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Different Induction of Two Plasminogen Activator Inhibitor 1 mRNA Species by Phorbol Ester in Human Hepatoma Cells*

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In man, the plasminogen activator inhibitor 1 (PAI-1) gene codes for two mRNA species, one of 3.2 kilobases (kb) and the other of 2.4 kb. We report that the protein kinase C activating phorbol ester, phorbol 12-myristate-13-acetate (PMA), causes a different induction of the two PAI-1 mRNA species in the human hepatoma cell line, HepG2. Upon addition of 100 nM PMA, the level of the 3.2-kb PAI-1 mRNA species increased to 25-fold after 3 h, and then declined rapidly. The level of the 2.4-kb species increased more slowly and reached a maximal 18-fold stimulation after 6 h, followed by a gradual decrease towards control levels. Run-on analysis showed that PMA induces a transient 40-fold increase in PAI-1 gene transcription rate. The relative concentration of the two PAI-1 mRNA species in the nuclei of PMA-treated HepG2 cells shifted towards the 2.4-kb form, suggesting that changes in transcription termination site and/or post-transcriptional nuclear processing might contribute to their different accumulation. Also, the two mRNAs differ in turnover rate, with a half-life of about 0.85 h for the 3.2-kb form and a half-life of about 2.5 h for the 2.4-kb form.

By itself, cycloheximide had no effect on PAI-1 gene transcription rate or PAI-1 mRNA levels in HepG2. When added 1 h prior to PMA, however, cycloheximide prevented the induction of PAI-1 mRNA, which suggests that PMA exerts its stimulating transcriptional activity through a newly synthesized regulatory protein. When cycloheximide was added 2 h after PMA, when the PAI-1 gene transcription rate was maximally increased, the two PAI-1 mRNAs reached even higher levels than with PMA alone and maximal mRNA levels were maintained for a much longer period (up to 8 h). Thus, ongoing protein synthesis is required for both the induction and the transient nature of the PMA-induced PAI-1 mRNA accumulation.

We conclude that the differential accumulation of the two PAI-1 mRNAs by PMA in serum-starved HepG2 cells is due both to changes in transcription termination and/or post-transcriptional nuclear processing and to differences in half-life between the two mRNAs in a process that requires ongoing protein synthesis.

Plasminogen activation provides an important source of localized proteolytic activity, not only during fibrinolysis (Collen, 1980), but also during ovulation (Beers *et al.*, 1975), cell migration (Strickland *et al.*, 1976), tumor invasion and metastasis (Ossowski and Reich, 1983), hormone processing (Virji *et al.*, 1980), collagenase activation (Danø *et al.*, 1985), angiogenesis (Gross *et al.*, 1983), and a variety of other physiological processes. Precise regulation of plasminogen activator (PA)[1] activity thus constitutes a critical feature of many biological processes. This control may occur at various levels, including the synthesis and secretion of PAs, and the interaction with specific PA inhibitors, PAIs (Sprengers and Kluft, 1987). PAI-1 is the major PAI found in blood, and has been shown to be expressed in many body tissues (Quax *et al.*, 1990; Erickson *et al.*, 1990). It is a 50-kDa glycoprotein, belonging to the serpin family (Pannekoek *et al.*, 1986; Ny *et al.*, 1986), and a specific inhibitor of both tissue-type PA and urokinase-type PA. PAI-1 has also been detected in platelet releasates and in the conditioned media of a variety of cells, including endothelial cells, hepatocytes, smooth muscle cells, and several tumor cell lines (see Sprengers and Kluft, 1987). Recently, PAI-1 has been identified as a matrix protein (Rheinwald *et al.*, 1987; Pöllänen *et al.*, 1987; Knudsen *et al.*, 1987).

The biosynthesis of PAI-1 appears to be highly regulated. For example, PAI-1 synthesis can be induced by endotoxin (Colucci *et al.*, 1985; Emeis and Kooistra, 1986), inflammatory mediators (Emeis and Kooistra, 1986; Van Hinsbergh *et al.*, 1988; Van den Berg *et al.*, 1988; Schleef *et al.*, 1988; Sawdey *et al.*, 1989), glucocorticoids (Gelehrter *et al.*, 1983; Andreasen *et al.*, 1987), insulin (Alessi *et al.*, 1988; Kooistra *et al.*, 1989), and phorbol ester (Mayer *et al.*, 1988). Understanding of the complex regulation of PAI-1 biosynthesis at the molecular level is complicated by the existence in human cells of two distinct PAI-1 mRNA species, one of 3.2 and one of 2.4 kb (Ginsburg *et al.*, 1986; Ny *et al.*, 1986). Both PAI-1 mRNAs have an identical coding region, but differ in the length of their 3′-untranslated region, possibly as a result of alternative polyadenylation (Loskutoff *et al.*, 1987; Bosma *et al.*, 1988). Interestingly, the 3.2-kb species, but not the 2.4-kb species, contains an AU-rich sequence, which has been associated with message lability (Shaw and Kamen, 1986; Caput *et al.*, 1986; Fort *et al.*, 1987). Although several authors have noticed a variation in the relative concentrations of the two mRNAs between different experiments (Van den Berg *et al.*, 1988; Schleef *et al.*, 1988; Lucore *et al.*, 1988), the reason for this variation was not clear, and no study has previously been performed to address the regulatory aspects of the occurrence of the two PAI-1 mRNA forms.

We observed that the protein kinase C activator, phorbol

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[1] The abbreviations used are: PA, plasminogen activator; PAI-1, plasminogen activator inhibitor 1; PMA, phorbol 12-myristate 13-acetate; CHX, cycloheximide; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate.

12-myristate-13-acetate (PMA), strongly enhances the levels of the two PAI-1 mRNA species in the human hepatoma cell line, HepG2. A striking aspect of this induction is that the levels of the two messengers each increase with a different time profile and to a different extent. Further experiments were directed at the role of transcriptional activation and mRNA stability in determining the levels of the two PAI-1 mRNA species. We report that PMA induces a strong, but transient increase in the transcription of the PAI-1 gene and an accelerated decay of the 3.2-kb species. Both processes require *de novo* RNA and protein synthesis, as suggested by experiments with inhibitors of transcription and translation.

EXPERIMENTAL PROCEDURES

Materials—Hybond N filters, random primer kit (Multiprime DNA labeling system), ^{32}P-labeled dCTP and UTP, and [^{35}S]methionine were obtained from Amersham Corp. Cycloheximide (CHX), actinomycin D, α-amanitin, and PMA were purchased from Sigma. Stock solutions of PMA (10^{-4} M) were prepared in ethanol. 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) was obtained from Boehringer Mannheim. Other materials used in the methods described below have been specified in detail in the relating references.

Cell Culture Experiments—The human hepatoma cell line HepG2 (Knowles *et al.*, 1980) was cultured in Dulbecco's modification of Eagle's medium, supplemented with 10% (v/v) fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and glutamine (2 mM) under 5% CO_2, 95% air atmosphere at 37 °C (Havekes *et al.*, 1983). For the experiments, confluent cells were used. Fresh complete medium was added to the cells 40 h before the start of the experiments. 16 h prior to the experiments, the medium was changed to Dulbecco's modified Eagle's medium supplemented with 0.1% (w/v) human serum albumin, glutamine, penicillin, and streptomycin. Cells were incubated with test compounds for various times up to 24 h. Preparation of nuclei for RNA isolation was performed as described by Marzluff and Huang (1984). In this procedure, highly purified nuclei are prepared by centrifugation through a dense solution of sucrose. Isolated nuclei used in this study were clean as judged by microscopy and were contaminated with less than 1% of cytoplasmic RNA as deduced from the amount of glyceraldehyde-3-phosphate dehydrogenase mRNA found in the nuclei as compared to that in total cellular RNA preparations, with yields of nuclei exceeding 50%.

Protein Synthesis—Overall protein synthesis was determined by measuring the incorporation of [^{35}S]methionine into the 10% (w/v) trichloroacetic acid-precipitable fraction of radiolabeled conditioned media and cell lysates.

mRNA Analysis—Total cellular RNA and nuclear RNA were isolated according to the method of Chomczynski and Sacchi (1987). 4–10 μg of RNA, as determined spectrophotometrically, was subjected to gel electrophoresis in formaldehyde-agarose gels (Maniatis *et al.*, 1982). After electrophoresis, the RNA was transferred to Hybond N according to the instructions of the manufacturer. Prehybridization and hybridization were done at 65 °C in 0.5 M NaH_2PO_4/Na_2HPO_4 (pH 7.2), 7% (w/v) SDS, 1 mM EDTA (modified from Church and Gilbert, 1984). Hybridization was performed with cDNA probes (1 ng/ml) labeled to approximately 5×10^8 cpm/μg of DNA by the random primer method. After hybridization, the filters were washed twice for 15 min at 65 °C, with respectively: 2 × SSC, 1% SDS; 1 × SSC, 1% SDS, and 0.1% SSC, 1% SDS. Quantification was performed by cutting the bands, and counting them in scintillation fluid or by exposing the membranes to Kodak X-AR5 film with an intensifying screen. The relative intensities of the bands were determined by densitometric scanning.

The probes used were a 2.5-kb *Eco*RI PAI-1 cDNA fragment (Van den Berg *et al.*, 1988), a 1.2-kb *Eco*RI PAI-2 cDNA fragment provided by Dr. E. K. O. Kruithof: (University of Lausanne Medical School, Lausanne, Switzerland) (Schleuning *et al.*, 1987), and a 1.2-kb *Pst*I cDNA fragment of rat glyceraldehyde-3-phosphate dehydrogenase, a kind gift from Dr. R. Offringa: (University of Leiden, Leiden, The Netherlands) (Fort *et al.*, 1988). All probes were isolated from low melting agarose and purified using the gene clean kit (Bio 101, Inc.).

Run-on Analysis—Preparation of nuclei for run-on analysis was performed as described by Greenberg and Ziff (1984). After isolation, the nuclei were resuspended in glycerol buffer (50 mM Tris, pH 8.3, 40% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA), frozen in liquid nitrogen, and stored at −80 °C until use. Per run-on assay, approximately 5×10^6 nuclei were used. The elongation reaction and RNA isolation were performed according to Nevins (1987). In short, the nuclei were incubated for 20 min at 30 °C in a 200-μl reaction buffer, containing: 20 mM Tris (pH 7.9), 20% (v/v) glycerol, 140 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM each of ATP, CTP, GTP, 2 μM [α-^{32}P] UTP (800 Ci/mM (1 Ci = 3.7×10^{10} Bq), 10 mM creatine phosphate, 20 units/ml creatine kinase, and 1000 units/ml RNasin. At the end of the incubation, 800 μl of HSB buffer (10 mM Tris, pH 7.4, 0.5 M NaCl, 50 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 units/ml DNase I) was added, followed by the addition of 1 ml of extraction buffer (10 mM Tris, pH 7.4, 1% SDS, 20 mM EDTA) as soon as the viscosity had disappeared. The RNA was isolated by hot phenol extraction. Contaminating DNA was further degraded by a DNase treatment for 20 min at 37 °C in 20 mM Tris (pH 7.7), 1 mM $MgCl_2$, and DNase I, 50 μg/ml. After phenol extraction, the RNA was incubated for 20 min in ice-cold 0.2 M NaOH. The solution was then neutralized with HEPES buffer (pH 7.4). To remove unincorporated label, repeated ethanol precipitations with high salt were performed.

Hybridization of Labeled RNA to DNA—DNA (2 μg) was blotted onto Hybond N filter using a minifold filtration apparatus in alkali blotting solution (1.5 M NaCl, 0.25 M NaOH). Prehybridization and hybridization were performed at 65 °C in 0.5 M NaH_2PO_4/Na_2HPO_4 (pH 7.2), 7% SDS, 1 mM EDTA. The RNA was hybridized for 65 h. After hybridization, the filters were washed several times with 2 × SSC and treated with RNase and proteinase K as described (Nevins, 1987). Quantification was carried out by exposing the filters to Kodak X-AR5 film followed by densitometric scanning.

RESULTS

Time Course of PMA-induced Increases in PAI-1 mRNA in HepG2—Treatment of HepG2 cells for 8 h with 0.01–200 nM PMA showed a dose-dependent increase in PAI-1 mRNA levels, reaching a maximal stimulation at 100–200 nM (results not shown). Fig. 1 represents the time course of the transient

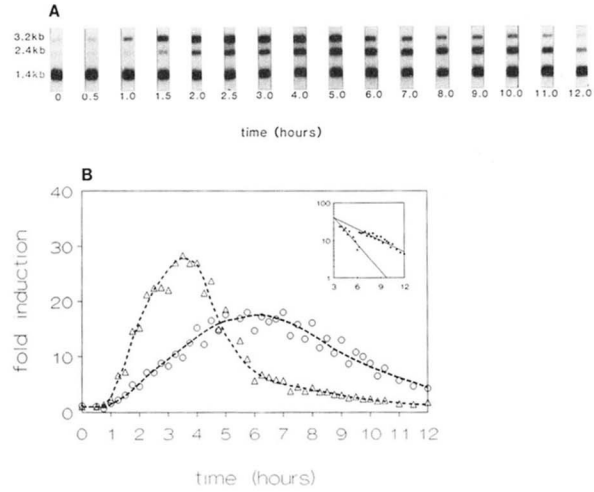

FIG. 1. **Time course of the induction of PAI-1 mRNA by PMA in HepG2 cells.** HepG2 cells were preincubated with serum-free medium for 16 h, and then treated with PMA (100 nM) in serum-free medium for the times indicated. RNAs were isolated, and 5 μg of total cellular RNA was analyzed by Northern blotting followed by hybridization to ^{32}P-labeled cDNA fragments of human PAI-1 and glyceraldehyde-3-phosphate dehydrogenase (*A*). Quantification of the 3.2 (△) and 2.4 (○) kb PAI-1 mRNA species was performed by cutting the bands and counting them in a liquid scintillation counter. The amount of each mRNA species present at the various time points is presented as -fold induction of the amount present at $t = 0$ (*B*). In the *inset* the decline of both mRNA species on a semi-log scale is shown.

increase of the two PAI-1 mRNAs, with lengths of 3.2 and 2.4 kb, by 100 nM PMA. After a lag period of 45 min, the level of both mRNAs starts to increase, but with a different time profile and to a different extent. The 3.2-kb species is maximally 25- to 30-fold stimulated after about 3 h, and then rapidly declines again to the prestimulatory level. The 2.4-kb messenger reaches maximally 18 times control values after 6 h, and then declines, more slowly than the 3.2-kb species, to basal levels. In the same experiment no significant effect of PMA on glyceraldehyde-3-phosphate dehydrogenase mRNA content in the cells was seen (Fig. 1*A*).

Run-on Analysis—To investigate whether the induction of the PAI-1 mRNAs by PMA is at the level of transcription, nuclear run-on experiments were performed. For that purpose, nuclei from HepG2 cells that had been incubated with PMA for various times, were isolated. Nascent nuclear transcripts were elongated in the presence of [α-^{32}P]UTP and hybridized to various cDNA probes immobilized on Hybond N membranes. As shown in Fig. 2, PMA causes a strong and transient increase in the transcription of the PAI-1 gene. As early as 30 min after the addition of PMA to HepG2, the transcription of the PAI-1 gene is enhanced. After 2 h the transcription reaches its maximal 40-fold induction, followed by a rapid return back to prestimulatory levels at 8 h after PMA addition. In control cells, and in cells incubated with CHX for 4 h, PAI-1 transcripts stayed at the low prestimulatory level (not shown). PMA incubation had no effect on the transcription of the glyceraldehyde-3-phosphate dehydrogenase gene. No detectable signal was given by the PAI-2 cDNA and the pUC 18 probe, which served as negative controls. In the presence of the RNA polymerase II inhibitor, α-amanitin (10 μg/ml) no transcription of the PAI-1 or glyceraldehyde-3-phosphate dehydrogenase gene was seen (results not shown).

Analysis of PAI-1 mRNAs in Nuclei from PMA-treated HepG2 Cells—To investigate the possibility that differential accumulation of the 3.2- and 2.4-kb mRNAs is the result of a change in polyadenylation site or another post-transcriptional nuclear process, we analyzed PAI-1 mRNA levels in nuclei from HepG2 cells incubated for various times with PMA. As shown in Fig. 3, the ratio between the 3.2-kb form and the 2.4-kb form in the nuclei also shifts in time towards the 2.4-kb form, albeit to a lesser extent than that in total cellular RNA: at 2 h, the ratio between the two forms is about 2:1 in both the nuclear and cellular RNA preparations, whereas after 6 h, this ratio is 1:2 and 1:7, respectively. This suggests that differential nuclear processing of the two PAI-1 transcripts might at least partly contribute to the differential accumulation of the two PAI-1 mRNA species.

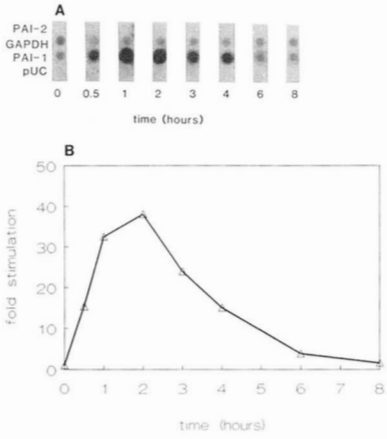

FIG. 2. **Analysis of the PAI-1 gene transcription rate after PMA stimulation.** HepG2 cells were incubated with PMA (100 nM) for various times up to 8 h, and then nuclei were isolated. Run-on assays were performed by elongation of the nuclear transcripts in the presence of [α-^{32}P]UTP. The ^{32}P-labeled RNA was isolated and hybridized to plasmid DNA of: PAI-1; PAI-2, as a eukaryotic negative control; glyceraldehyde-3-phosphate dehydrogenase, as a control for variation in mRNA labeling; and, as a prokaryotic control, pUC. All probes were blotted on Hybond N using a minifold filtration apparatus. The radioactivity is visualized by autoradiography (*A*). The intensity of the *dots* is determined by densitometric scanning (*B*). Values are given relative to corresponding $t = 0$ values, and are means of two independent experiments.

Stability of the Two PAI-1 mRNAs—To assess the role of mRNA stability in the observed changes in the relative levels of the two PAI-1 mRNAs, we determined their half-lives from the data presented in Figs. 1 and 2. On the assumption that PAI-1 mRNA synthesis is negligible after 6 h (Fig. 2), a half-life of 2.5 h can be calculated for the 2.4-kb mRNA species from its subsequent decline (see *inset* of Fig. 1). Very similar half-life times were calculated from experiments in which the disappearance of the 2.4-kb mRNA form was determined following addition of actinomycin D (5 μg/ml), an inhibitor of total DNA transcription, or DRB (200 μM), a specific inhibitor of RNA polymerase II (Mittleman *et al.*, 1983), at 4 h after induction with PMA (results not shown). For the 3.2-kb messenger a more rapid decline is seen, with an apparent half-life time of 0.85 h between 4 and 6 h after PMA addition (see *inset* of Fig. 1). This half-life time for the 3.2-kb mRNA species is likely to be a slight overestimation of the actual half-life, since PAI-1 mRNA synthesis is still elevated during this period. However, the 3.2-kb mRNA levels in the following period, when PAI-1 gene transcription rates had returned to basal levels, were too low to allow accurate estimates. Blocking of RNA synthesis by the addition of actinomycin D or DRB 2 h after PMA could not be used in these experiments, since these compounds blocked the rapid decay of the 3.2-kb mRNA, thereby keeping this messenger at the high induced level (results not shown). Due to the low preinduction levels of the both PAI-1 mRNAs, no determination of stability was possible before induction with PMA.

Effect of Cycloheximide on the Induction Profile of PAI-1 mRNA—Although CHX alone had no effect on PAI-1 gene transcription (see above), several papers suggest that inhibition of protein synthesis may lead to PAI-1 mRNA accumulation (Mayer *et al.*, 1988; Van den Berg *et al.*, 1988). We therefore tested the effect of CHX (10 μg/ml) on PAI-1 mRNA levels in HepG2, both under basal and PMA-stimulated conditions, when protein synthesis was found to be inhibited for over 95%. In control cells, incubation with CHX up to 12 h neither altered total PAI-1 mRNA levels nor did

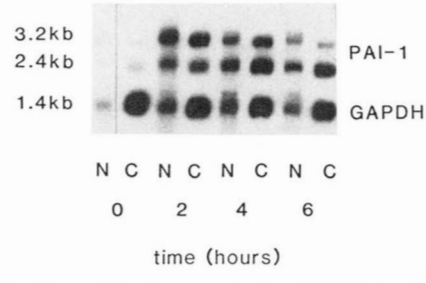

FIG. 3. **Northern blotting analysis of PAI-1 mRNA induction by PMA in HepG2 cells and nuclei.** HepG2 cells were preincubated with serum-free medium for 16 h, and then treated with PMA (100 nM) in serum-free medium for the times indicated. 4 μg of RNA isolated from nuclei (*N*) or cells (*C*) was analyzed by Northern blotting followed by hybridization to ^{32}P-labeled DNA fragments of human PAI-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

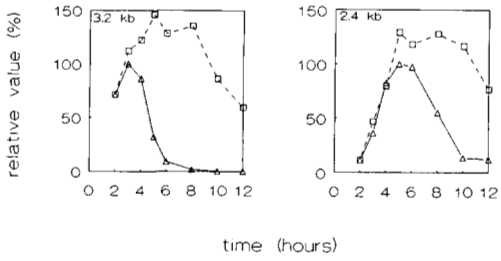

FIG. 4. **Effect of the protein synthesis inhibitor, cycloheximide, on the induction of PAI-1 mRNA by PMA.** HepG2 cells were preincubated with serum-free medium for 16 h, and then treated with PMA (100 nM). 2 h after PMA addition, CHX (10 μg/ml) was added. At various times (2–12 h after PMA addition), RNAs were isolated and analyzed by Northern blot hybridization. The Northern blots were exposed to Kodak X-AR film, and the bands were quantified by densitometric scanning. The amounts found of each mRNA are given as percentage values of the maximally enhanced levels in the presence of PMA alone, *i.e.* the amount of the 3.2-kb species at 3 h and of the 2.4-kb species at 6 h. PMA (△); PMA + CHX (□).

it change the relative concentration of the two PAI-1 mRNA forms. Thus, in unstimulated HepG2 cells, addition of CHX has no effect on the stability or synthesis of PAI-1 mRNAs. When CHX was added 1 h prior to PMA, the rapid induction of both mRNA species was completely suppressed (results not shown). However, when CHX was added two h after PMA, when transcription rate is maximal (see Fig. 2), both messengers reach even higher levels than with PMA alone, and maximal mRNA levels are maintained for a longer period (Fig. 4). In fact, the induction profiles of the two species become very similar. Whereas with PMA alone, the 3.2-kb species reaches its peak value after 3 h and then declines with a half-life of about 0.85 h, in the presence of both PMA and CHX, the 3.2-kb mRNA continues to increase, reaching 1.5-fold maximal PMA-induced values after 5 h. This plateau level is maintained up to 8 h, and then the 3.2-kb PAI-1 mRNA level slowly declines. The 2.4-kb mRNA species reaches its peak value after 6 h with PMA alone. In the presence of both PMA and CHX, the 2.4-kb species reaches (a slightly higher) maximal level after 6 h again, but the decline of this species is delayed: the fall in the 2.4-kb mRNA concentration starts after 10 h. Thus, ongoing protein synthesis plays a crucial role in both the induction of PAI-1 gene transcription by PMA and the course of the induction profile.

DISCUSSION

In man, two distinct PAI-1 mRNA species are found, one of 3.2 and one of 2.4 kb (Ginsburg *et al.*, 1986; Ny *et al.*, 1986), which differ in the length of their 3′-untranslated region, possibly as a result of alternative polyadenylation (Loskutoff *et al.*, 1987; Bosma *et al.*, 1988). In this paper we show that treatment of HepG2 cells with PMA resulted in a strong and transient increase in both PAI-1 mRNAs, but each species with a different induction profile: at early time points the 3.2-kb species predominates, whereas at later time points the 2.4-kb species is more abundant. It was found that PAI-1 mRNA accumulation was preceded by a strongly increased rate of transcription and that it was dependent on *de novo* protein synthesis: CHX, when added 1 h prior to PMA, completely inhibited the accumulation of PAI-1 mRNA. Thus the induction of PAI-1 mRNA levels may be dependent on the synthesis of transcription factors, which enhance PAI-1 gene expression.

We have found that PMA also affects the ratio between the two PAI-1 mRNAs in the nuclei: in time a shift towards the 2.4-kb species is seen (Fig. 3). This could be the result of a change in the termination site of PAI-1 gene transcription, regulation at the level of poly(A) site selection, or another post-transcriptional nuclear event. It is interesting in this respect that the induction of PAI-1 expression in HepG2 is accompanied by a transient increase in c-*myc* mRNA.[2] PAI-1 gene expression has been shown to be regulated by c-myc protein, which may act at the level of RNA export, splicing, or nuclear RNA turnover (Prendergast and Cole, 1989; Prendergast *et al.*, 1990).

Besides nuclear post-transcriptional processes, the different stability of the two PAI-1 mRNAs may contribute to the different induction profile of the two PAI-1 mRNAs: after PMA addition to HepG2, the 3.2-kb species shows a half-life time of about 0.85 h, which is at least three times shorter than the 2.5 h calculated for the 2.4-kb messenger. The enhanced lability of the 3.2-kb species in comparison to the 2.4-kb species may be due to the presence of an AU-rich sequence in the 3′-untranslated region of the 3.2-kb form. Such a sequence, absent in the 2.4-kb PAI-1 mRNA, has been reported to be associated with the rapid turnover of the mRNAs coding for lymphokines, cytokines, and proto-oncogenes (Shaw and Kamen, 1986; Caput *et al.*, 1986; Fort *et al.*, 1987). Recently, a cytosolic protein has been identified that binds specifically to RNA molecules containing an AU-rich sequence (Malter, 1989), and therefore could be involved in the rapid degradation of the AU sequence containing mRNAs. Whether or not such a protein is also responsible for the rapid degradation of the 3.2-kb PAI-1 mRNA in PMA-treated HepG2 cells cannot be deduced from our experiments.

The 2.4-kb PAI-1 mRNA is only found in humans and higher primates: the extra putative polyadenylation site in the PAI-1 gene generating this smaller mRNA has been recently acquired during primate evolution (Cicila *et al.*, 1989). The appearance of a second messenger form in humans, which lacks an AU-rich sequence in the 3′-untranslated region, can be anticipated to have physiological consequences: stimulation of PAI-1 gene expression may result in prolonged biosynthesis of PAI-1, and, consequently, in prolonged inhibition of plasminogen activation.

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