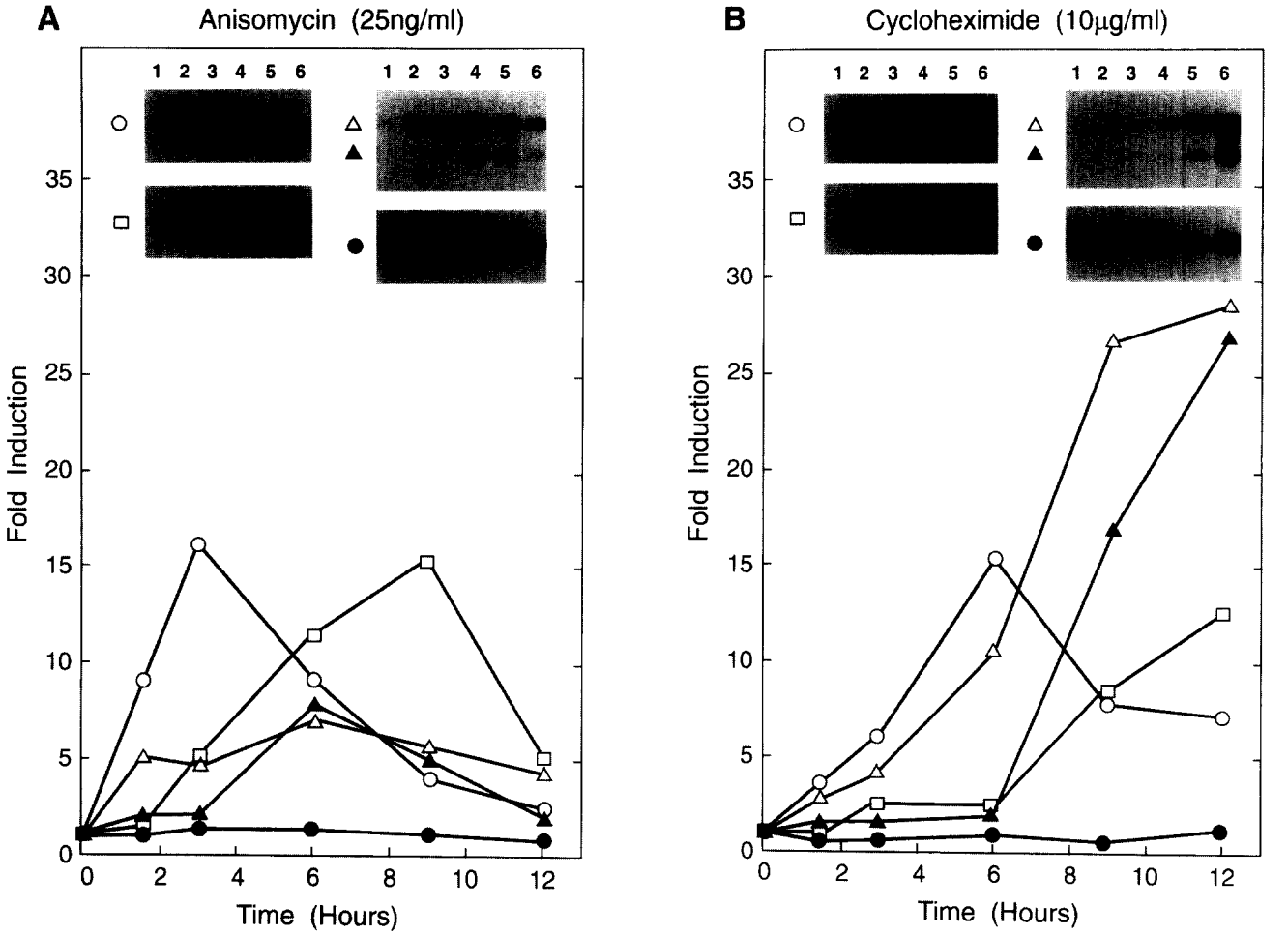

Fig. 3. Time-dependent effects of anisomycin and cycloheximide on the induction of uPAR, uPA and PAI-1 mRNA in A549 cells. Cells were cultured, harvested and analyzed by RNA blotting as described in the legend to Fig. 1, except that the cells were treated with either 25 ng/ml anisomycin (A) or 10 μg/ml cycloheximide (B) for the times indicated. The membranes were hybridized with a randomly primed ^{32}P-labeled uPAR cDNA probe (○), and, after stripping, rehybridized with a uPA cDNA probe (□), a PAI-1 cDNA probe (△, 3.4 kb; ▲, 2.4 kb) or a GAPDH cDNA probe (●). The relative amounts of uPAR, uPA and PAI-1 mRNA at each time point were estimated by spectrophotometric scanning of autoradiograms of the RNA blots hybridized with the uPAR, uPA or PAI-1 probes after normalization against the corresponding relative amounts of GAPDH mRNA. mRNA levels at time 0 were set equal to 1, and subsequent points indicate fold induction. The numbers in the insets indicate untreated cells (lane 1) and cells treated with either 25 ng/ml of anisomycin or 10 μg/ml of cycloheximide for 1.5 h (lane 2), 3 h (lane 3), 6 h (lane 4), 9 h (lane 5) or 12 h (lane 6).

emerges with respect to their effects on specific gene expression (Fig. 1A–C).

In A549 cells, cycloheximide (lane 2), anisomycin (lane 3) and emetine (lane 4) strongly induced uPAR mRNA and both the 3.4-kb and 2.4-kb PAI-1 transcripts and weakly induced uPA mRNA. Puromycin (lane 5) modestly induced only PAI-1 mRNA.

In the fibrosarcoma cell lines tested (8387 and HT-1080), anisomycin (lanes 8 and 13) induced uPAR, uPA and PAI-1 mRNA. Cycloheximide had no effect in HT-1080 cells (lane 12), whereas 8387 cells responded with an increase in both PAI-1 and uPA mRNA and a modest increase in uPAR mRNA (lane 7). Emetine did not affect any of the mRNAs in HT-1080 cells (lane 14) but induced the mRNA levels of uPAR, uPA and PAI-1 in 8387 cells (lane 9). Puromycin decreased the basal level of all mRNAs tested in both 8387 and HT-1080 cells (lanes 10 and 15).

In the rhabdomyosarcoma cell line RD, a strong increase in uPAR, uPA and PAI-1 mRNA levels was observed after treatment with cycloheximide (lane 17), whereas anisomycin (lane 18) increased only the level of PAI-1 mRNA; only minor effects, or none, were detected with the other inhibitors.

Ethidium bromide staining of the gel and blot showed comparative loading of intact RNA for each cell line (data not shown), and rehybridization with a GAPDH probe showed that the level of GAPDH mRNA was not affected by treatment with the various inhibitors of protein synthesis in any of the cell lines (Fig. 1D). The quantitative results of the densitometric scanning of the autoradiograms of the RNA blots are summarized in Table 1. Because the most pronounced effects were found with uPAR and PAI-1 mRNA expression in A549 cells, this cell line was selected for more detailed analysis.

3.2. *Dose-dependent effects of inhibitors of protein synthesis on synthesis and induction of uPAR mRNA in A549 cells*

A549 cells were treated with increasing concentrations of each of the four inhibitors for 6 h. [^{35}S]methionine incorpora-

tion into TCA-precipitable cellular proteins showed that anisomycin (Fig. 2A), cycloheximide (Fig. 2B), and emetine (data not shown) had an almost identical effect on total protein synthesis: 90% inhibition was achieved at 250 ng/ml. For puromycin, 2 μg/ml was required for 90% inhibition (data not shown). SDS-polyacrylamide gel electrophoresis of TCA-precipitated proteins, followed by autoradiography, showed that all the inhibitors blocked synthesis of all labeled proteins in a dose-dependent manner (data not shown).

No detectable effect on the uPAR mRNA level was observed by concentrations of cycloheximide <500 ng/ml (Fig. 2B, inset B′, lanes 3–5), whereas higher concentrations caused a dose-dependent increase in the uPAR mRNA (lanes 6–8). A very different dose-dependent pattern of uPAR mRNA induction was observed after treatment with anisomycin: a 9-fold increase in uPAR mRNA was observed at 10 ng/ml (Fig. 2A, inset A′, lane 3), reaching a 25-fold maximum at 100 ng/ml (lane 5), followed by a gradual decrease in the response at higher concentrations (lanes 6–8). Identical dose-dependent induction of uPA and PAI-1 mRNA was obtained after cycloheximide and anisomycin treatment (data not shown). In contrast, puromycin only slightly induced uPAR, uPA and PAI-1 mRNA at very high concentrations (50 and 100 μg/ml) (data not shown).

3.3. Time-dependent effects of inhibitors of protein synthesis on uPAR, uPA and PAI-1 mRNA levels in A549 cells

Total RNA was extracted from untreated A549 cells and from cells treated with each of the inhibitors for various times. After 1.5 h of anisomycin treatment (25 ng/ml), a signal for uPAR was visible and increased to a maximum of 15-fold after 3 h of treatment, followed by a slow decline to the basal level after 12 h (Fig. 3A). Cycloheximide (10 μg/ml) increased the uPAR mRNA level to 15-fold after 6 h, followed by a slow decline (Fig. 3B).

Only a modest effect of anisomycin on uPA mRNA level was observed for the first 6 h of treatment, followed by a maximal 15-fold increase after 9 h; after cyclohexi mide treatment a maximal 13-fold increase was observed after 12 h (Fig. 3A,B).

In untreated A549 cells the signal for PAI-1 mRNA was barely detectable (Fig. 3A,B). After 1.5 h of treatment with anisomycin (25 ng/ml), a 5-fold increase in the 3.4-kb transcript for PAI-1 was detected, whereas no effect on the 2.4-kb transcript was detected for up to 3 h of treatment. The level for both PAI-1 transcripts increased 6- to 7-fold after 6 h, followed by a decline. A similar, although more potent, induction pattern in the PAI-1 transcripts was observed after treatment with 100 ng/ml of anisomycin (data not shown). A more potent induction of the 3.4-kb transcript was observed after treatment with cycloheximide for 6 h, and induction increased for up to 12 h; the 2.4-kb transcript began to increase after 9 h (Fig. 3B).

4. Discussion

The cell-specific and gene-specific effects of four inhibitors of protein synthesis were examined to determine whether induction of the mRNAs for uPA, uPAR and PAI-1 is a general effect related to the blocking of protein synthesis by the inhibitors, or if it can also be caused by other effects of the drugs. Cycloheximide acts on the 60S ribosomal subunit to inhibit initiation, elongation and termination of protein synthesis and, like emetine and anisomycin, to stabilize polysomes. Anisomycin acts on the 60S subunit to block peptide formation, and emetine acts on the 40S subunit to block translocation. Puromycin is an analog of the 3′ end of aminoacyl tRNA and degrades polysomes [15].

From the results shown in Fig. 1 and Table 1, it is clear that inhibition of protein synthesis per se is not sufficient for induction of the specific mRNA. At concentrations that completely blocked protein synthesis, cycloheximide, anisomycin and emetine strongly induced the uPAR and PAI-1 mRNA levels in A549 cells, whereas puromycin induced a 5-fold increase only in the 3.4-kb PAI-1 mRNA. The fact that puromycin did not induce uPAR mRNA in A549 cells shows that blocking of protein synthesis is not sufficient for induction of a gene that is induced by other inhibitors of protein synthesis. In both fibrosarcoma cell lines tested (HT-1080 and 8387), cycloheximide did not affect the basal uPAR mRNA level, whereas in RD cells it induced a strong increase in uPA, uPAR and both PAI-1 mRNAs. Generally, the most pronounced inductive effects were obtained with cycloheximide, anisomycin and emetine, whereas only weak inductive or even reductive effects were obtained with puromycin. Although cycloheximide, anisomycin and emetine all stabilize mRNA by trapping it in polysomes, thereby shielding the RNA from cytoplasmic ribonucleases, only cycloheximide increased the levels of uPA, uPAR and PAI-1 mRNA in RD cells. Thus, a very complex and not fully understood induction pattern was observed after treatment with the various inhibitors.

The positive effect of protein synthesis inhibitors is not restricted to cancer cell lines. Cycloheximide was previously found to increase the level of uPAR mRNA in A549, U937 and HCT116 cells [11,16,22] and the level of PAI-1 mRNA in the human fibroblast cell line WI-38 [10]; however, it had no effect on the PAI-1 mRNA level in HepG2 cells for up to 12 h of treatment [23]. Although cycloheximide treatment for 6 h had no effect on PAI-1 mRNA in HT-1080 cells in this study, a previous study showed a positive effect after 16 h [24], and yet another study showed that cycloheximide induced PAI-1 mRNA in HT-1080 cells within 4–8 h [9]. These results show that even in the same cell line, various results can be obtained, depending on the source of the cell line. This variability suggests that cell-specific mechanisms are involved in the induction. Cell specificity is further supported by the finding in the present study that in A549 cells, cycloheximide, anisomycin and emetine all induced uPAR and PAI-1, whereas in RD cells, cycloheximide induced uPA, uPAR and PAI-1 mRNA, anisomycin induced only PAI-1 mRNA, and emetine had no effect.

As shown in Fig. 2B, cycloheximide increased the uPAR mRNA level in a dose-dependent manner only at concentrations at which ≥90% inhibition of protein synthesis was observed, whereas anisomycin strongly increased uPAR mRNA even at a concentration as low as 10 ng/ml, at which only partial inhibition of protein synthesis was observed (Fig. 2A). The maximal effect of anisomycin on uPAR mRNA was seen at 100 ng/ml, at which 70% inhibition of total protein synthesis was detected. Recent results obtained in C3H 10T1/2 cells showed that anisomycin, and to a lesser extent cycloheximide, but not emetine and puromycin, may act directly as an agonist in the phorbol ester and epidermal growth factor signal transduction pathway for c-*fos* and c-*jun* [11,12].

The results reported here suggest that cycloheximide and anisomycin mediate their effects on the various components of the plasminogen activation system in A549 cells through different mechanisms, although not identical to those described for c-*fos* and c-*jun* in C3H 10T1/2 cells; cycloheximide did not induce uPAR and PAI-1 mRNA at concentrations <500 ng/ml. It will be interesting to test the effect of protein synthesis inhibitors on c-*fos* and c-*jun* mRNA expression in A549 cells.

Analysis of the time-dependent effects of anisomycin and cycloheximide showed that both inhibitors induced a fast and potent, although transient, induction of uPAR mRNA, whereas their effect on uPA mRNA was delayed, with a maximal effect after 9 or 12 h, respectively. Similarly, the time-dependent effects of anisomycin and cycloheximide on PAI-1 mRNA induction were different. Both inhibitors induced the strongest and fastest increase in the 3.4-kb transcript, whereas the 2.4-kb transcript increased with slower kinetics, consistent with previous reports [10,25]. However, the effect of anisomycin was transient, with a maximal 6–7-fold effect after 6 h, whereas cycloheximide induced a prolonged effect that increased for at least 12 h. As shown in Fig. 1C and Table 1, the strongest increase in all tested cell lines was observed with the 3.4-kb transcript of PAI-1, suggesting that the two transcripts are separately regulated by the various inhibitors of protein synthesis. uPA, uPAR, and the 3.4-kb PAI-1 transcript, but not the 2.4-kb transcript, contain an AUUUA-rich sequence in the 3′ noncoding region that has been found in a number of short-lived mRNAs that encode inflammatory response mediators [26,27]. This sequence, together with additional sequences within the c-*fos* gene, confers instability to the c-*fos* transcript [28]. In the uPA mRNA, three instability-determining sites have been identified in the 3′ noncoding region [29]. Taken together, these data and the data reported here strongly support the idea that inhibitors mediate their effects on different genes through different molecular mechanisms.

Further experiments are needed to elucidate the detailed molecular mechanisms by which the inhibitors mediate their inductive effects. Nuclear run-on assays demonstrated only a minor effect on the transcription of uPAR, uPA and PAI-1 genes in A549 cells after treatment with cycloheximide, anisomycin or emetine (data not shown), whereas cycloheximide transiently increased uPA gene transcription in macrophages [30]. In other systems, an increase in mRNA stability has been reported after inhibition of protein synthesis [31], but it remains to be analyzed if the inhibitors have a similar effect on uPA, uPAR and PAI-1 mRNA.

In summary, it was found that induction of uPA, uPAR and PAI-1 mRNA by inhibitors of protein synthesis was dependent on the gene, the cell line and the type of inhibitor. Furthermore, inhibition of protein synthesis per se was not sufficient for induction. Further studies are needed to elucidate the detailed molecular mechanisms underlying the induction, or lack of induction of specific genes by protein synthesis inhibitors.

Acknowledgements: The excellent technical assistance of Kirsten Lund Jakobsen, John Post and Anne-Margrethe Poulsen is gratefully acknowledged. I thank Mary McKenney for editing the manuscript. This work was supported by the Danish Biotechnology Program, Center for Medical Biotechnology, the Danish Cancer Society, and Fru Astrid Thaysens Legat for Lægevidenskabelig Grundforskning.

References

[1] Danø, K., Andreasen, P.A., Grøndahl-Hansen, J., Kristensen, P., Nielsen, L.S. and Skriver, L. (1985) Adv. Cancer. Res. 44, 139–266.
[2] Mignatti, P. and Rifkin, D.B. (1993) Physiol. Rev. 73, 161–195.
[3] Mayer, M. (1990) Clin. Biochem. 23, 197–211.
[4] Andreasen, P.A., Georg, B., Lund, L.R., Riccio, A. and Stacey, S.N. (1990) Mol. Cell. Endocrinol. 68, 1–19.
[5] Blasi, F., Conese, M., Møller, L.B., Pedersen, N., Cavallaro, U., Cubellis, M.V., Faziola, F., Hernandez-Marrero, L., Limongi, P., Munõz-Cannoves, P., Resnati, M., Tittinen, L., Sidenius, N., Soravia, E., Soria, M.R., Stoppelli, M.P., Talarico, D., Teesalu, T. and Valcamonica, S. (1994) Fibrinolysis 8, Suppl. 1, 182–188.
[6] Ellis, V., Behrendt, N. and Danø, K. (1991) J. Biol. Chem. 266, 12752–12758.
[7] Laiho, M. and Keski-Oja, J. (1989) Cancer Res. 49, 2533–2553.
[8] Stoppelli, M.P., Verde, P., Grimaldi, G., Locatelli, E.K. and Blasi, F. (1986) J. Cell Biol. 102, 1235–1241.
[9] Medcalf, R.L., Van den Berg, E. and Schleuning, W.D. (1988) J. Cell Biol. 106, 971–978.
[10] Lund, L.R., Riccio, A., Andreasen, P.A., Nielsen, L.S., Kristensen, P., Laiho, M., Saksela, O., Blasi, F. and Danø, K. (1987) EMBO J. 6, 1281–1286.
[11] Lund, L.R., Rønne, E., Roldan, A.L., Behrendt, N., Rømer, J., Blasi, F. and Danø, K. (1991) J. Biol. Chem. 266, 5177–5181.
[12] Herschman, H.R. (1991) Annu. Rev. Biochem. 60, 281–319.
[13] Mahadevan, L.C. and Edwards, D.R. (1991) Nature 349, 747–748.
[14] Edwards, D.R. and Mahadevan, L.C. (1992) EMBO J. 11, 2415–2424.
[15] Pestka, S. (1971) Annu. Rev. Microbiol. 25, 487–562.
[16] Lund, L.R., Rømer, J., Rønne, E., Ellis, V., Blasi, F. and Danø, K. (1991) EMBO J. 10, 3399–3407.
[17] Lund, L.R., Georg, B., Nielsen, L.S., Mayer, M., Danø, K. and Andreasen, P.A. (1988) Mol. Cell. Endocrinol. 60, 43–53.
[18] Roldan, A.L., Cubellis, M.V., Masucci, M.T., Behrendt, N., Lund, L.R., Danø, K., Appella, E. and Blasi, F. (1990) EMBO J. 9, 467–474.
[19] Verde, P., Stoppelli, M.P., Galeffi, P., Di Nocera, P. and Blasi, F. (1984) Proc. Natl. Acad. Sci. USA 81, 4727–4731.
[20] Andreasen, P.A., Riccio, A., Welinder, K.G., Douglas, R., Sartorio, R., Nielsen, L.S., Oppenheimer, C., Blasi, F. and Danø, K. (1986) FEBS Lett. 209, 213–218.
[21] Fort, P., Marty, L., Piechaczyk, M., el Sabrouty, S., Dani, C., Jeanteur, P. and Blanchard, J.M. (1985) Nucleic Acids Res. 13, 1431–1442.
[22] Wang, Y., Jones, C.J., Dang, J., Liang, X., Olsen, J.E. and Doe, W.F. (1994) FEBS Lett. 353, 138–142.
[23] Bosma, P.J. and Kooistra, T. (1991) J. Biol. Chem. 266, 17845–17849.
[24] Andreasen, P.A., Pyke, C., Riccio, A., Kristensen, P. Nielsen, L.S., Lund, L.R., Blasi, F. and Danø, K. (1987) Mol. Cell. Biol. 7, 3021–3025.
[25] Mayer, M, Lund, L.R., Riccio, A., Skouv, J., Nielsen, L.S., Stacey, S.N., Danø, K. and Andreasen, P.A. (1988) J. Biol. Chem. 263, 15688- 15693.
[26] Caput, D., Beutler, B., Hartog, K., Thayer, R., Brown-Shimer, S. and Cerami, A. (1986) Proc. Natl. Acad. Sci.USA 83, 1670–1674.
[27] Shaw, G. and Kamen, R. (1986) Cell 46, 659–667.
[28] Shyu, A.-B., Belasco, J.G. and Greenberg, M.E. (1991) Genes Dev. 5, 221–231.
[29] Nanbu, R., Menoud, P.-A. and Nagamine, Y. (1994) Mol. Cell. Biol. 14, 4920–4928.
[30] Collart, M.A., Belin, D., Vassalli, J.-D., De Kossodo, S. and Vassalli, P. (1986) J. Exp. Med. 164, 2113–2118.
[31] Stimac, E., Groppi, V.E., Jr. and Coffino, P. (1984) Mol. Cell. Biol. 4, 2082–2090.