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Activation of Endogenous c-*fos* Proto-Oncogene Expression by Human T-Cell Leukemia Virus Type I-Encoded p40^{tax} Protein in the Human T-Cell Line, Jurkat

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We examined the ability of the *trans*-acting factor p40^{tax} of human T-cell leukemia virus type I (HTLV-I), which is thought to be a crucial molecule in T-cell transformation by HTLV-I, to activate expression of a set of endogenous cellular genes related to T-cell proliferation. For this purpose, we established a subclone (JPX-9) of Jurkat cells that was stably transfected with an expression plasmid containing the p40^{tax} gene, whose expression is definitively dependent on heavy-metal ions. Expression of the interleukin-2 receptor α chain in JPX-9 cells was induced in response to the induction of p40^{tax} expression, as has been demonstrated by others in transient transfection experiments with Jurkat cells. In addition, we found that significant enhancement of expression of the nuclear proto-oncogene c-*fos* was closely associated with expression of p40^{tax}. Continuous enhancement in the level of c-*fos* mRNA was observed in the presence of p40^{tax}. In contrast, mRNA levels of other nuclear proto-oncogenes (c-*myc*, c-*myb*, and c-*jun*) were not appreciably affected by the expression of p40^{tax}. These results suggest that (i) in addition to the interleukin-2–interleukin-2 receptor system, cellular genes such as c-*fos*, which regulate normal T-cell growth, are also activated directly or indirectly by p40^{tax} and (ii) p40^{tax}-induced modulation of gene expression plays a crucial role in T-cell transformation by HTLV-I.

Human T-cell leukemia virus type I (HTLV-I) is a human retrovirus known to be an etiological agent of adult T-cell leukemia (ATL) (16, 33, 59) and to transform normal human T cells in vitro (26, 34, 58). The mechanism by which HTLV-I induces leukemogenesis or cell transformation is still unclear.

Unlike many other acutely transforming animal retroviruses, HTLV-I has no typical oncogene (48). Furthermore, since the HTLV-I proviral genome is integrated randomly into the cellular genome in leukemic cells of ATL, the promoter insertion model for retrovirus-induced leukemogenesis may not be applicable to ATL (46). On the other hand, it has been shown that HTLV-I has a unique gene, designated pX (48). The pX gene encodes at least three distinct products, one of which, p40^{tax}, functions as a *trans*-acting transcriptional activator for its own enhancer in a long terminal repeat (10, 47, 51). It has also been reported that p40^{tax} can activate noncognate viral enhancers (41, 50). These findings led us to postulate that the *trans*-acting function of p40^{tax} may play a central role in HTLV-I-induced cell transformation or leukemogenesis by means of activating certain cellular genes that regulate T-cell growth.

In this regard, activation of interleukin-2 (IL-2) and IL-2 receptor α chain (IL-2Rα) genes by p40^{tax} has been documented (8, 17, 23, 40, 49). These genes are considered candidates for the key target genes that p40^{tax} acts on (54) because the IL-2–IL-2R system is known to be the central system for normal T-cell growth (6) and abnormal, constitutive expression of IL-2Rα is observed in almost all HTLV-I-transformed cell lines and ATL leukemic cells (15, 54, 55, 57). IL-2 production is, however, rarely observed in HTLV-I-transformed cell lines and ATL leukemic cells (2, 3). In addition, we have no direct evidence proving that unregulated constitutive expression of IL-2Rα alone is sufficient to induce transformation in normal T cells. On the other hand, in transgenic mice carrying the p40^{tax} gene, development of tumors of multiple tissues was observed (29). Hence, one may assume that p40^{tax} causes another alternation in expression of cellular genes which is decisive to the immortal growth of T cells. But the effect of p40^{tax} on expression of cellular genes other than the IL-2 and IL-2Rα genes has not been investigated in depth.

In normal T cells, sequential expression of several proto-oncogenes, such as c-*fos*, c-*myc*, and c-*myb*, is observed during lectin-stimulated mitogenesis (36), indicating that these proto-oncogene products may be involved in activation and proliferation of T cells. It is therefore possible that some of these proto-oncogenes or other cellular genes are also activated by p40^{tax}, resulting in abnormal cell growth in HTLV-I-infected T cells.

In this present study, we examined the effect of p40^{tax} on expression of nuclear proto-oncogenes by using a Jurkat clone stably transfected with a heavy-metal-inducible p40^{tax} expression plasmid. The levels of mRNA of the cellular genes tested were compared before and after induction of p40^{tax}. Our results show that expression of the endogenous c-*fos* gene is facilitated by p40^{tax} as well as the IL-2Rα gene. In contrast, the expression of other nuclear proto-oncogenes, i.e., c-*myc*, c-*myb*, and c-*jun*, was not appreciably affected by p40^{tax}.

MATERIALS AND METHODS

Cells. The human acute lymphocytic leukemia T-cell line Jurkat (a gift from N. Kondo, Ajinomoto Co., Inc.) was maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, and antibiotics at 37°C under 7% CO_2 in air. The HTLV-I-producing cell line MT-2 was maintained under the same conditions.

Plasmids and transfection. The plasmids and transfection procedures used have been described elsewhere (30). The p40^{tax} expression plasmid pMAXRHneo-1 is a derivative of pMAXneo (31, 32), which contains a coding region for p40^{tax}

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preceded by a murine metallothionein promoter unit and the bacterial aminoglycoside phosphotransferase (*neo*) gene driven by the simian virus 40 early promoter. Although p40^{tax} from pMAXRHneo-1 is a fused protein carrying seven amino acids from the murine metallothionein gene at the amino terminal, its activity in transactivating the HTLV-I enhancer is indistinguishable from that of the authentic product (our unpublished result). pMAXneo/M is a mutant plasmid in which the frameshift mutation is introduced in the coding region of p40^{tax} in pMAXneo (31). To obtain stable transfectant cell lines, plasmids were introduced into Jurkat cells by the electroporation method. Stable transfectants were then isolated by selection with G418 (600 μg/ml as the active moiety; GIBCO Laboratories), followed by cloning by the limiting-dilution method. For the transient transfection assay, the plasmid was transfected into cells by the DEAE-dextran method as described previously (31).

Immunofluorescence. Cells were washed with phosphate-buffered saline, smeared on a glass slide, and fixed with cold acetone-methanol (1:1) for 20 min. p40^{tax} was detected by the anticomplement immunofluorescence method (37). Cells were first incubated with a mixture of anti-p40^{tax}-positive human serum (final dilution, 1:10) and fresh anti-p40^{tax}-negative human serum (complement source; final dilution, 1:10) at 37°C for 1 h, washed with phosphate-buffered saline, and then incubated with rhodamine-labeled goat antibody to human complement (Organon Teknika) at 37°C for 30 min. To visualize IL-2Rα molecules, cells were serially incubated with monoclonal antibody H-31 (10 μg/ml), specific to human IL-2Rα (56), at 37°C for 30 min, with a biotinylated goat antibody specific to mouse immunoglobulin (Organon Teknika) at 37°C for 30 min, and with fluorescein-labeled avidin D (Vector Laboratories) at room temperature for 20 min, with a phosphate-buffered saline wash after each step.

Immunoblotting. Cells were lysed with a sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis sample buffer consisting of 2% SDS, 5% 2-mercaptoethanol, 50 mM Tris hydrochloride (pH 7.5), and 10% glycerin. After boiling for 5 min, the cell lysate was subjected to electrophoresis, using 10% SDS-polyacrylamide gel, by the method of Laemmli (19). Proteins separated by electrophoresis were electrically transferred onto a Durapore filter (Millipore Corp.). p40^{tax} was detected by incubating the blot serially with anti-p40^{tax}-positive human serum (1:200 dilution, room temperature, 1 h) and ^{125}I-labeled sheep antibody specific to human immunoglobulin G (Amersham Corp.) (0.5 μCi/ml, room temperature, 1 h) as recommended by the supplier. After extensive washing, the blot was exposed to X-ray film (X-AR; Eastman Kodak Co.) with an intensifying screen at −70°C.

Northern (RNA) blotting and probes. Total cellular RNA was isolated from cells by extraction with guanidinium thiocyanate, and Northern blot hybridization was performed as described previously (42). The IL-2Rα probe was a 0.4-kilobase (kb) *Pst*I fragment from pIL-2R-6 (14). The c-*myc* probe was a 1-kb *Cla*I-*Eco*RI fragment encompassing the third exon of human c-*myc* from pMyc6514 (4). The *myb* probe was a 0.9-kb *Eco*RI-*Bam*HI fragment of v-*myb* from pmyb (5). The c-*fos* probe was a 2.1-kb *Eco*RI fragment from pSPT-fos (53). The pX probe was a 0.5-kb *Mlu*I-*Stu*I fragment of the pX region from pXS6 containing a 1.5-kb pX fragment at the *Nco*I site converted from the *Sma*I site of pUC9. The probe for the glyceraldehyde-3-phosphate dehydrogenase gene was a 1.2-kb *Pst*I fragment from pGAD-28 (9). The c-*jun* probe was a 52-base synthetic oligodeoxynucleotide derived from the putative DNA-binding domain encoded by the c-*jun* gene (nucleotides 1039 to 1190; 1). Plasmids pMyc6514, pmyb, and pSPT-fos were provided by the Japanese Cancer Research Resources Bank. Probes were labeled with random primers and [α-^{32}P]dCTP (Amersham) except for the c-*jun* probe, which was end labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP.

RESULTS

Establishment and characterization of a Jurkat clone inducibly expressing p40^{tax}. We used the T-cell line Jurkat because this cell line retains its original nature as a helper T cell with regard to the inducibility of both IL-2 and IL-2Rα in response to extracellular stimuli (12, 21) and because leukemic cells of ATL and HTLV-I-transformed cell lines have the cell surface phenotype of helper T cells (15, 55).

To make stable transfectants containing the inducible p40^{tax} gene, we introduced a metallothionein promoter-driven p40^{tax} expression plasmid, pMAXRHneo-1, into Jurkat cells by the electroporation method. After selection with G418 and repeated cloning by limiting dilution, we obtained a transfected clone that expressed little p40^{tax} spontaneously at either the mRNA or protein level but began to express it markedly upon treatment with $CdCl_2$. This clone was designated as JPX-9. The level of $CdCl_2$-induced p40^{tax} expression was dose dependent, and the maximal level, which was comparable to that in MT-2, an HTLV-I-producing cell line, was observed at $CdCl_2$ concentrations of 10 to 30 μM (Fig. 1A). Toxicity of $CdCl_2$ to JPX-9 cells was seen at concentrations higher than 30 μM. Immunofluorescence studies with anti-p40^{tax}-positive serum demonstrated that the nuclei of up to half of the JPX-9 population were stained after treatment with $CdCl_2$ (Fig. 2a), whereas no staining was observed in the absence of $CdCl_2$ treatment (data not shown).

As a control transfectant we used JPX/M, which was similarly generated by introduction of a mutant plasmid, pMAXneo/M, into Jurkat cells, followed by selection with G418. JPX/M was apparently polyclonal, because it was derived from a mass culture in which many G418-resistant cell colonies were observed. No p40^{tax} antigen was detected by immunofluorescence analysis in JPX/M cells even after treatment with $CdCl_2$ (data not shown) despite induction of transcript from the introduced plasmid by $CdCl_2$ (see Fig. 5A).

We tested the activity of p40^{tax} induced by $CdCl_2$ to transactivate the HTLV-I enhancer. A reporter plasmid, pCHL4 (31), which contains the chloramphenicol acetyltransferase (CAT) gene linked to the HTLV-I long terminal repeat, was transiently transfected into cells, and CAT activity was determined as described previously (31). An approximately 13-fold increase in CAT activity was induced by treatment of JPX-9 cells with $CdCl_2$. In contrast, no increase in CAT activity was found in parental Jurkat cells and JPX/M cells even after $CdCl_2$ treatment (Fig. 1B). These results indicated that a functional p40^{tax} was induced in JPX-9 cells by $CdCl_2$ treatment.

The functional product of p40^{tax} in JPX-9 cells was also confirmed by examining the induction of expression of the IL-2Rα gene, which has been reported to be transactivated by transient introduction of the p40^{tax} gene to some T-cell lines, including Jurkat (8, 17, 23, 49). JPX-9 cells were treated with $CdCl_2$, and expression of p40^{tax} and IL-2Rα was examined by double immunofluorescence staining. Expression of IL-2Rα was seen only in cells that expressed p40^{tax} (Fig. 2), suggesting that induction of the IL-2Rα gene by

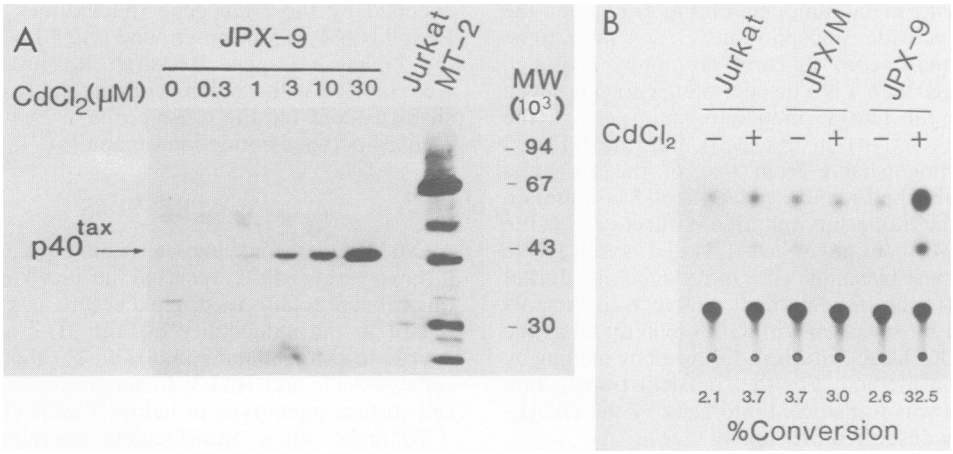

FIG. 1. Induction of p40^{tax} by $CdCl_2$ in JPX-9 cells. (A) Dose-dependent induction of p40^{tax} protein in JPX-9 cells by $CdCl_2$. JPX-9 cells were incubated with various concentrations of $CdCl_2$ at 37°C for 24 h and harvested for examination of the expression of p40^{tax} protein. Cells (2×10^5 per lane) were lysed directly with SDS sample buffer. After boiling for 5 min, the cell lysate was subjected to SDS-polyacrylamide gel electrophoresis (10% gel) and immunoblot analysis, using anti-p40^{tax}-positive human serum and ^{125}I-labeled anti-human immunoglobulin G. The blot was then exposed to X-ray film with an intensifying screen at −70°C. (B) *trans*-Activating activity of p40^{tax} induced in JPX-9 cells. JPX-9, JPX/M, and Jurkat cells were transfected with pCHL4 by the DEAE-dextran method. After 12 h of incubation, the culture was divided in half, and each half was cultured for a further 24 h in the presence (+) or absence (−) of 10 μM $CdCl_2$. The cells were then harvested for the CAT assay. CAT activity is expressed as the percentage of [^{14}C]chloramphenicol converted into acetylated forms.

p40^{tax} occurs other than through a paracrine manner involving some humoral factor(s), which is induced by p40^{tax}. In parental Jurkat cells and JPX/M cells, expression of IL-2Rα was not detected either before or after treatment with $CdCl_2$ (data not shown). These results show that JPX-9 cells retained the characteristics of the parental Jurkat cells in that they could express T-cell-specific genes in response to p40^{tax}. Therefore, JPX-9 cells can be considered useful in studies of the function of p40^{tax} as a modulator of cellular gene expression in T cells.

Effect of p40^{tax} on expression of proto-oncogenes. We next examined the effects of p40^{tax} on expression of a set of nuclear proto-oncogenes that are known to be activated by T-cell activators such as phytohemagglutinin in normal T cells (36). JPX-9 cells were cultured in the presence of 20 μM $CdCl_2$ and harvested at the indicated times. Levels of gene expression were determined by Northern blot hybridization. Expression of the p40^{tax} gene was induced within 2 h after addition of $CdCl_2$ and reached a nearly maximal level within 4 to 6 h (Fig. 3A). Similarly, the amount of p40^{tax} protein was nearly maximal after 4 to 6 h (Fig. 3B). Expression of the IL-2Rα gene was undetectable until 6 h but became detectable within 9 h after addition of $CdCl_2$. A similar time lag between appearance of p40^{tax} and onset of IL-2Rα gene expression was observed in other experiments.

We also found a significant increase in the level of expression of c-*fos* mRNA. The level of c-*fos* mRNA began to rise 4 h after addition of $CdCl_2$; the maximal level was seen at 6 to 9 h, after which the level declined. In contrast, the levels of mRNAs of c-*myc* and c-*myb* oncogenes were not significantly affected by induction of p40^{tax}. We also examined the effect of p40^{tax} on expression of the c-*jun* proto-oncogene, which has been shown to be activated in fibroblasts in response to various stimuli that give rise to activation of the c-*fos* gene. No appreciable change in the level of c-*jun*

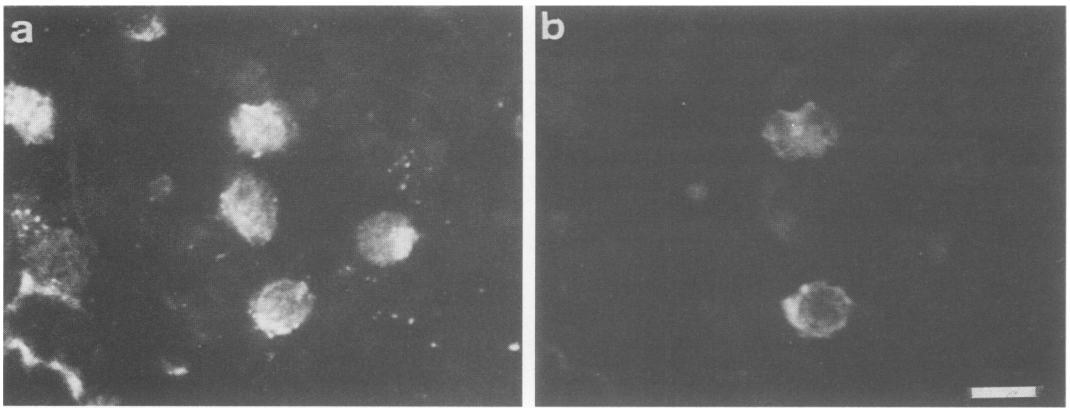

FIG. 2. Immunofluorescence analysis of p40^{tax} and IL-2Rα. JPX-9 cells were incubated in the presence of 10 μM $CdCl_2$ for 20 h, harvested, fixed with cold acetone-methanol, and doubly stained for p40^{tax} (a) and IL-2Rα (b) with anti-p40^{tax}-positive human serum and anti-IL-2Rα monoclonal antibody H-31, respectively, as described in Materials and Methods. The same cells were observed by fluorescent microscopy with appropriate filters for rhodamine (a) and fluorescein (b). Bar, 10 μm.

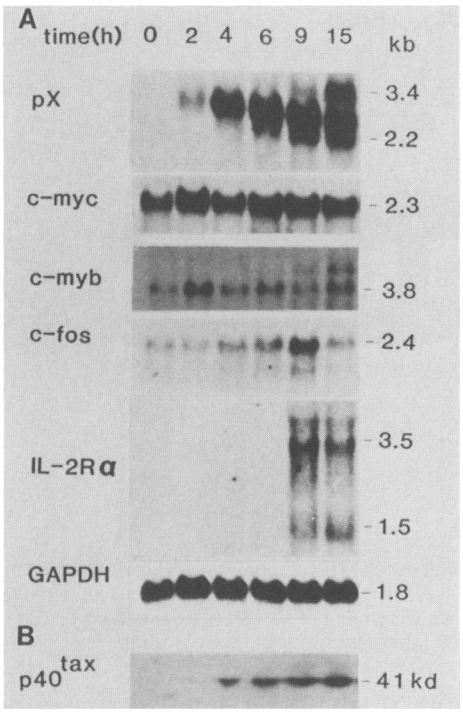

FIG. 3. Northern blot analysis of expression of nuclear proto-oncogenes after induction of p40^{tax} in JPX-9 cells. (A) Growing JPX-9 cells were recovered and suspended in fresh medium containing 20 μM $CdCl_2$ at 10^6 cells per ml. At the indicated times, total RNA was isolated from the cells and analyzed (15 μg per lane) for relative levels of mRNAs with radiolabeled probes. The relative amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was monitored to check the amount of total RNA applied to each lane. Sizes of the mRNAs are shown at the right. (B) A portion (2×10^5 cells per lane) of the same cell sample was analyzed for p40^{tax} expression by immunoblotting. kd, Kilodaltons.

mRNA after induction of p40^{tax} in JPX-9 cells was seen (Fig. 4).

Since the c-*fos* gene generally responds rapidly to various stimuli, the fact that enhancement of c-*fos* expression always followed the appearance of p40^{tax} suggested that this enhancement may have been due to the *trans*-acting function of p40^{tax} but not to the direct effect of $CdCl_2$. To confirm this notion, we examined the effect of $CdCl_2$ treatment on the expression of c-*fos* mRNA in control cells. The c-*fos* mRNA level in JPX-9 cells reproducibly increased after treatment with $CdCl_2$ for 6 to 9 h in several experiments (Fig. 4 and 5A). In contrast, parental Jurkat cells and control transfectant JPX/M cells, in which expression of the mutant p40^{tax} gene was profoundly induced at the mRNA level, reproducibly showed no significant change in the level of c-*fos* mRNA upon treatment with $CdCl_2$ for at least 6 to 9 h after addition of $CdCl_2$ (Fig. 5A). However, the possibility that activation of the c-*fos* gene by $CdCl_2$ depends on the clones used could not be completely ruled out. To test this, we further examined the correlation between expression of p40^{tax} and increase in the c-*fos* mRNA level in newly isolated subclones of JPX-9 cells. Enhancement of c-*fos* expression by $CdCl_2$ was observed in subclones that expressed p40^{tax} in response to $CdCl_2$, whereas no increase in c-*fos* expression was seen in subclones that did not express the p40^{tax} gene (Fig. 5B). These results led us to conclude that the increased expression of the c-*fos* gene observed in $CdCl_2$-treated JPX-9 cells was mediated by the functional p40^{tax} protein.

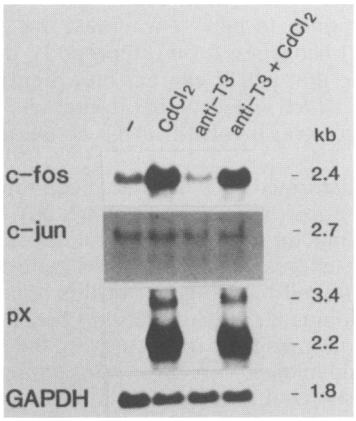

FIG. 4. Effects of anti-T3 antibody on c-*fos* mRNA levels. JPX-9 cells were incubated in medium alone or in medium containing $CdCl_2$ (20 μM), anti-T3 antibody (0.5 μg/ml), or both. Cells were harvested 9 h later, and total RNA was analyzed (25 μg per lane) for relative amounts of c-*fos*, c-*jun*, and pX mRNAs by Northern blotting.

Anti-T3 antibody, which significantly augmented induction of IL-2Rα expression by p40^{tax} (data not shown), exhibited no effect on either the basal or the enhanced level of c-*fos* mRNA in JPX-9 cells (Fig. 4).

Effects of long-term treatment with $CdCl_2$ on c-*fos* and IL-2Rα expression. Since induction of IL-2Rα expression and enhancement of c-*fos* expression are transient in a normal environment, we were interested in investigating whether p40^{tax} can activate the IL-2Rα or c-*fos* gene continuously. To do this, JPX-9 cells were cultured for several days in the presence of $CdCl_2$ at a concentration of 10 μM, which was nontoxic and still effective in the induction of p40^{tax}; at the indicated times, the levels of c-*fos* and IL-2Rα mRNAs were determined and compared with those of untreated JPX-9 cells. Expression of the p40^{tax} gene was maintained at a substantially constant level in the presence of $CdCl_2$ throughout the cultivation; after removal of $CdCl_2$, the level declined (Fig. 6). Similarly, a constant level of expression of the IL-2Rα gene was seen in the presence of $CdCl_2$; after removal of $CdCl_2$, the level declined. On the other hand, the level of c-*fos* mRNA became highly elevated at 9 h after the addition of $CdCl_2$, but then, unlike the IL-2Rα mRNA level, it decreased even in the presence of $CdCl_2$ (Fig. 6). This reduction may be explained, at least in part, by the negative-feedback regulatory function of the c-*fos* protein itself (45). Thereafter, however, a slight but definitely significant increase (two- to fourfold) in the c-*fos* mRNA level was observed in cells treated with $CdCl_2$ as compared with untreated control cells (Fig. 6). These results indicate that p40^{tax}, if it is constitutively expressed, can continuously activate expression of both the IL-2Rα and c-*fos* genes.

DISCUSSION

The *trans*-acting function of HTLV-I p40^{tax} in viral and cellular genes has been investigated mainly through transient cotransfection experiments in which a p40^{tax} expression plasmid and plasmids containing regulatory regions of test genes linked to reporter genes such as the CAT gene are transfected together into various types of cell lines. These experiments have revealed that p40^{tax} can transactivate enhancers of several viral and cellular genes such as those of simian virus 40 (41), human immunodeficiency virus type 1

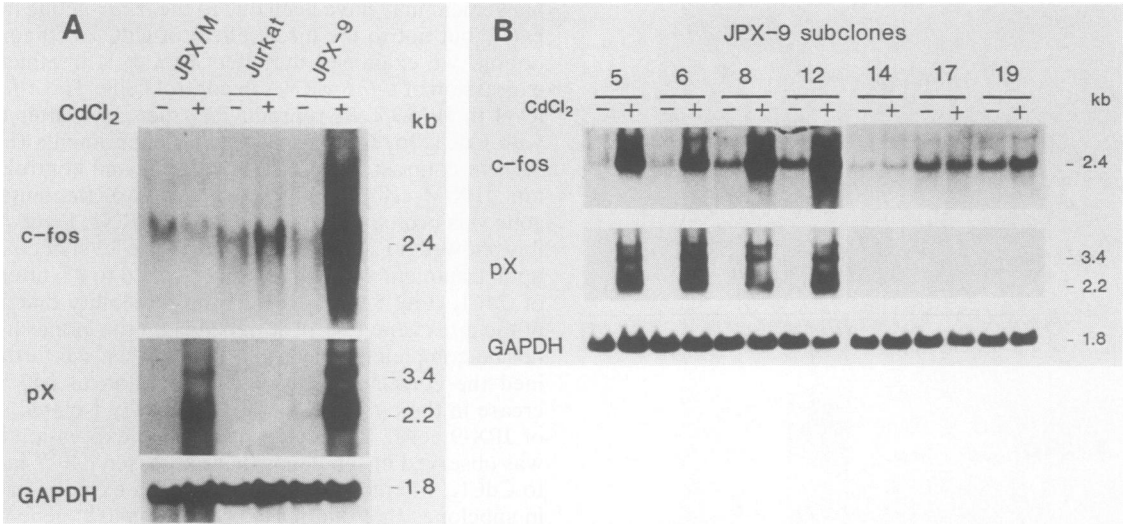

FIG. 5. Effects of $CdCl_2$ treatment on levels of c-*fos* mRNA in JPX-9 and control cells. (A) JPX-9, parental Jurkat, and JPX/M cells were incubated in the presence (+) or absence (−) of 20 μM $CdCl_2$ for 6 h. Cells were harvested, and total RNA was isolated and analyzed (25 μg per lane) for relative levels of c-*fos* mRNA by Northern blotting. (B) JPX-9 subclones were incubated with (+) or without (−) 20 μM $CdCl_2$ for 9 h and harvested to determine relative levels of c-*fos* mRNA (25 μg of total RNA per lane) by Northern blotting.

(50), IL-2 (8, 23, 49), granulocyte-macrophage colony-stimulating factor (25), IL-3 (25), IL-4 (25), and IL-2Rα (8, 23, 49). On the other hand, it has also been reported that the results obtained from transient cotransfection assays are not necessarily applicable to endogenous genes (25). Therefore, to evaluate the role of $p40^{tax}$ in T-cell transformation, it is important to examine the effect of $p40^{tax}$ on endogenous gene expression in T cells. However, with the exception of IL-2 and IL-2Rα genes, the effects of $p40^{tax}$ on expression of endogenous genes has not been investigated in detail (17, 40).

In general, transfection efficiency is unacceptably low in such human lymphoid cells as T-cell lines; therefore, it is difficult to quantitatively examine the cellular changes induced by $p40^{tax}$ in transient transfection experiments. To overcome this difficulty, we established a stable transfectant clone in which expression of $p40^{tax}$ is completely inducible by heavy-metal treatment. This clone thereby allowed us to carry out a quantitative analysis and kinetics study of the $p40^{tax}$-induced expression of cellular genes.

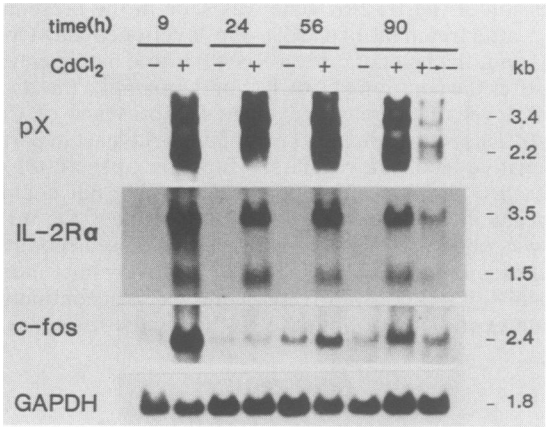

FIG. 6. Effects of long-term treatment with $CdCl_2$ on mRNA levels of IL-2Rα and c-*fos* genes. JPX-9 cells were incubated with (+) or without (−) 10 μM $CdCl_2$ for the indicated periods. Total RNA was analyzed (25 μg per lane) for relative levels of IL-2Rα and c-*fos* mRNAs by Northern blotting. The total medium was exchanged for fresh medium with or without 10 μM $CdCl_2$ 56 h after initiation of the culture. At that time, the $CdCl_2$-containing culture was divided in half, and the two halves were further cultured in the presence (+) or absence (+→−) of $CdCl_2$ for 34 h.

In this study, we examined the effects of $p40^{tax}$ on expression of some oncogenes involved in cell growth: c-*myc*, c-*fos*, c-*myb*, and c-*jun*. These oncogenes are nuclear proto-oncogenes and are known to respond well to various extracellular activating stimuli such as lectins, growth factors, and phorbol esters in various types of cells, including T cells (13, 18, 20, 36, 42, 52). We chose these oncogenes as targets of $p40^{tax}$ because of their high levels of responsiveness to extracellular stimuli and because of an assumption that $p40^{tax}$ can mimic such extracellular stimuli in part by interacting with a cellular component(s) that is involved in transduction of the activating signals from the cell membrane to nuclear target genes. Our results clearly show that the c-*fos* gene responds to $p40^{tax}$, whereas, unexpectedly, the other nuclear oncogenes tested apparently do not. These results indicate that $p40^{tax}$ can act on a limited number of cellular genes, which suggests that it interacts with a particular cellular factor(s) involved in the activation of specific genes.

HTLV-I-transformed cells have not been reported to have a high level of c-*fos* expression. Such a study is under way, but it may be difficult to draw a general conclusion because basal levels of expression of the c-*fos* gene seem to vary from cell line to cell line (Fig. 5). In this regard, an abnormality in development of the thymus has been independently observed both in transgenic mice carrying the $p40^{tax}$ expression plasmid and in those carrying a c-*fos* expression plasmid (29, 39). These results suggest that $p40^{tax}$ may activate continuously the c-*fos* gene even in in vivo tissue such as that of the thymus, resulting in abnormal development of the thymus in transgenic mice carrying the $p40^{tax}$ gene.

Several lines of evidence demonstrate that the c-*fos* product is implicated in cell growth (38), differentiation (28), and development (27). It has also been reported that overexpres-

sion of the c-*fos* gene results in cell transformation in fibroblasts (24). Nevertheless, no lymphoid malignancy has been observed in transgenic mice carrying the c-*fos* expression plasmid, although a high level of c-*fos* mRNA has been found in lymphoid tissues such as splenocytes (39). Thus, the question of whether continuous activation of the c-*fos* gene can directly cause transformation of normal human mature T cells, which are the natural targets of HTLV-I, is still controversial. Recently, c-*fos* protein has been shown to associate with several nuclear proteins (43), and the major *fos*-associated protein has been identified as the sequence-specific transcription factor AP-1 (the product of the c-*jun* gene), which is known to mediate the action of the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (7, 35, 44). Furthermore, it has been demonstrated that the c-*fos* product stimulates AP-1-responsive genes (7, 44), and it is also expected to participate in formation of the transcription machinery responsible for other genes (44). Therefore, continuous activation of the c-*fos* gene by p40^{tax}, if this is the case in normal T cells, may cause unregulated activation of some genes, such as AP-1-responsive genes, resulting in direct transformation of cells or maintenance of cells in an activated state such as occurs with 12-*O*-tetradecanoylphorbol-13-acetate-treated cells, which may be a crucial step required for complete transformation.

The molecular mechanism by which p40^{tax} transactivates its target genes is still unclear. Recent studies have indicated that p40^{tax} functions in at least two distinct ways (41): for HTLV-I itself, and for IL-2Rα, simian virus 40, and human immunodeficiency virus type 1. In the latter case, exertion of this *trans*-acting activity has been shown to occur through a tissue-specific transcription factor, NF-κB (22). In fact, significant enhancement of NF-κB activity in JPX-9 cells was induced by $CdCl_2$ treatment (our unpublished data). Another nuclear transcription factor, cyclic AMP-responsive-element binding factor, is shown to be involved in p40^{tax}-dependent activation of the HTLV-I enhancer (M. Nakamura, M. Niki, K. Ohtani, and K. Sugamura, submitted for publication). However, it is still unclear how p40^{tax} activates these factors and whether these factors are used in activation of the c-*fos* gene by p40^{tax}. Identification of the primary target molecule of p40^{tax} should lead to a much better understanding of the molecular basis of the p40^{tax} function. Inducible transfectants of the p40^{tax} expression plasmid, such as JPX-9, will be useful for this purpose.

During the preparation of this article, we learned of a recent report demonstrating the ability of p40^{tax} to transactivate the c-*fos* gene in a transient transfection assay (11).

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