Kinetic analysis of Human T-cell Leukemia Virus type 2 expression in chronically- infected cells and patient PBMCs

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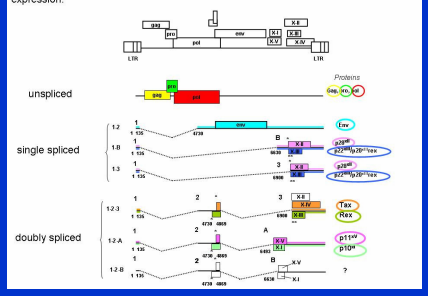
INTRODUCTION

The elucidation of the viral gene expression profile provides useful information in assessing the function of specific viral genes in the process of infection and cellular transformation. HTLV-2 pattern of mRNAs expression produces three major classes of mRNAs: unspliced genomic mRNA for Gag, protease and Pol proteins; singly spliced mRNAs encoding Env and the accessory proteins p28, p22/p20-1 and -2; and a doubly spliced mRNA for the regulatory proteins Tax, Rex and for the p10/p11 and p? accessory ones (Ref.1 and Fig. 1). To date, very little information has been obtained on the temporal regulation of different HTLV-2 transcripts expression in infected cells. Aim of this study was to investigate the kinetics of gene expression from HTLV-2 infected cell lines and from PBMCs of HTLV-2B infected subjects. The expression profile and kinetics of the different transcripts were analysed by real time RT-PCR using splice-junction-specific primers.

Fig 1: Representation of HTLV-2 proviral DNA organisation, alternative splicing and expression.



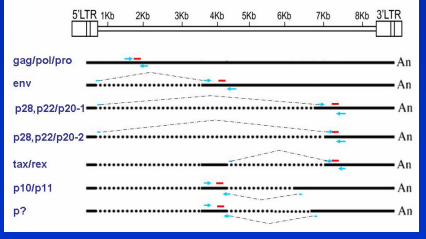
METHODS

The levels of different transcripts expression was evaluated in different HTLV-2 chronically infected cell lines in log phase of growth.

The levels of expression of three major classes of HTLV-2 mRNAs were measured at different time points in BJAB-Gu infected human B-cell line and in IL-2 stimulated PBMCs from HTLV-2B infected patients. In order to synchronise viral expression, BJAB-Gu cells were plated at 1/10 of optimally growing concentration and analysed every 24 hours. Cryopreserved PBMCs were plated and analysed at closer time points.

Total RNA was subjected to reverse transcription, resulting cDNAs were amplified by real time quantitative PCR using different combinations of splice-junction-specific primers pair and probes (Fig. 2). Transcripts copy number were normalised to that obtained for the 18SrRNA.

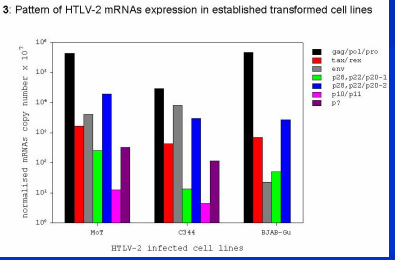
Fig 2: Schematic HTLV-2 genome organisation, alternative splicing and location of primers and probes used to amplify the different cDNAs.



RESULTS

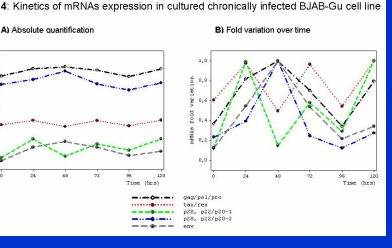
This approach was used to first determine the steady-state levels of expression for the different viral transcripts in three different cell lines in log phase of growth (Fig. 3). Experiments performed indicated that *gag/pol* is the most abundant transcript. The expression level of *env* was comparable in the two T-cell lines, Mo-T and C344, infected by the 2A subtype, and was considerably higher than in the B-cells infected with HTLV-2B subtype, where *p10/p11* and *p?* transcripts were below the limit of detection.

Fig. 3: Pattern of HTLV-2 mRNAs expression in established transformed cell lines



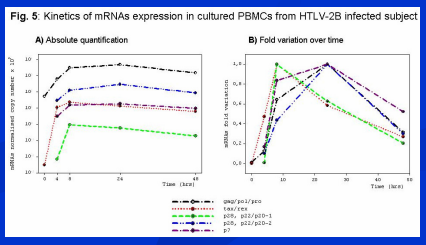
We next investigated the kinetics of viral transcripts expression in infected BJAB-Gu cells. As in the previous experiment, the absolute copy number of *gag/pol* was the highest over the time period analysed (Fig. 4A). Among the accessory transcripts, *p28,p22/p20-2* was the most abundant while other regulatory and accessory genes were lower. The analysis of fold variation, reported in Fig. 4B, indicated that *tax/rex* and *p28, p22/p20-1* showed a biphasic profile with an early peak at 24 hours and a second one at 72 hours, whereas the transcripts *gag/pol, env* and *p28,p22/p20-2* were expressed later.

Fig. 4: Kinetics of mRNAs expression in cultured chronically infected BJAB-Gu cell line



The kinetics of gene expression also was analysed from *ex-vivo* PBMCs of HTLV-2B infected subjects. Fig. 5 shows a typical pattern of expression. Also in this case, among the mRNAs species, *gag/pol* was consistently the most abundant transcript, *p28, p22/p20-2* was approximately 15 fold lower than *gag/pol*, followed by *tax/rex* and *p?* that were present at approximately 25 fold lower than the unspliced mRNA coding for *gag/pol* (Fig. 5A). Very low levels of expression were found for *p28, p22/p20-1*, while *env* and *p10/p11* transcripts were below the limit of detection. In Fig. 5B the fold variation analysis showed that the first mRNAs expressed were *tax/rex* and *p28,p22/p20-1* with a peak at 4 hours followed by all the other transcripts that showed a later peak at 24 hours.

Fig. 5: Kinetics of mRNAs expression in cultured PBMCs from HTLV-2B infected subject



These results indicate that *tax/rex* is the earliest transcript expressed, while the other genes, coding for accessory and structural proteins, are expressed in a later phase of the viral cycle.

CONCLUSIONS

The expression of different HTLV-2 genes follows a distinct timing both in infected cell lines and PBMCs isolated from infected patients. The transcript *tax/rex* is the first to be expressed, thus indicating that it is necessary at the beginning of the infection cycle to transactivate and regulate viral and cellular transcripts. These results also suggest that the control of viral gene expression is highly regulated both in its kinetics and expression level.

REFERENCES

1. Expression and characterization of proteins produced by mRNAs spliced into the X region of the human T-cell leukemia/lymphotropic virus type II. Ciminale V. *et al.* Virology. 1995 Jun 1;209(2):445-56.