The second pX product $p27^{x\text{-}III}$ of HTLV-1 is required for gag gene expression

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The human T-cell leukemia virus type-1 (HTLV-1) contains a unique pX region, which encodes the gene products $p40^{x}$, $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$. $p40^{x}$ is required for transcriptional trans-activation, whereas $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$ have no such function. Transfection of pX expression plasmids containing different combinations for the three gene products into cells integrated with HTLV-1 proviruses defective in pX expression revealed that both $p40^{x}$ and $p27^{x\text{-}III}$ are required for expression of the gag protein and accumulation of gag mRNA. These observations suggest that the pX product $p40^{x}$ activates transcription and $p27^{x\text{-}III}$ controls the level of gag mRNA by post-transcriptional modulation.

pX gene *HTLV-1* *Gene product* *Gene expression* *Post-transcriptional regulation*

1. INTRODUCTION

Human T-cell leukemia virus type 1 (HTLV-1) [1,2] is thought to be involved in leukemogenesis in adult T-cell leukemia (ATL) [2–5]. The unique pX region between the *env* and 3′LTR of HTLV-1 [6] codes for three proteins, $p40^{x}$ [7–10], $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$ [11], and one of these, $p40^{x}$ also termed *tat*-1, was shown to be a transcriptional trans-activator which can activate the viral long terminal repeat (LTR) [12–18] as well as the genes for interleukin 2 (IL-2) and its receptor (IL-2R) in T cells [19,20]. Thus, $p40^{x}$ is believed to be essential for efficient viral replication and induction of abnormal proliferation of infected T-lymphocytes. The other two gene products, $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$ are not required for the trans-activation of the LTR-CAT construction [16–18], IL-2 or IL-2R genes [20] and their roles have not been well elucidated to date. In this work, we found that $p27^{x\text{-}III}$ along with $p40^{x}$ is required for expression of the gag gene from the integrated proviral genome.

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2. MATERIALS AND METHODS

2.1. *Transfection*

FL45 cells (6×10^5) were seeded on 60 mm plate and transfected the following day with 6 μg of plasmid DNA by the calcium phosphate method described [13]. After 40 h incubation, the cells were divided into two groups and subjected to either indirect immunofluorescence staining or to extraction of RNA and proteins.

2.2. *RNA and protein analyses*

Total cellular RNA was extracted with vanadyl complexes by the procedure of Berger and Birkenmeier [21]. The poly(A) RNA was denatured with 2.2 M formaldehyde, electrophoresed in 1% agarose gel, and transferred onto nitrocellulose filter. RNA on the filter was hybridized with the ^{32}P-labeled gag probe (see fig.1A) at 42°C under the conditions of 50% formamide and 0.6 M NaCl.

Viral antigens were characterized by blot procedure using serum from an ATL patient.

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3. RESULTS

Since the second and third products of the pX region of HTLV-1, $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$, were not required for trans-activation of the LTR-CAT [17,18], cellular IL-2 and IL-2R genes [20], we tested the effect of the pX proteins on the expression of genes in the HTLV provirus. A defective provirus genome $HTLV_{GAGPX}$ was constructed by a deletion of the sequence between the *Bgl*II site located at the 5′-region of *pol* and the *Bam*HI site at the 3′-region of *env* of the λATK-1 clone (fig.1A) [6]. This defective provirus, $HTLV_{GAGPX}$, has the capacity to produce the gag proteins and the smallest pX protein $p21^{x\text{-}III}$, but not the two other major pX proteins $p40^{x}$ and $p27^{x\text{-}III}$ nor pol and env proteins, since the initiation codons for expression of $p40^{x}$ and $p27^{x\text{-}III}$ were deleted (fig.1A).

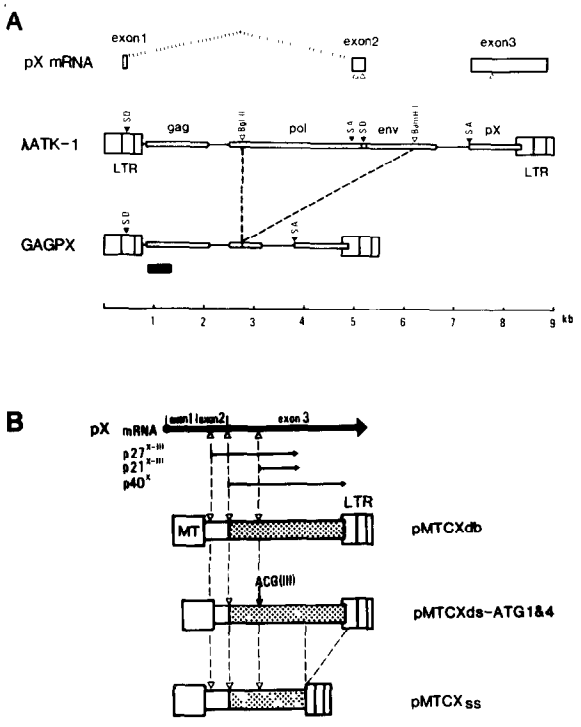

Fig.1. Schematic illustration of HTLV-1 proviruses, λATK-1 and GAGPX (A), and of plasmids for pX expression (B). (A) S.D. and S.A., splicing donor and acceptor sites; (Δ) initiation codons; thick horizontal bar represents DNA sequences used for hybridization probe. (B) Construction of pX plasmids. The cDNA sequence of pX mRNA were inserted under the metallothioneine promoter (MT) with various modifications. Details for pMTCXdb and pMTCXds-ATG1&4 were described in [18]. pMTCXss has a large deletion from *Stu*I (no.8033 from the 5′-end of λATK-1) to *Sma*I (no.8310). (Δ) Initiation codons.

$HTLV_{GAGPX}$ DNA along with a gene conferring neomycin resistance was transfected into FL cells, a human amnion cell line, and a stable cell line FL45 was isolated by G418 selection. Southern blot analysis of FL45 DNA showed multicopy integration of $HTLV_{GAGPX}$ (not shown). Neither viral mRNA nor proteins were detected in the FL45 cells by Northern blot analysis, indirect immunofluorescence staining or Western blot analysis (not shown).

The FL45 cell line was transfected with a plasmid, pMTCXdb [20], containing the wild type coding sequence for the pX proteins, the transcription of which is controlled by a metallothionein promoter (fig.1B). After 2 days, the transfected cells were stained by the indirect immunofluorescence method using monoclonal antibody against a gag protein p19. Several percent of transfected cells were stained strongly, indicating production of gag proteins. The gag protein expressed was characterized by Western blot assay. As shown in fig.2A (lane a), a gag precursor Pr53 was detected in cells transfected with pMTCXdb. These results clearly demonstrate that a proviral genome defective in pX expression can express its *gag* gene when the pX functions are supplied exogenously. Transfection with a plasmid, pMTCXss, containing a single large deletion in the coding sequence for $p40^{x}$ yielded no expression of the gag gene lending support to the conclusion that $p40^{x}$ trans-activation is required for gag expression.

On the other hand, a mutant plasmid pMTCXds-ATG1&4 [20], which can code for $p40^{x}$ but not for $p27^{x\text{-}III}$ and $p21^{x\text{-}III}$ (fig.1B), was also found to be unable to induce gag gene expression (fig.2A, lane b). Normal expression of $p40^{x}$ in these cells was confirmed by the activation of pLTR-CAT and also by Western blot analysis. Failure of $p40^{x}$ alone to induce gag gene expression was unexpected since this gene product is capable of activating transcription from the viral LTR [16–18]. To verify the requirement of $p27^{x\text{-}III}/p21^{x\text{-}III}$ for expression of the gag gene, a complementation assay was carried out: two plasmids, pMTCXds-ATG1&4 and pMTCXss,

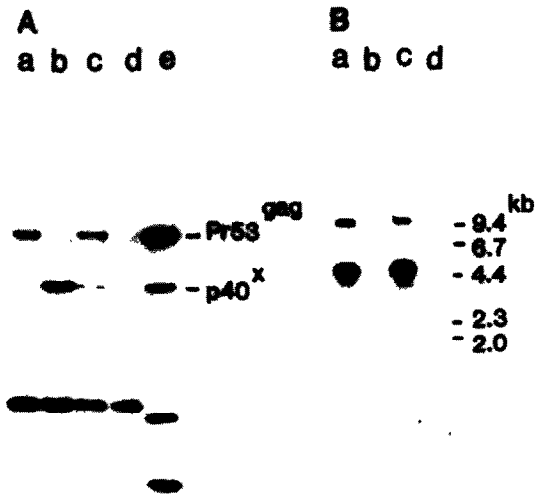

Fig.2. Effect of $p27^{x\text{-}III}$ expression on the levels of gag protein and mRNA. (A) Detection of *gag* gene products: whole cell lysate (30 μg proteins) was subjected to 12.5% polyacrylamide gel electrophoresis followed by blotting analysis using an ATL patient serum. Lysates of FL45 cells transfected with 6 μg of pMTCXdb (a), pMTCXds-ATG1&4 (b), pMTCXds-ATG1&4 plus pMTCXss (c) and pMTCXss (d) were analysed. Lysate of HUT102 cells (e). (B) Detection of the gag mRNA: poly(A) RNA extracted from 3×10^6 cells were analysed by the Northern blot procedure using the gag probe (see fig.1A). RNA samples were prepared from the same cells as described in (A).

which can code for $p40^x$ and $p27^{x\text{-}III}/p21^{x\text{-}III}$, respectively, were cotransfected into FL45 cells. Expression of the gag precursor Pr53 was observed (fig.2A, lane c). This result clearly indicates that $p40^x$ alone is not sufficient and a second product, $p27^{x\text{-}III}$ or $p21^{x\text{-}III}$, is required for gag gene expression. However, transfection of pMTCXds-ATG1 ($p40^x$ and $p21^{x\text{-}III}$) could not induce the gag expression, thus it was suggested that $p27^{x\text{-}III}$ is the required second factor.

To elucidate which process is modulated by $p27^{x\text{-}III}$, gag mRNA in transfected FL45 cells was analysed by the Northern blot procedure using the gag sequence as a probe. In conjunction with the expression of Pr53, two distinct gag mRNA species (5.0 and 9.7 kb) were observed in FL45 cells transfected with the wild type plasmid, but not in cells with plasmid unable to express $p27^{x\text{-}III}$ (fig.2, lanes a and b). The 5.0 kb band corresponds to a transcript from the defective provirus sequence $HTLV_{GAGPX}$, and the 9.7 kb may represent a transcript from tandemly integrated proviral sequences. Complementation of plasmid lacking in expression of $p27^{x\text{-}III}$ with a $p27^{x\text{-}III}$ expression plasmid rescued the gag mRNA (fig.2B, lane c). These results clearly indicate that $p27^{x\text{-}III}$ plays a critical role in regulation of gag mRNA levels. No gag mRNA was detected without $p40^x$ even in the presence of $p27^{x\text{-}III}$ (fig.2B, lane d).

4. DISCUSSION

In this study, we have presented evidence that $p27^{x\text{-}III}$, a second pX protein of HTLV-1, is required for expression of the gag gene and also for accumulation of the mRNA. From these observations, it was concluded that $p27^{x\text{-}III}$ affects the mRNA levels. For activation of transcription from the LTR in pLTR-CAT, cellular IL-2 or IL-2R genes, $p40^x$ alone is sufficient [17,18,20]. However, for gag gene expression, both $p40^x$ and $p27^{x\text{-}III}$ were found to be required. Since $p40^x$ can activate transcription from the LTR [12–18], even from the integrated form of the LTR (J.I., unpublished), it is reasonable to assume that $p27^{x\text{-}III}$ modulates the post-transcriptional process, which eventually affects the gag mRNA levels. The process modulated by $p27^{x\text{-}III}$ could be splicing, transport, or stabilization of mRNA precursors or the mRNA itself. A similar requirement for $p27^{x\text{-}III}$ was observed on other proviral constructions, thus it should not be an artifact of the $HTLV_{GAGPX}$ construction in particular (not shown).

Post-transcriptional regulation was also demonstrated on HIV (HTLV III/LAV) by Sodroski et al. [22]. They reported, however, that gag and env expression were affected by the *art* gene, but that their mRNA levels were not. Therefore, the mode of function of $p27^{x\text{-}III}$ seems to differ from the *art* of HIV.

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