Post-transcriptional regulation of H-ferritin gene expression in human monocytic THP-1 cells by protein kinase C

Jong-Hwei S. PANG, Chia-Jung WU and Lee-Young CHAU*

Division of Cardiovascular Research, Institute of Biomedical Sciences, Academia Sinica, Nankang, Taipei, 11529, Taiwan, R.O.C.

The mRNA coding for H-ferritin was highly induced in human monocytic THP-1 cells following treatment with phorbol 12-myristate 13-acetate (PMA). The induction was detected at 3 h, reached maximal levels at 12 h, and was sustained for up to 48 h subsequent to PMA exposure. PMA-induced up-regulation of H-ferritin gene expression was also observed in other leukaemic cell lines, HL60 and U937, but not in non-leukaemic cell types, including human fibroblasts, endothelial cells and smooth muscle cells. The effect of PMA could be completely blocked by the protein kinase C inhibitor, H-7. Furthermore, treatment of THP-1 cells with bacterial phospholipase C also produced a marked increase in expression of H-ferritin mRNA, suggesting the activation of protein kinase C was responsible for the accumulation of mRNA. Nuclear run-off experiments demonstrated that PMA did not increase the transcriptional rate of the H-ferritin gene. In contrast, the half-life of the H-ferritin mRNA measured in the presence of actinomycin D was greatly prolonged in PMA-treated cells. The induction of H-ferritin mRNA by PMA required no protein synthesis. Conversely, treatment of THP-1 cells with protein synthesis inhibitor, cycloheximide, resulted in a 4–5-fold increase in H-ferritin mRNA. The increase in the stability of the H-ferritin mRNA was also observed in cells treated with cycloheximide. Taken together, these results suggest that the stability of H-ferritin mRNA in THP-1 is subjected to regulation via a protein kinase C-mediated phosphorylation on existing putative protein factor(s).

INTRODUCTION

Ferritin, a multimeric iron-storage protein found widely distributed throughout the living creatures, consists of a hollow protein shell with a capacity for storing up to 4500 iron atoms in its central cavity as a ferric–oxyhydroxyphosphate complex [1–3]. This unique structure allows the ferritin molecule to sequester iron in a soluble, bioavailable and non-toxic form despite differences in cell type and cytological location. Each ferritin molecule consists of 24 subunits of two types, heavy (H) and light (L), which can combine in highly variable ratios depending upon the cellular requirement for iron [1–3].

Regulation of H- and L-ferritin gene expression can occur at multiple levels. In addition to the presence of excessive iron, increased ferritin gene expression has also been observed in other circumstances, including cell differentiation, proliferation, inflammation and specific hormonal stimulation [4–11]. Studies focused on iron-induced gene expression has provided a significant insight into the regulation of ferritin at the translational level. It has been shown that both the 5′-untranslated regions of H- and L-ferritin mRNAs contain a conserved 28-nucleotide iron regulatory element (IRE) [12], which interacts with a 90 kDa repressor protein [13,14]. Increased intracellular iron concentration has been shown to cause the release of this protein and permits, in a co-ordinate manner, extensive ferritin synthesis at the translational level [15,16]. In some cell types, iron has also been shown to up-regulate the transcription of the L-ferritin gene, without affecting the transcription of H-ferritin [17,18]. Furthermore, recent studies have revealed that regulation of H-ferritin gene expression by the cytokine tumour necrosis factor and hormone thyrotropin occurs at the transcriptional level [10,19]. Nevertheless, very little is known about the mechanism(s) involved in the differential regulation of the expression of H- and L-ferritin genes at the post-transcriptional level.

In previous studies, it has been shown that induction of the differentiation of human leukaemic cells results in an increase in the H/L ratio of ferritin gene expression [20,21]. However, the molecular mechanism underlying the differential regulation of ferritin gene expression in leukaemic cells was not well understood. To address this issue, in the present study, we used the human monocytic THP-1 cell line as a cell model to elucidate the regulation of ferritin gene expression during differentiation of monocytes toward macrophages induced by phorbol 12-myristate 13-acetate (PMA). It was of interest to find that the half-life of H-ferritin mRNA, but not L-ferritin mRNA, was markedly prolonged in PMA-treated THP-1 cells. Furthermore, the increase in H-ferritin mRNA stability appeared to be mediated by a phosphorylative event activated by protein kinase C (PKC) in a cell-type specific manner.

EXPERIMENTAL

Cell cultures

Human THP-1, U937 and HL60 cells were grown in RPMI 1640 medium supplemented with 10% (v/v) fetal-calf serum. Human embryonic lung fibroblast NTU-1 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal-calf serum. Human umbilical vein endothelial cells (HUVECs) were subcultured in EGM™ medium supplied from Clonetics. Human aortic vascular smooth muscle cells (HA-VSMCs) were subcultured in SmGM™ medium (Clonetics). Cells were incubated in a humidified atmosphere of 95% air/5% CO_2 at 37 °C. For PMA treatment, suspension cell cultures were grown to a density of 1×10^6 cells/ml and other cultured cells were grown to confluency.

Abbreviations used: H7, 1-(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride; PMA, phorbol 12-myristate 13-acetate; H, heavy; L, light; IRE, iron regulatory element; PKC, protein kinase C; HUVEC, human umbilical vein endothelial cell; HA-VSMC, human aortic vascular smooth muscle cell; GAPDH, glyceraldehyde-3-phoshate dehydrogenase; CHX, cycloheximide.

* To whom correspondence should be addressed.

CDNA probes

The cDNA clones for H-ferritin (1.1 kb), L-ferritin (1.3 kb), and β-globin (0.9 kb) were originally isolated from a human aortic cDNA library constructed in bacteriophage λgt11 [22]. The cDNA insert of each phage clone was amplified by PCR using a pair of λgt11 primers flanking the *Eco*RI cloning site. Amplified cDNA was electrophoresed on a 1 % agarose gel and excised from the gel. The cDNA was then extracted and purified from the gel using a DNA purification kit from Qiagen Inc. To prepare cDNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 1 μg of the total RNA isolated from THP-1 cells was reverse-transcribed and an aliquot of the cDNA was subjected to PCR using two GAPDH-specific primers [23]. The amplified cDNA fragment (0.55 kb) was then purified as described above.

RNA isolation and Northern blotting

Cells (5×10^6) incubated with or without various agents for the indicated times were harvested and lysed using guanidine isothiocyanate solution [24]. Total RNA was isolated by a one-step phenol–chloroform–isoamyl alcohol extraction method described by Chomczynski and Sacchi [24]. Equal amounts of RNAs were loaded on to a 1 % agarose gel containing 6.6 % formaldehyde and electrophoresed for 2 h at 120 V. The RNAs were transferred on to Hybond-N membrane (Amershan) using vacuum blotting apparatus. After UV-cross-linking, the RNAs on the membranes were prehybridized with a solution containing 50 % formamide, 1 % SDS, 1 M NaCl, 10 % dextran sulphate and 0.1 mg/ml salmon sperm DNA at 42 °C for at least 1 h. Denatured [α-^{32}P]dCTP-labelled DNA probe, prepared by random priming (Amersham), was added directly to the prehybridization solution and the hybridization was continued overnight. Membranes were washed once at 50 °C with 2 × SSC (1 × SSC consisted of 150 mM NaCl, 15 mM sodium citrate, pH 7.0) containing 1 % SDS for 30 min followed by 0.2 × SSC/1 % SDS for another 30 min at 50 °C. Membranes were then exposed to X-ray film for 6 h or until the signals were visible.

Nuclear run-off assay

Cells (2×10^7) treated with or without 100 ng/ml PMA for 24 h were harvested and washed twice with ice-cold PBS. The cell pellet was resuspended in 1 ml of lysis buffer containing 10 mM Tris/HCl, pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$ and 0.5 % (v/v) Nonidet P-40. After incubation at 4 °C for 5 min, nuclei were collected by centrifugation at 500 ***g*** for 5 min at 4 °C and washed once with lysis buffer. Nuclei were then resuspended in 100 μl of 50 mM Tris/HCl, pH 8.3, containing 0.1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol and 40 % glycerol. *In vitro* transcription reaction was conducted at 30 °C for 30 min in buffer containing 10 mM Tris/HCl, pH 8.0, 5 mM $MgCl_2$, 300 mM KCl, 1.5 mM ATP, 1.5 mM CTP, 1.5 mM GTP and 100 μCi of [α-^{32}P]UTP. The radiolabelled RNA was isolated by the guanidine isothiocyanate extraction as described above. Samples of radiolabelled RNA (1×10^6 c.p.m./ml) were hybridized to nylon membrane slot-blotted with 5 μg of the tested cDNAs. The cDNAs for GAPDH and β-globin were used as the internal and negative controls, respectively. The hybridization conditions were the same as those described for the Northern blot except that 0.1 mg/ml yeast tRNA was included in the prehybridization solution instead of salmon sperm DNA. After washing, the membrane was exposed to X-ray film for 3 days.

RESULTS

Induction of ferritin gene expression by PMA in THP-1 cells

The regulation of ferritin gene expression during monocyte–macrophage differentiation was examined by treating the human monocytic leukaemia cell line THP-1 with PMA for various intervals, followed by Northern blot analysis of the H- and L-ferritin mRNA levels in the cells. As shown in Figure 1, the level of H-ferritin mRNA was increased approximately 3-fold by PMA treatment for 3 h, reached the maximal level (> 10-fold) at 12 h and maintained this level up to 48 h. In contrast, the level of L-ferritin mRNA was not significantly altered during the 48 h period. Since the mRNA levels of commonly used internal controls, such as GAPDH and β-actin, in THP-1 cells were decreased by PMA treatment (results not shown), the ethidium bromide-stained RNA gel was displayed to show the equal loading of RNA samples. It is known that PMA elicits many different responses throughout a variety of cells and leads to altered cellular functions [25]. To determine whether the induction of the H-ferritin gene is cell-type specific or merely a general effect of PMA, other non-leukaemic human cells, including human lung fibroblasts (NTU-1), aortic smooth muscle cells (HA-VSMCs) and umbilical endothelial cells (HUVECs), were also treated with PMA for 24 h and the levels of both H- and L-ferritin mRNAs were examined. As illustrated in Figure 2(A), PMA did not enhance the expression of either the H- or the L-ferritin gene in these cell lines. In contrast, however, the H- but not L-ferritin mRNA in other leukaemic cell lines, such as HL60

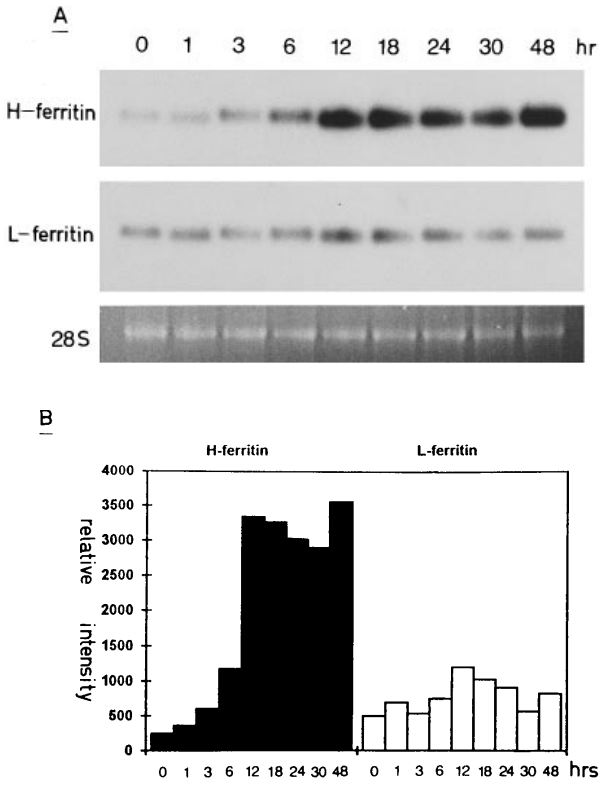

Figure 1 Time course of PMA-induced ferritin mRNA accumulation in THP-1 cells

(**A**) THP-1 cells were treated with PMA (100 ng/ml) for various times as indicated. RNA was isolated for Northern blot analysis with ^{32}P-labelled H- or L-ferritin cDNA. (**B**) Densitometric scanning result of the autoradiogram.

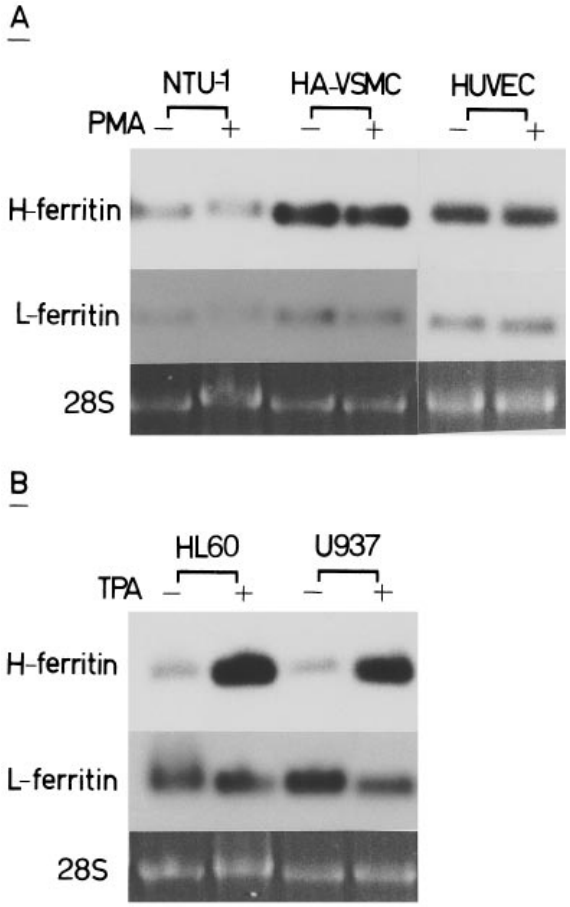

Figure 2 Cell-type-specific induction of H-ferritin mRNA accumulation by PMA in leukaemic cell lines

(**A**) Northern blot analysis of H- and L-ferritin mRNA expression in NTU-1 cells, HA-VSMCs and HUVECs treated with PMA (100 ng/ml) for 24 h. (**B**) Induction of H-ferritin mRNA accumulation in leukaemic HL60 and U937 cells treated with PMA for 24 h.

and U937, was increased subsequent to PMA treatment (Figure 2B). These results demonstrate that the induction of H-ferritin gene expression by PMA may be leukaemic cell-specific.

PMA-induced H-ferritin gene expression was mediated by PKC

To examine whether the induction of H-ferritin gene expression by PMA is mediated by PKC, THP-1 cells were treated together with PMA and various concentrations of the PKC inhibitor, H7 [1-(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride] [26], and the level of H-ferritin mRNA assessed. As shown in Figure 3(A), H7 inhibited the PMA-induced H-ferritin mRNA increment in a dose-dependent manner. At a concentration of 30 μM, H7 completely attenuated the effect of PMA. When the concentration of H7 exceeded 30 μM, the constitutive expression level of H-ferritin was also suppressed. To test further the role of PKC in up-regulation of H-ferritin gene expression, THP-1 cells were treated with *Bacillus cereus* phospholipase C for 24 h and the mRNA level of H-ferritin measured. As shown in Figure 3(B), treatment of cells with phospholipase C, which leads to generation of diacylglycerol and the subsequent activation of PKC [25], also resulted in a prominent increase in H-ferritin mRNA without affecting L-ferritin mRNA levels.

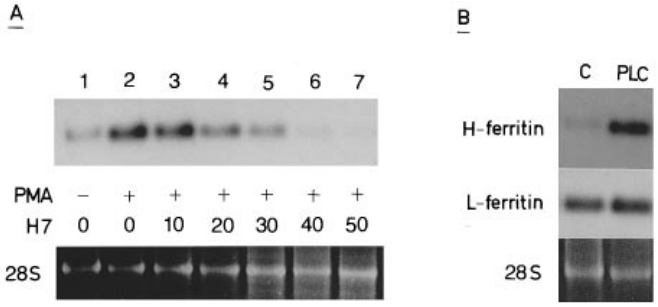

Figure 3 (A) Inhibitory effect of PKC inhibitor, H7, on the PMA-induced H-ferritin mRNA accumulation in THP-1 cells and (B) effect of phospholipase C on ferritin gene expression

(**A**) Cells were preincubated with various concentrations of H7 as indicated for 15 min followed by treatment of PMA (100 ng/ml) for 24 h. Total RNA was then isolated for Northern blot analysis. (**B**) THP-1 cells were treated with *Bacillus cereus* phospholipase C (1 unit/ml) for 24 h. The levels of H- and L-ferritin mRNA were then analysed by Northern blot.

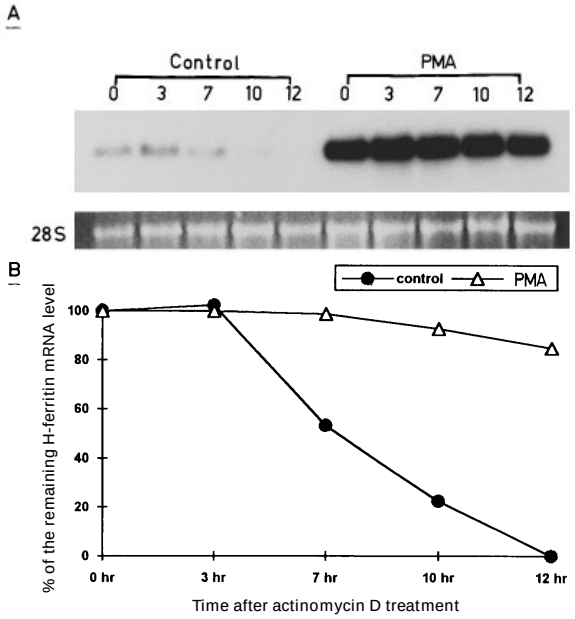

Figure 4 Analysis of H-ferritin mRNA stability in control and PMA-treated THP-1 cells

(**A**) THP-1 cells were treated with or without PMA (100 ng/ml) for 12 h prior to the addition of actinomycin D (10 μg/ml). Cells were harvested at indicated time points following the actinomycin D treatment and RNA was isolated for Northern blot analysis. (**B**) H-ferritin mRNA level expressed as a percentage of the initial value was plotted versus time after actinomycin D treatment.

Collectively, these results strongly support the involvement of PKC in the induction of H-ferritin gene expression in THP-1 cells.

The increased H-ferritin gene expression induced by PMA resulted from the prolonged half-life of the mRNA

To examine further the biochemical mechanism underlying the induction of H-ferritin gene expression by PKC, nuclei were isolated from both control cells and PMA-treated cells and the rate of H-ferritin gene transcription measured by a nuclear run-

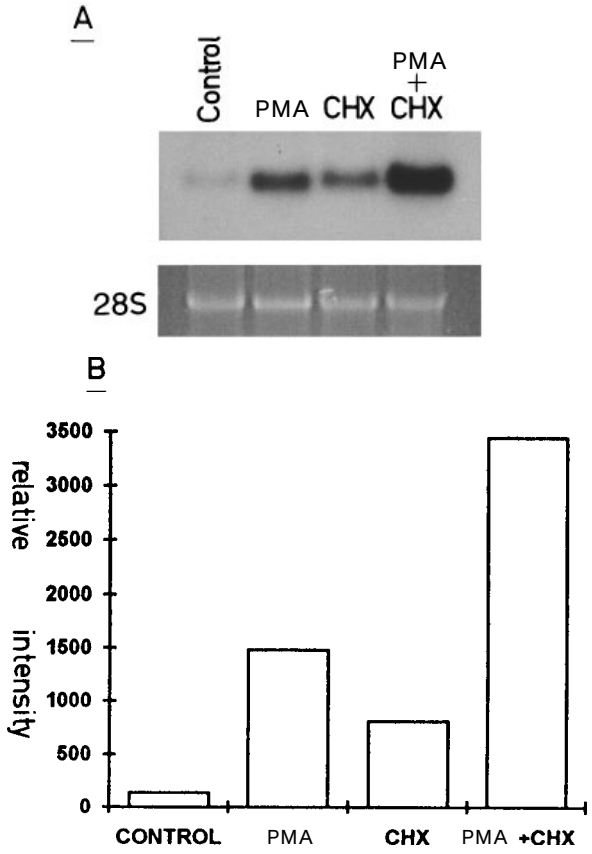

Figure 5 Synergistic effect of PMA and CHX on the induction of H-ferritin gene expression in THP-1 cells

THP-1 cells were treated with PMA (100 ng/ml), CHX (10 μg/ml), or PMA and CHX together for 24 h. Cells were harvested and the H-ferritin mRNA level analysed.

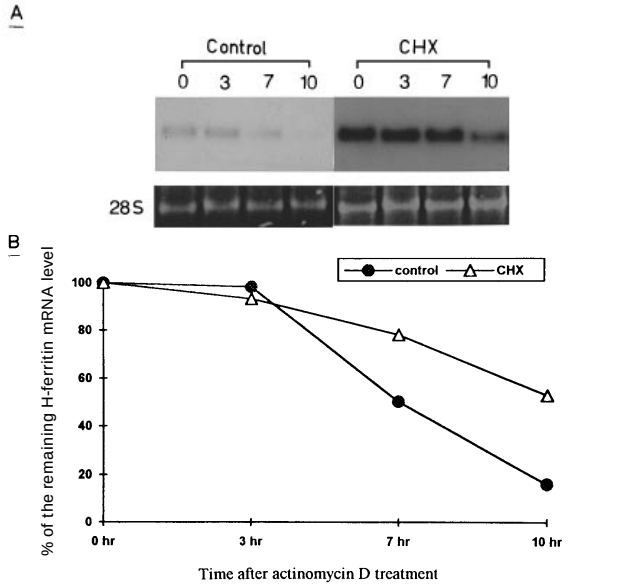

Figure 6 Prolonged H-ferritin mRNA stability in CHX-treated THP-1 cells

(**A**) THP-1 cells were treated with or without CHX (10 μg/ml) for 24 h before the addition of actinomycin D (10 μg/ml). At indicated time points, RNA was isolated for Northern blot analysis. (**B**) H-ferritin mRNA level expressed as a percentage of the initial value was plotted versus time after actinomycin D treatment.

off experiment. It was found that THP-1 cells treated with PMA did not significantly alter the rate of transcription for either H- or L-ferritin genes (results not shown), indicating that the increase in H-ferritin gene expression in THP-1 cells by PMA is not regulated at the transcriptional level. A further experiment was carried out to assess the stability of the H-ferritin transcript in the presence of the RNA synthesis inhibitor, actinomycin D. Total RNA from either control cells or PMA-treated cells was isolated at various intervals subsequent to the addition of actinomycin D and the level of H-ferritin mRNA analysed. As illustrated in Figure 4, the half-life of H-ferritin transcript in control cells was around 7 h. In contrast, the half-life of the H-ferritin mRNA in PMA-treated cells was extended beyond 12 h. This result clearly demonstrates that increased levels of H-ferritin mRNA in PMA-treated cells were due to the prolonged stability of the transcript.

Cycloheximide (CHX) superinduced the H-ferritin gene expression increased by PMA in THP-1 cells

The prolonged half-life of H-ferritin mRNA in PMA-treated THP-1 cells is presumably caused by the inhibition of the H-ferritin mRNA degradation. Since protein factors have largely been shown to be involved in the process of RNA degradation [27,28], the effect of the protein synthesis inhibitor, CHX, on the PMA-induced H-ferritin gene expression was also examined. It was found that addition of CHX at any time points throughout the 12 h treatment of PMA in THP-1 cells did not lower the increased level of the H-ferritin mRNA (results not shown). This result ruled out the possibility that the prolonged half-life of the H-ferritin transcript was due to the newly synthesized protein which protects the H-ferritin mRNA from degradation. Conversely, it was noted that the level of H-ferritin mRNA in PMA-treated cells was further increased by treatment of CHX in a time-dependent manner, implying that inhibition of protein synthesis can somehow superinduce the PMA-induced H-ferritin gene expression in THP-1 cells. To confirm this observation, cells were treated with CHX in the absence or presence of PMA for 24 h and the H-ferritin mRNA level was assessed. As shown in Figure 5, treatment of THP-1 cells with CHX alone also induced the H-ferritin mRNA, though to a lesser degree compared with that in PMA-treated cells. When THP-1 cells were treated with PMA and CHX in concert, the H-ferritin mRNA increased synergistically.

CHX prolonged the half-life of H-ferritin mRNA

To further test whether the inhibition of protein synthesis by CHX leads to the stabilization of H-ferritin mRNA, the half-life of H-ferritin transcript in cells treated with CHX was also examined. As shown in Figure 6, the half-life of the H-ferritin transcript in cells treated with CHX was increased to 10 h compared with that of the control cells (7 h). The result clearly demonstrates the involvement of a labile protein factor in the degradation of H-ferritin mRNA.

DISCUSSION

In the present study, we have demonstrated that PMA specifically induced in a cell-type-specific manner the accumulation of H-ferritin mRNA, but not L-ferritin mRNA, in human leukaemic THP-1 cells. The PMA-induced H-ferritin mRNA increment

resulted from the stabilization of the mRNA but not the increase in transcriptional rate. Activation of PKC appeared to be responsible for the PMA effect. H-7, a PKC inhibitor, not only abolished the H-ferritin mRNA accumulation induced by PMA, but also affected the basal expression level of H-ferritin gene expression, implicating kinase activity as being required for the maintenance of the constitutive expression of H-ferritin in THP-1 cells. Furthermore, the observation that the protein synthesis inhibitor CHX, superinduced the H-ferritin gene expression implicates the involvement of labile protein factor(s) in the regulation of H-ferritin transcript turnover. Nevertheless, when PMA and CHX, were added together to the cell culture, the increase in H-ferritin mRNA was greater than the additive effect, suggesting that these two agents may act through different mechanisms.

Although the underlying mechanisms that regulate the mRNA stability of different genes have unique features, it has been shown that specific RNA sequences located mainly within the untranslated region of each gene bind to specific protein factors and play a role in the turnover of messages [27–30]. For example, the transferrin receptor mRNA contains in its 3′-untranslated region an iron-responsive element, which resembles the IRE in the 5′-untranslated region of ferritin mRNA, to facilitate mRNA degradation in the presence of iron [31]. Furthermore, studies on many cellular labile mRNAs, such as oncogene and cytokine mRNAs, have revealed the presence of AUUUA pentamer in their 3′-untranslated regions [32,33]. Specific proteins (AUBF) binding to the AU motif were identified and shown to modulate the rate of the mRNA degradation [34–38]. Subsequent studies have further demonstrated that the activity of AUBF is subjected to regulation by redox switch and phosphorylation, which mediates PMA- and ionophore-induced labile mRNA stabilization [39]. Recently, down-regulation of an RNA-binding activity to a unique sequence at the 3′-untranslated region of mammalian ribonucleotide reductase R2 mRNA has also been shown to be responsible for the PMA-induced alteration in R2 message stability [40]. Although the present study does not provide direct evidence to demonstrate the binding of specific factor to the H-ferritin mRNA, it does clearly show that the turnover rate of H-ferritin message in THP-1 cells is regulated by a mechanism which involves the PKC-mediated phosphorylation of a pre-existing putative factor, which in turn leads to the stabilization of H-ferritin mRNA.

It is well documented that both L- and H-ferritin mRNAs contain a conserved IRE on their 5′-untranslated region to respond to iron regulation at the translational level [12–16]. However, the post-transcriptional regulation on their mRNA stabilities appears to be regulated by different mechanisms or factors in THP-1 cells. It is conceivable that the preferential expression of H-ferritin gene in myelocytic cells undergoing differentiation is associated, at least in part, with the alteration in the turnover rate of the H-ferritin message. It would be of great interest to know whether the PKC-mediated phosphorylation is also responsible for the increased H-ferritin expression in leukaemic cells following differentiation induced by other agents. Since activation of PKC is a common pathway in cells following activation by various stimuli, the cell-type-specific effect of PMA on H-ferritin gene induction may be due to the presence of specific protein factors in THP-1 cells. Nevertheless, the possibility of the differential expression of PKC isoenzymes in different cell types [41] and the involvement of specific PKC isoenzyme in the regulation of H-ferritin gene induction cannot be ruled out. Further studies are required to clarify these issues.

In summary, the present study demonstrates for the first time that the alteration in message stability by PKC-mediated phosphorylation plays an important role in the regulation of H-ferritin gene expression in leukaemic cells. The identification of the potential target for PKC as well as the corresponding *cis*-element on the H-ferritin mRNA appears to be essential for further understanding the molecular mechanism underlying the regulation of the turnover of H-ferritin in leukaemic cells.

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REFERENCES

1 Theil, E. C. (1987) Annu. Rev. Biochem. **56**, 289–315
2 Theil, E. C. (1990) Adv. Enzymol. Relat. Areas Mol. Biol. **63**, 421–449
3 Rice, D. W., Ford, G. G., White, J. L., Smith, J. M. A. and Harrison, P. (1983) in Structure and Function of Iron Storage and Transport Proteins (Urushizaki, I., Aisen, P., Listowski, I. and Drysdale, J. W., eds.), pp. 11–16, Elsevier, Amsterdam
4 McCarty, G. A., Chan, A. K., Choy, B. K. and Wright, J. A. (1990) J. Biol. Chem. **265**, 7539–7547
5 Stevens, P. W., Dodgson, J. B. and Engel, J. D. (1987) Mol. Cell Biol. **7**, 1751–1758
6 Torti, S. V., Kwak, E. L., Miller, S. C., Miller, L. L., Ringold, G. M., Myambo, K. B., Young, A. P. and Torti, F. M. (1988) J. Biol. Chem. **263**, 12638–12644
7 Colucci-D'Amoto, L., Ursini, M. V., Colletta, G., Cirafici, A. and de Franciscis, V. (1989) Biochem. Biophys. Res. Commun. **165**, 506–511
8 Wei, Y., Miller, S. C., Tsuji, Y., Torti, S. V. and Torti, F. M. (1990) Biochem. Biophys. Res. Commun. **169**, 289–296
9 Chazenbalk, G. D., Wadsworth, H. L. and Rapoport, B. (1990) J. Biol. Chem. **265**, 666–670
10 Liau, G., Chan, L. M. and Feng, P. (1991) J. Biol. Chem. **266**, 18819–18826
11 Miller, L. L., Miller, S. C., Torti, S. V., Tsuji, Y. and Torti, F. M. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 4946–4950
12 Hentze, M. W., Caughman, S. W., Rouault, T. A., Barriocanal, J. G., Dancis, A., Harford, J. B. and Klausner, R. D. (1987) Science **238**, 1570–1573
13 Leibold, E. A. and Munro, H. N. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 2171–2175
14 Haile, D. J., Rouault, T. A., Harford, J. B., Kennedy, M. C., Blondin, G. A., Beinert, H. and Klausner, R. D. (1992) Proc. Natl. Acad. Sci. U.S.A. **89**, 11735–11739
15 Klausner, R. D., Rouault, T. A. and Hardford, J. B. (1993) Cell **72**, 19–26
16 Munro, H. N. (1990) J. Cell. Biochem. **44**, 107–115
17 Cairo, G., Bardella, L., Schiaffonati, L., Arosio, P., Levi, S. and Bernelli-Zazzera, A. (1985) Biochem. Biophys. Res. Commun. **133**, 314–321
18 White, K. and Munro, H. N. (1988) J. Biol. Chem. **263**, 8938–8942
19 Kwak, E. L., Larochelle, D. A., Beaumont, C., Torti, S. V. and Torti, F. M. (1995) J. Biol. Chem. **270**, 15285–15293
20 Chou, C.-C., Gatti, R. A., Fuller, M. F., Concannon, P., Wong, A., Chada, A., Davis, R. C. and Salser, W. A. (1986) Mol. Cell. Biol. **6**, 566–573
21 Beaumont, C., Jain, S. K., Bogard, M., Nordmann, Y. and Drysdale, J. (1987) J. Biol. Chem. **262**, 10619–10623
22 Pang, J.-H. S., Jiang, M.-J., Chen, Y.-L., Wang, F.-W., Wamg, D. L., Chu, S.-H. and Chau, L.-Y. (1996) J. Clin. Invest. **97**, 2204–2212
23 Chau, L.-Y., Peck, K., Yen, H.-H. and Wang, J.-Y. (1994) Biochem. J. **301**, 911–916
24 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
25 Nishizuka, Y. (1988) Nature (London) **334**, 661–665
26 Hodaka, H., Inagaki, M., Kawamoto, S. and Sasaki, Y. (1984) Biochemistry **23**, 5036–5041
27 Klausner, R. D. and Harford, J. B. (1989) Science **246**, 870–872
28 Atwater, J. A., Wisdom, R. and Verma, I. M. (1990) Annu. Rev. Genet. **24**, 519–541
29 Carter, B. Z. and Malter, J. S. (1991) Lab. Invest. **65**, 610–621
30 Chen, F. Y., Amara, F. M. and Wright, J. A. (1993) EMBO J. **12**, 3977–3986
31 Mullner, E. W. and Kuhn, L. C. (1988) Cell **52**, 893–901
32 Shaw, G. and Kamen, R. (1986) Cell **46**, 659–667
33 Brawerman, G. (1989) Cell **57**, 9–10
34 Malter, J. S. (1989) Science **246**, 664–666
35 Bohjanen, P. R., Petryniak, B., June, C. H., Thompson, C. B. and Lindsten, T. (1991) Mol. Cell. Biol. **11**, 3288–3295
36 Brewer, G. (1991) Mol. Cell. Biol. **11**, 2460–2466
37 Gillis, P. and Malter, J. S. (1991) J. Biol. Chem. **266**, 3172–3177
38 Vakalopoulou, E., Schaack, J. and Schenk, T. (1991) Mol. Cell. Biol. **11**, 3355–3364
39 Malter, J. S. and Hong, Y. (1991) J. Biol. Chem. **266**, 3167–3171
40 Amara, F. M., Chen, F. Y. and Wright, J. A. (1994) J. Biol. Chem. **269**, 6709–6715
41 Hug, H. and Sarre, T. F. (1993) Biochem. J. **291**, 329–343

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