ELSEVIER

FEBS Letters 338 (1994) 47–52

FEBS 13553

FEBS LETTERS

# Induction of protein-tyrosine phosphatase LC-PTP by IL-2 in human T cells

## LC-PTP is an early response gene

Masaaki Adachi[a,*], Masuo Sekiya[a], Masaho Ishino[b], Hiroko Sasaki[b], Yuji Hinoda[a], Kohzoh Imai[a], Akira Yachi[a]

[a]*Department of Internal Medicine (Section 1), and* [b]*Division of Biochemistry in Cancer Research Institute, Sapporo Medical University School of Medicine, Sapporo 060, Japan*

Received 18 October 1993; revised version received 8 December 1993

**Abstract**

Tyrosine phosphorylation has been implicated in interleukin 2 (IL-2)-induced growth signaling and the phosphorylation levels are regulated by the balance of tyrosine kinase and tyrosine phosphatase activities. Here, we demonstrate the rapid activation of a leukocyte tyrosine phosphatase LC-PTP (HePTP) gene expression by IL-2 in an IL-2 dependent human T cell ILT-Mat. Accumulation of LC-PTP mRNA appeared at 1 h and peaked at 6 h after IL-2 stimulation, simultaneous with the G1 to early S phase, and the induction of LC-PTP mRNA did not require protein synthesis. LC-PTP protein increased approximately 6-fold at 8 h after IL-2 stimulation. Nuclear run-on assays showed that the induction of LC-PTP mRNA expression is mostly due to transcriptional activation. These data suggest that LC-PTP is an early response gene and its protein seems to be a crucial molecule which regulates the tyrosine phosphorylation level during T cell proliferation.

*Key words:* Protein-tyrosine phosphatase; IL-2; Early response gene; Signal transduction

## 1. Introduction

Accumulating evidence suggests that tyrosine kinase activity is essential for some cellular responses, i.e. proliferation, differentiation, and transformation. In T-cells, IL-2 stimulation promotes the progression of competent cells from the G1 phase to the S phase and has been shown to result in increased tyrosine phosphorylation of cellular substrates; this is usually initiated by $p56^{lck}$ [1].

Protein tyrosine phosphatases (PTPs) reverse the action of PTKs by removal of the phosphotyrosyl groups from target proteins and thus have been thought to function as a counter-regulator of PTKs. However, recent advances in characterization of PTPs reveal complicated cross-talk between PTPs and PTKs. Over-expression of cytoplasmic PTP1B can suppress the tumorigenesis of *erbB2*-transformed NIH3T3 cells [2], whereas overexpression of transmembrane PTPα enhances the tyrosine kinase (PTK) activity of c-*src* and leads to a transformed phenotype [3]. Importantly, CD45 dephosphorylates $p56^{lck}$ to activate its PTK [4] and also has some key role in T-cell activation via TCR, which requires tyrosine phosphorylation events [5]. These findings strongly suggest some PTPs activate specific PTKs and specifically trigger increased protein tyrosine phosphorylation in intracellular signal transduction. It follows that PTPs must be strictly regulated while they actuate cellular responses to extracellular signals just as PTKs are.

Recently, we and others cloned a nontransmembrane PTP cDNA (LC-PTP [6,7], HePTP [8]) which is preferentially expressed in T-cells and induced by some mitogens [6,8]. This particular expression pattern suggests LC-PTP may play a crucial role in T-cells. We further investigated LC-PTP gene induction and its regulation following IL-2 stimulation. We report here that LC-PTP is an early response gene and the induction leads to increase of its protein. This strongly suggests LC-PTP seems to regulate tyrosine phosphorylation level of protein(s) crucial for T cell proliferation.

## 2. Materials and methods

### 2.1. Reagents and cell culture

ILT-Mat cells were maintained in RPMI-1640 with 10% fetal calf serum (FCS) containing 400 U/ml IL-2 (kindly provided by Takeda

*Corresponding author. Fax: (81) (11) 613 1141.


*SSDI* 0014-5793(93)E1470-7

Chemical Pharmacy, Japan). For factor deprivation experiments, cells were washed twice, and resuspended without IL-2 for 30–36 h. Following IL-2 stimulation, RNA expression levels, nuclear run-on assay, cell cycle status, and protein synthesis were analyzed. For analysis of the effect of protein synthesis on LC-PTP gene expression, ILT-Mat cells were cultured with 10 μg/ml of cycloheximide (CHX) (Sigma). Human peripheral blood mononuclear cells (PBMC) were isolated from a normal donor by Ficoll–Hypaque centrifugation. The composition of PBMC populations, determined by indirect immunofluorescence with cell-sorter analysis, was 71.8% $CD3^+$, 38.5% $CD4^+$, 32.6% $CD8^+$, 67.0% $TCR\alpha\beta^+$, 17.2% $CD19^+$, 2.8% $IL\text{-}2R^+$. The $CD3^+$ cells were enriched by the incubation in CD3 antibody-covered CELLector flasks (Applied Immune Sciences Inc., Menlo Park, CA) for 48 h following the instructions of the manufacturer. The enriched T cells (90.2% $CD3^+$, 0.3% $CD19^+$, 26.6% $IL\text{-}2R^+$) were activated by IL-2 after incubation in 10% FCS-RPMI-1640 for 24 h.

*2.2. Northern blot analysis*

Ten μg of the total RNAs extracted from ILT-Mat cells were separated in 1% agarose formaldehyde gels by electrophoresis and transferred to nitrocellulose filters. The filters were hybridized with $^{32}P$-labeled various cDNA fragments as probes; the 0.6-kb 5′ region of the human LC-PTP cDNA clone [6], the 2.2-kb human full-length c-*myc* cDNA [9], the 0.3-kb human PTP1B cDNA fragment [7,10] and the β-actin cDNA probe [11]. The hybridization conditions were 50% formamide, 2 × SSC (1 × SSC is 150 mM NaCl/15 mM trisodium citrate), 2.5 × Denhart's solution, 0.1% SDS, 2.5% dextran sulfate, and denatured salmon testes DNA (100 μg/ml). The same blots were used to test the LC-PTP, c-*myc*, PTP1B and β-actin transcripts under identical conditions. The amount of hybridization was quantified by scanning the autoradiographs with a densitometer (Model d1650, Bio-Rad) or laser densitometry using an NIH image program.

*2.3. Cell cycle analysis*

The ILT-Mat cells processed under the above conditions were evaluated for cell cycle status following the modified protocol CycleTEST, Becton Dickinson [12]. The cells were fixed with ethanol for 30 min at −20°C, washed, and incubated with 1 mg/ml of ribonuclease A for 30 min at 37°C, followed by addition of an ice-cold solution containing propidium iodide to stain the nuclei (final propidium concentration, 125 μg/ml). The fluorescence in each nucleus was measured with FACScan and the proportions of the cells in each phase of the cycle were determined by analysis with the computer program CellFIT (Becton Dickinson).

*2.4. Nuclear run-on transcription assays*

A total of $1 \times 10^7$ to $2.5 \times 10^7$ ILT-Mat cells were suspended in 500 μl of lysis buffer (10 mM Tris pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP40), washed twice with lysis buffer, suspended in 100 μl of storage buffer (50 mM Tris pH 8.3, 40% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA) and stored at −80°C. As essentially described by M.R. Stallcup et al. [13], 10 μg of linearized, denatured plasmid DNAs containing LC-PTP, c-*myc* and β-actin cDNA fragments (described above) per slot were transferred to nitrocellulose using a slot blot apparatus (Hybri-slot Manifold, BRL), and prehybridized overnight in hybridization solution (50% Formamid, 5 × SSC, 50 mM Na-phosphate pH 6.5, 1 × Denhardt and 250 μg/ml of salmon testis DNA). The nuclei were incubated with 100 μCi of [α-$^{32}$P]UTP in reaction buffer (10 mM Tris pH 8.0, 5 mM $MgCl_2$, 300 mM KCl and 5 mM ATP, CTP, GTP) and the newly synthesized RNA was extracted and hybridized in the same buffer at 42°C for approximately 48 h. No distinct hybridization to plasmid DNA alone was detected on any of the filters (data not shown). Each transcript was scanned by laser densitometry and its gray scale unit was determined by NIH image program [14].

*2.5. Antibodies*

A region of the LC-PTP cDNA encoding amino acids 100–360 was isolated and cloned into the bacterial expression plasmid pMAL-cRI. Maltose binding protein (MAL) fusion protein was purified using amylose resin (NEB) and eluted using 10 mM maltose in accordance with manufacturer's protocol. Anti-LC-PTP sera were generated in New Zealand white male rabbits using approximately 100 μg of the purified bacterial recombinant pMAL-LC-PTP fusion protein.

*2.6. Preparation of recombinant baculovirus and lysates from baculovirus-infected insect cells*

The full-length LC-PTP gene was cloned as an *Xba*I 1.6 kb fragment into pBlueBac (Invitrogen). Recombinant baculoviruses encoding the LC-PTP gene were isolated by standard procedure using the kit from Invitrogen. Sf9 cells were infected with recombinant or wild type baculoviruses, harvested 2 days after infection, washed, and resuspended in lysis buffer, which consisted of 20 mM Tris-HCl pH 7.6, 50 mM NaCl, 1 mM dithiothreitol, 1% NP40 and 1 mM PMSF.

*2.7 Western blot analysis*

Human gastric cancer cell line JRST, T-cell leukemia cell lines Jurkat and ILT-Mat were washed and suspended in 100 μl of lysis buffer (100 mM NaCl, 2 mM EDTA, 10 mM sodium orthovanadate, 1 mM PMSF, 1% NP40 and 50 mM Tris pH 7.2). The protein concentrations of these lysates were calculated with Protein Assay (Bio-Rad) and each lysate was incubated for 2 h with 20 μl of anti-LC-PTP-agarose. Following extensive washing, immunoprecipitated proteins were subjected to SDS-PAGE and transferred to Immobilon PVDF (Millipore). The blots were incubated with blocking solution containing 3% BSA, 10 mM Tris pH 8.2, 140 mM NaCl, and 0.01% $NaN_3$. Then, they were incubated with 2 μg/ml of anti-LC-PTP antibody or pre-immunized rabbit IgG for 2 h in washing solution (150 mM NaCl, 10 mM Tris pH 7.5 and 0.01% Tween 20) with 2% FCS, followed by an additional 1 h of incubation with alkaline phosphatase-conjugated monoclonal anti-

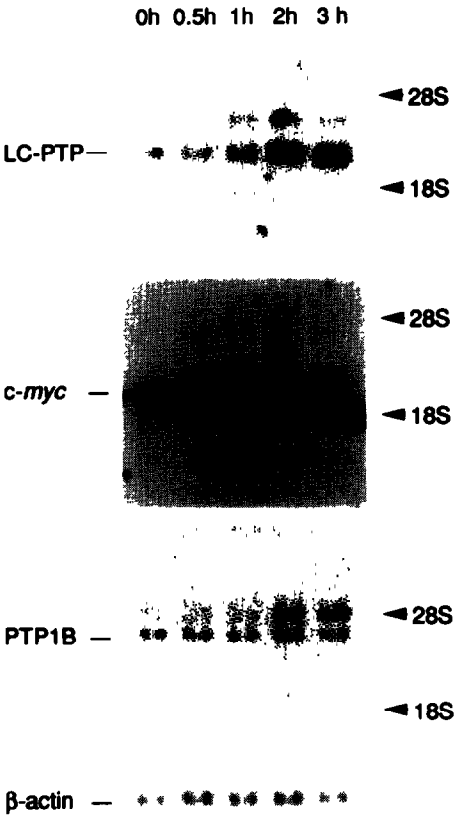


Fig. 1. Expressions of LC-PTP, c-*myc*, and PTP1B genes in ILT-Mat cells after IL-2 stimulation. After 36 h of IL-2 deprival, ILT-Mat cells were stimulated with IL-2 for various periods. The same total RNA samples from the ILT-Mat cells were loaded on 1% formaldehyde agarose gel and analyzed by Northern blot for LC-PTP, c-*myc*, and PTP1B transcripts. After the first autoradiography, the same filters were rehybridized with $^{32}$P-labeled β-actin cDNA probe which was used as a control. The positions of ribosomal RNA markers, which showed equal amount of RNA loaded (data not shown), are indicated to the right of the blots.

rabbit IgG antibody (Sigma). The blots were developed by a standard alkaline phosphatase method.

*2.8. Immunoprecipitations*

Following starvation of ILT-Mat cells for 24 h in IL-2-depleted medium, the cells were washed twice with methionine-free medium containing dialyzed 10% FCS, then metabolically labeled for 8 h with [$^{35}$S]cysteine and [$^{35}$S]methionine (sp.act. 1,000 Ci/mmol, NEN). Prior to the harvest, IL-2 was added to the medium at the indicated periods. Whole-cell lysates were prepared (see above) and immunoprecipitated with 20 $\mu$l of 50% suspension of anti-LC-PTP-agarose. Following extensive washing, resulting complexes were subjected to SDS-PAGE 8% gel electrophoresis. The gels were fixed, treated with ENLIGHTENING (NEN) and dried for autoradiography.

## 3. Results

### *3.1. LC-PTP mRNA expression is induced by IL-2*

The human ILT-Mat is an IL-2-dependent T-cell line carrying human T-cell leukemia virus type I; the cells express the IL-2R$\alpha/\beta$ heterodimer [15]. After 30–36 h deprival of IL-2, the cells (viability > 90%) were stimulated by saturation with IL-2. RNA blot analysis showed that IL-2 was capable of inducing LC-PTP mRNA. The induction was detectable at 1 h and peaked at 9 h after IL-2 addition (Figs. 1 and 3A). As reported previously [16], IL-2 has a similar effect on the steady-state level of c-*myc* mRNA and the induced mRNA was already detectable at 30 min following the stimulation, peaked at 2 h, and decreased slowly thereafter (Figs. 1 and 3A). In contrast, the closely related nontransmembrane PTP1B transcripts did not vary greatly (Fig. 1). We also observed that LC-PTP mRNA expression was induced at 3 h by IL-2 in normal human CD3$^+$ cells, which had been activated by an anti-CD3 antibody (Fig. 2).

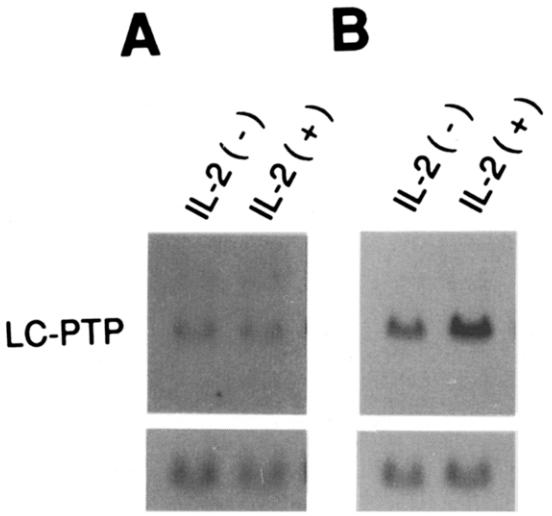


Fig. 2. Induction of LC-PTP gene expression in normal T-cells after IL-2 stimulation. Normal T-cells were stimulated with IL-2 for 3 h, after culture in anti-CD3-coated flask for 2 days, following by an additional 1 day of culture in 10% FCS-RPMI-1640 (B). Mononuclear cells from the same donor were stimulated by IL-2 for 3 h without CD3 stimulation (A). The same total RNA samples were loaded on 1% formaldehyde agarose gel and analyzed by Northern blot for LC-PTP transcripts. After the first autoradiography, the same filters were rehybridized with $^{32}$P-labeled $\beta$-actin cDNA probe which was used as a control.

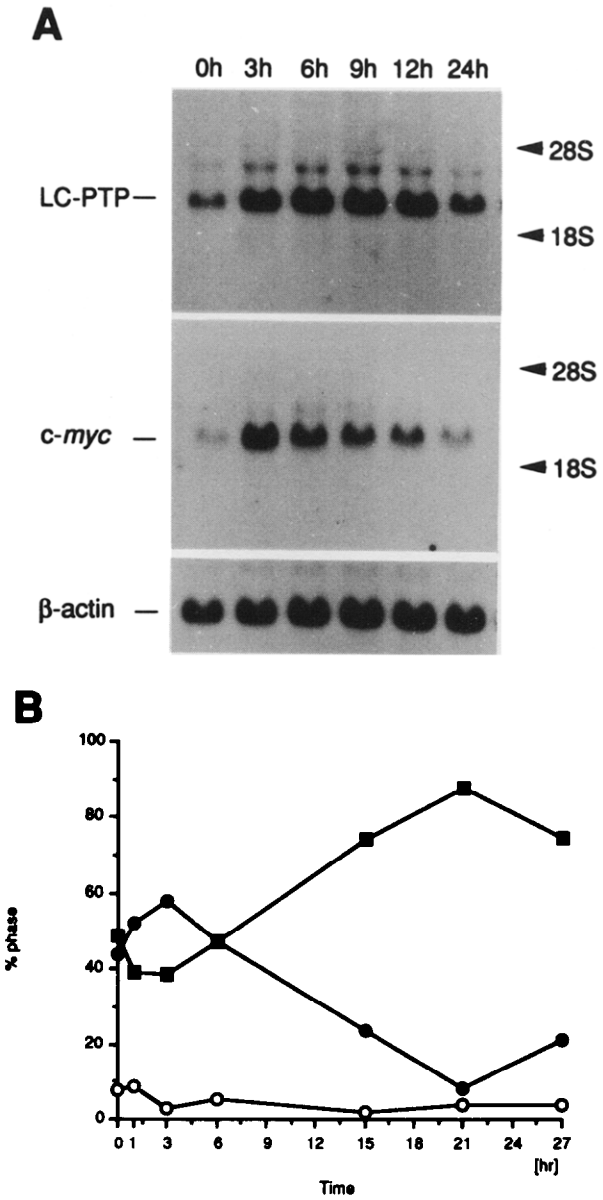


Fig. 3. (A) Expressions of LC-PTP and c-*myc* genes in ILT-Mat cells for long periods after IL-2 stimulation. The same total RNA samples loaded on 1% formaldehyde agarose gel were analyzed by Northern blot for LC-PTP, c-*myc*, and $\beta$-actin transcripts. The positions of ribosomal RNA markers, which showed equal amount of RNA loaded (data not shown), are indicated to the right of the blots. (B) Cell cycle analysis of ILT-Mat cells after IL-2 treatment. Cells were harvested at various times after IL-2 addition, stained with propidium iodide, and analyzed by flow cytometry. The calculated percentages (means of two independent experiments) of G1 phase (●), S phase (■) and G2/M phase (○) are plotted.

Forty-five percent of the ILT-Mat cells starved for 36 h were arrested in the G1 phase of the cell cycle shown as time zero in Fig. 3B. After IL-2 stimulation, they began to replicate DNA within 5–6 h, showed a maxi-

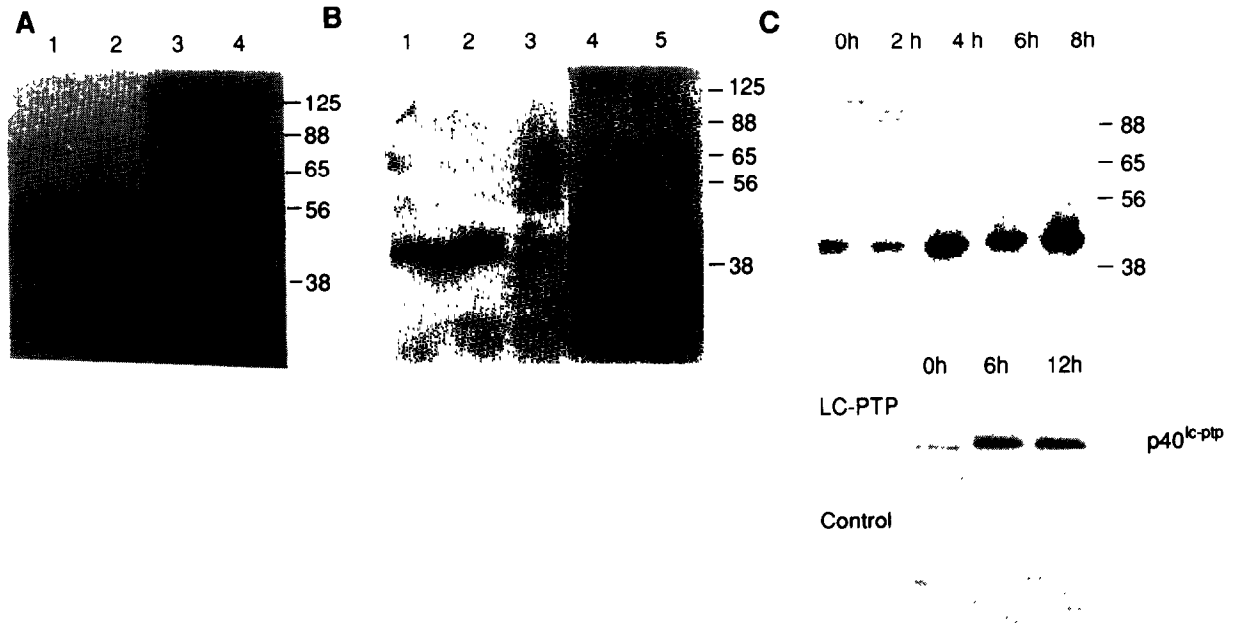


Fig. 4. Expression of $p40^{lc\text{-}ptp}$. Western blot analysis of $p40^{lc\text{-}ptp}$ in Sf9 cells (A) (lanes: 1 and 3, Sf9 cells infected with baculovirus expressing full-length LC-PTP cDNA; 2 and 4, Sf9 cells infected with wild-type baculovirus), or human cell lines (B) (lanes: 1, Jurkat; 2 and 4, ILT-Mat; 3 and 5, JRST). Each cell lysate (400 $\mu$g) was immuno-precipitated with anti-LC-PTP-agarose and the precipitates were loaded on 10% SDS-PAGE gels. Control serum showed no distinctive band (lanes: 3 and 4 in (A); lanes: 4 and 5 in (B)). (C) Metabolic labeling of $p40^{lc\text{-}ptp}$ after IL-2 stimulation. ILT-Mat cells were deprived of IL-2 for 24 h, then washed twice with methionine-free medium, and incubated with addition of [$^{35}$S]cysteine and [$^{35}$S]methionine for 8 h without IL-2 (0 h). At the indicated periods (1–8 h) prior to harvest, IL-2 (400 U/ml) was added. Each fraction ($3 \times 10^6$ cells) was immunoprecipitated with the anti-LC-PTP-agarose and the precipitates were loaded on a 8% SDS-PAGE gel, treated with ENLIGHTENING and dried for autoradiography. Minor bands above $p40^{lc\text{-}ptp}$ are nonspecific, which was confirmed by Western blot analysis (bottom panel), and a slight decrease of $p40^{lc\text{-}ptp}$ at 6 h after IL-2 stimulation was not seen in other two independent experiments. Molecular weight of marker proteins are given in kDa.

mum percentage of cells in S phase at 21 h, and thereafter reentered G1 (Fig. 3B). Although the cells appeared to grow in a less synchronous manner compared with fibroblast cells, the induction of LC-PTP gene by IL-2 seems to occur at G1 to early S phase, while the c-*myc* gene was induced simultaneously with an accumulation of cells at the early G1 phase (Fig. 3).

### 3.2. *LC-PTP protein level is elevated by IL-2*

The open reading frame of LC-PTP cDNA encodes a ~ 40-kDa protein composed of 360 amino acids [6]. In order to identify the product of LC-PTP mRNA ($p40^{lc\text{-}ptp}$) expressed in human T-cells, we generated the anti-$p40^{lc\text{-}ptp}$ antibody (see section 2). The antibody detected $p40^{lc\text{-}ptp}$ in baculovirus-infected Sf9 cells expressing full-length LC-PTP cDNA (lane 1), whereas no distinctive band appeared in the cells infected with wild-type virus (lane 2) (Fig. 4A). Consistent with the previous data showing exclusive expression of LC-PTP mRNA in hematopoietic cells [6,8], $p40^{lc\text{-}ptp}$ was detected in the hematopoietic cells; Jurkat (lane 1) and ILT-Mat (lane 2), but not in non-hematopoietic cells; JRST (lane 3) (Fig. 4B).

ILT-Mat cells were deprived of IL-2 for 24 h, then the washed cells were metabolically labeled with [$^{35}$S]methionine and [$^{35}$S]cysteine for 8 h. At the indicated periods prior to harvest, IL-2 was added to the cells. The increase of $p40^{lc\text{-}ptp}$ appeared at 4 h and reached approximately 6-fold at 8 h after IL-2 addition (Fig. 4C). Western blot analysis also showed LC-PTP protein increased at 6 h after IL-2 stimulation (Fig. 4C, bottom). Thus, the transcriptional elevation of LC-PTP gene leads to increase of $p40^{lc\text{-}ptp}$ level in ILT-Mat cells.

### 3.3. *Mechanism of IL-2-induced LC-PTP transcriptional elevation*

A nuclear run-on assay was used to determine whether LC-PTP transcripts are increased in ILT-Mat cells because of elevation of the transcriptional activity. Nuclei were prepared from cells 0, 1, 3, and 6 h after exposure to IL-2. The nascent transcription of the LC-PTP gene was elevated (approximately 2-fold) at 1 h and elevated greatly at 6 h (approximately 6-fold) after IL-2 stimulation (Fig. 5), suggesting that the induction of LC-PTP transcripts following IL-2 stimulation was mostly due to transcriptional activation. As reported previously [17], the c-*myc* gene transcription was transiently activated (approximately 2-fold) at 3 h after IL-2 stimulation.

The effect of CHX treatment on LC-PTP mRNA levels was analyzed to determine whether the elevation of LC-PTP mRNA by IL-2 stimulation in ILT-Mat cells requires protein synthesis. After 36 h of IL-2 deprival, the induction of LC-PTP gene by IL-2 was not blocked by the addition of CHX (Fig. 6A). Thus, protein synthesis is not necessarily required for its induction by IL-2.

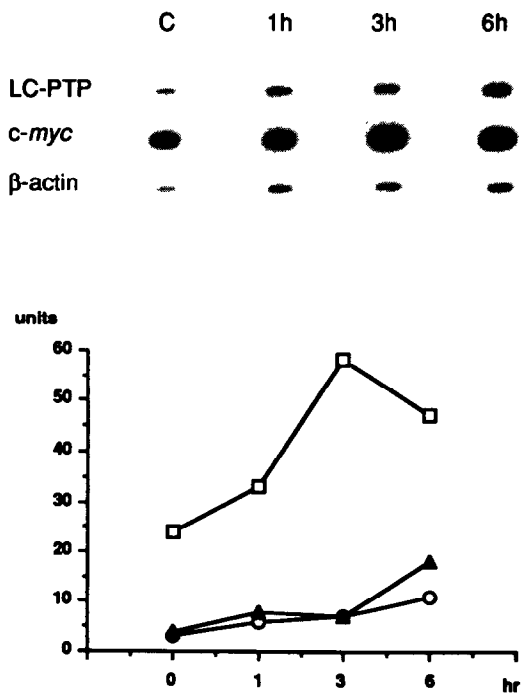


Fig. 5. Run-on analysis of IL-2 treated ILT-Mat cells. Nuclei were isolated, and nascent RNA transcripts were radiolabeled and hybridized with a nitrocellulose filter onto which various plasmid DNAs (LC-PTP, c-*myc*, and β-actin) had been slotted (top). Representative data of nascent RNA transcripts of LC-PTP (▲) and c-*myc* (□) genes plotted against time after IL-2 treatment in two independent experiments (bottom). Each signal was scanned by laser densitometry and the intensity was shown by gray scale unit which was determined using NIH scan image program.

Interestingly, CHX treatment enhanced LC-PTP mRNA expression at 6 h after CHX addition (Fig. 6B). This gives us an idea that LC-PTP mRNA levels seem to be actively controlled post-transcriptionally by some protein(s).

## 4. Discussion

We and others have identified a LC-PTP (HePTP) cDNA which is preferentially expressed in T-cells [6,8]. We present here, for the first time, evidence that IL-2 activates LC-PTP mRNA expression in ILT-Mat cells, suggesting that LC-PTP gene is an important target for the IL-2 signal. Following IL-2 stimulation, the LC-PTP transcripts increased simultanously with an accumulation of cells at the G1 to early S phase. The period of the LC-PTP gene activation is longer and delayed in comparison to c-*fos* and c-*myc* gene expressions [18,16]: transcription of LC-PTP peaked about 6–9 h after IL-2 stimulation and continued for several hours. Importantly, transcription of a closely-related nontransmembrane PTP1B was not altered by IL-2 stimulation (Fig. 1), and transmembrane PTP (CD45) was not either (unpublished observation, M. Sekiya and M. Adachi). This suggests that LC-PTP gene is specifically activated by IL-2 and implies a biological significance for LC-PTP in T-cell activation. It is also noteworthy that IL-2-induced LC-PTP gene activation was also seen in normal T cells activated by CD3 (Fig. 2).

We also demonstrate, for the first time, in this study that the product of LC-PTP gene is a protein with a molecular weight of 40 kDa ($p40^{lc\text{-}ptp}$). In addition, metabolic protein labeling of $p40^{lc\text{-}ptp}$ showed distinctly that $p40^{lc\text{-}ptp}$ was increased approximately 6-fold at 8 h after IL-2 stimulation.

In order to clarify the mechanisms of increase of LC-PTP mRNA and protein levels, following points were tested. An increase in the level of a given mRNA in a cell can result from increased transcription of the message, decreased degradation of the synthesized mRNA, or stabilization of the mRNA. The transcriptional rate of the LC-PTP gene, as assessed by nuclear run-on transcription assays, was significantly elevated after IL-2 stimulation (Fig. 5). We also examined the half-lives of LC-PTP mRNA in IL-2-induced proliferating and IL-2-deprived quiescent ILT-Mat cells. Two independent experiments showed that they were not significantly altered; the half-

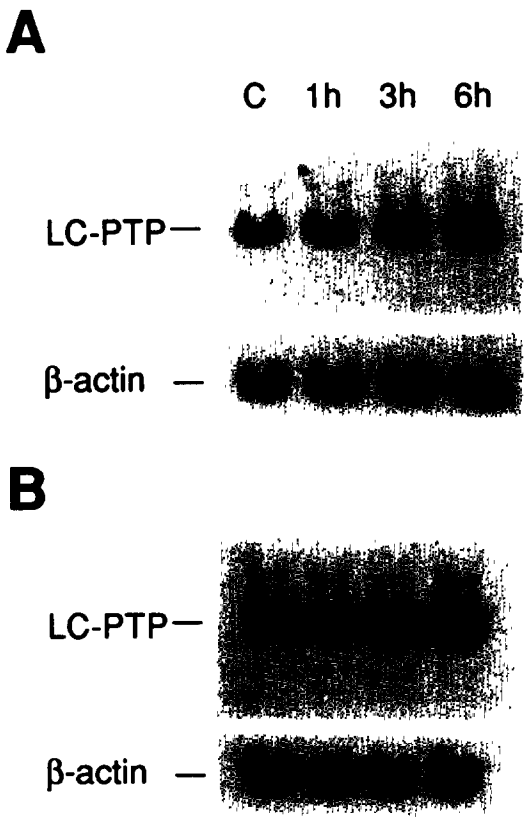


Fig. 6. Effect of CHX on LC-PTP transcripts in ILT-Mat cells. (A) LC-PTP transcripts of ILT-Mat cells treated with IL-2 and CHX simultaneously after 36 h of IL-2 deprival. (B) LC-PTP transcripts in ILT-Mat cells treated with CHX. The same total RNA samples from ILT-Mat cells at the indicated periods were loaded on 1% formaldehyde agarose gel and analyzed by Northern blot for LC-PTP transcripts. After the first autoradiography, the same filters were rehybridized with $^{32}$P-labeled β-actin cDNA probe which was used as a control.

lives of LC-PTP mRNA in proliferating and quiescent ILT-Mat cells were approximately 2.7 h and 2.2 h, respectively (data not shown), suggesting that stabilization of LC-PTP mRNA is unlikely as an explanation for the induction. It is noteworthy that the induction of the LC-PTP gene by IL-2 was not blocked, but super-induced in the presence of cycloheximide (Fig. 6), suggesting that the LC-PTP gene expression level may be regulated by some protein(s) with short half-lives. Taken together, the induction of LC-PTP mRNA is mostly due to transcriptional activation, but unknown regulation by some protein(s) also should be considered.

The fact that protein synthesis is not necessary for the IL-2-induced LC-PTP gene to be activated strongly suggests that the LC-PTP gene is a primary target of IL-2 signaling. Together with the kinetic analysis of LC-PTP mRNA, we conclude that the LC-PTP gene is one of (immediate) early genes which are characterized as nuclear proto-oncogenes or genes associated with cell cycle progression, such as c-*fos*, c-*jun*, c-*myc*, cyclins, and cdc2 kinase, whose gene expressions are independent of protein synthesis. Recently, a mitogen-induced gene (PAC-1) has been cloned from human T cells [19] and the predicted protein sequence has a similarity with phosphatases induced by mitogens or heat shock in human homolog (CL100) [20], fibroblast (3CH134) [21], and a vaccinia virus-encoded serine-tyrosine phosphatase (VH1) [22]. These (immediate) early response genes encode proteins which have minimal active site for PTP, but are different from typical PTPs exemplified by PTP1B or LC-PTP, suggesting that they may constitute a new structural family of PTP. In this context, LC-PTP is an unique early-response PTP.

Recently, a novel PTK *itk* (IL-2-inducible T-cell kinase) gene has been identified; its transcripts were found to be preferentially expressed in T-cells and increased 7-fold in 2 h in parallel with IL-2Rα transcripts after IL-2 stimulation [23]. Since LC-PTP is preferentially expressed in T cells and similarly induced by IL-2, it is of great interest to investigate whether $p40^{lc\text{-}ptp}$ is associated with $p72^{ntk}$. In any case, our data provide novel insights into IL-2 induced signaling pathways.

*Acknowledgements*: We thank Mr. Daniel Day (Sapporo Medical University, Sapporo, Japan) for his editorial assistance and Dr. Terukatsu Sasaki and Dr. Yumi Yamashita (Sapporo Medical University) for helpful suggestions. We are grateful to the Japanese Cancer Research Bank for providing us with the c-*myc* probe, Jurkat, and JRST. We are also grateful to Dr. Kazuo Suganuma (Tohoku University School of Medicine, Japan) for providing us with ILT-Mat cells, and Dr. Haruo Saito (Dana-Farber Cancer Institute, Boston) for providing us with CD45 cDNA probe. This work was supported by Grants-in-Aid for Cancer Research (A.Y., K.I., Y.H., M.A.), and for Scientific Research on Priority Areas (K.I.) from the Ministry of Education, Science and Culture, and Grants for Cancer Research from the Ministry of Health and Welfare (A.Y., K.I.), Japan.

## References

[1] Hatakeyama, M., Kono, T., Kobayashi, N., Kawahara, A., Levin, S.D., Perlmutter, M.R.M. and Taniguchi, T. (1991) Science 252, 1523–1528.
[2] Brown-Shimer, S., Johnson, K.A., Hill, D.E. and Bruskin, A.M. (1992) Cancer Res. 52, 478–482.
[3] Zheng, X.M., Wang, Y. and Pallen, C. (1992) Nature 359, 336–339.
[4] Ostergaard, H.L., Shackelford, D.A., Hurley, T.R., Johnson, P., Hyman, R., Sefton, B.M. and Trowbridge, I.S. (1989) Proc. Natl. Acad. Sci. USA 86, 8959–8963.
[5] Ledbetter, J.A., Tonks, N., Fischer, E.H. and Clark, E.A. (1989) Proc. Natl. Acad. Sci. USA 85, 8628–8632.
[6] Adachi, M., Sekiya, M., Isobe, M., Kumura, Y., Ogita, Z-I., Hinoda, Y., Imai, K. and Yachi, A. (1992) Biochem. Biophys. Res. Commun. 186, 1607–1615.
[7] Adachi, M., Sekiya, M., Arimura, Y., Takekawa, M., Itoh, Hinoda, Y., Imai, K. and Yachi, A. (1992) Cancer Res. 52, 737–740.
[8] Zanke, B., Suzuki, H., Kishihara, K., Mizzen, L., Minden, M., Pawson, A. and Mak, T.M. (1992) Eur. J. Immunol. 22, 235–239.
[9] Watt, R , Stanton, L.W., Marcu, K.B., Gallo, R.C., Croce, C.M. and Rovera, G. (1983) Nature 301, 725–728.
[10] Brown-Shimer, S., Johnson, K.A., Lawrence, J.B., Johnson, C., Bruskin, A.M., Green, N.R. and Hill, D.E. (1990) Proc. Natl. Acad. Sci. USA 87, 5148–5153.
[11] Nakajima-Iijima, S., Hamada, H., Reddy, P. and Kakunaga, T. (1985) Proc. Natl. Acad. Sci. USA 82, 6133–6137.
[12] Vindelov, L.L., Christensen, I.J. and Nissen, N.I. (1983) Cytometry 3, 323–327.
[13] Stallcup, M.R. and Washington, L.D. (1983) J. Biol. Chem. 258, 2802–2807.
[14] Masters, D.B., Griggs, C.T. and Berde, C.B. (1992) BioTechniques 12, 902–910.
[15] Takeshita, T., Goto, Y., Tada, K., Nagata, K., Asao, H. and Sugamura, K. (1989) J. Exp. Med. 169, 1323–1332.
[16] Reed, J.C., Nowell, P.C., Hoover, R.G. (1985) Proc. Natl. Acad. Sci. USA 82, 4221–4224.
[17] Greenberg, M.E. and Ziff, E.B. (1984) Nature 311, 433–438.
[18] Hatakeyama, M., Kawahara, A., Mori, H., Shibuya, H. and Taniguchi, T. (1992) Proc. Natl. Acad. Sci. USA 89, 2022–2026.
[19] Rohan, P.J., Davis, P., Moskaluk, C.A., Kearns, M., Krutzsch, H., Siebenlist, U. and Kelly, K. (1993) Science 259, 1763–1766.
[20] Keyse, S.M. and Emslie, E.A. (1992) Nature 359, 644–647.
[21] Charles, C.H., Abler, A.S. and Lau, L.F. (1992) Oncogene 7, 187–190.
[22] Guan, K., Broyles, S. and Dixon, J.E. (1991) Nature 350, 359–362.
[23] Siliciano, J.D., Morrow, T.A. and Desiderio, S.V. (1992) Proc. Natl. Acad. Sci. USA 89, 11194–11198.