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Rapid Communication

A novel, mouse mammary tumor virus encoded protein with Rev-like properties

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Abstract

We have identified a novel, multiple spliced, subgenomic mRNA species in MMTV producing cells of different origin containing an open reading frame encoding a 39-kDa Rev-like protein, Rem (regulator of expression of MMTV). An EGFP–Rem fusion protein is shown to be predominantly in the nucleolus. Further leptomycin B inhibits the nuclear export of nonspliced MMTV transcripts, implicating Rem in nuclear export by the Crm1 pathway in MMTV. Rem is thus reminiscent of the Rec protein from the related endogenous human retrovirus, HERV-K.

Keywords: MMTV; Rem; RNA-expression; Regulation; Rec; Rev

Introduction

Mouse mammary tumor virus (MMTV) has been classified as a simple retrovirus, associated with adenocarcinoma and T-cell lymphoma in mice. Unlike other simple retroviruses which have only three open reading frames (ORF) for structural (Gag, Env) and enzymatic (Pol) proteins, MMTV encodes accessory/regulatory factors such as Sag (Acha-Orbea and Palmer, 1991; Marrack et al., 1991) and Naf (Salmons et al., 1990). Moreover, multiple additional promoters have been described in the MMTV genome (Arroyo et al., 1997; Gunzburg et al., 1993; Miller et al., 1992). HERV-K is an endogenous human retrovirus, related to MMTV, and has been recently shown to encode Rec (Lower et al., 1993, 1995), a functional homologue of Rev and Rex of HIV and HTLV, respectively. The 14-kDa Rec protein, encoded by a double spliced RNA, contains a putative arginine-rich nuclear localization signal (NLS) and a putative nuclear export signal (NES), stabilizes incompletely spliced and unspliced viral mRNAs, and enhances their export to the cytoplasm. In contrast to HIV, where the first Rev coding exon is located between *pol* and *env*, the first Rec coding exon comprises nearly the entire signal peptide of HERV-K Env. Interestingly, there is a striking overall similarity between the Env regions of HERV-K and MMTV, both in their hydrophobicity profiles, and in the structure of their respective signal peptides. Moreover, the signal peptides of both HERV-K and MMTV contain an NLS (Hoch-Marchaim et al., 1998; Lower et al., 1995). Here we report the identification of a novel, double spliced MMTV transcript, analogous to Rec that encodes a regulatory protein, Rem.

Results

A novel double spliced MMTV-specific transcript

The MMTV-*env* gene was introduced into the expression vector pcDNA3 between the CMV promoter and the bovine

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growth hormone polyadenylation site to generate an MMTV-Env expression construct. Stable transfection of the resulting plasmid, pCMVenv, into MMTV permissive feline kidney cells, CrFK (Salmons et al., 1985) however, did not result in detectable Env expression, although stable transfection of a plasmid containing the complete MMTV provirus did (Salmons et al., 1985). RT-PCR analysis of mRNAs from pCMVenv transfected cells using primers designed to detect envelope transcripts (Fig. 1A) did not reveal the expected 2 kb fragment corresponding to the full-length *env* RNA, but rather a smaller product of about 0.9 kb was reproducibly detected (Fig. 1B, lane 2). Sequencing of the shorter product revealed that it results from a splicing event. This intra-*env* splicing using the same splice donor and acceptor sites has previously been described in mouse T-lymphoma cells (EL-4) for a Sag encoding transcript initiating from the promoter within the *env* gene (Miller et al., 1992).

PCR using primers located in the CMV promoter and the 3′ end of the *env* gene confirmed the presence of a complete expression cassette in the stably transfected cells (data not shown), suggesting that the vast majority of *env* RNA is spliced in these cells and that this event is responsible for the lack of Env protein expression.

Double spliced transcript is also present in MMTV-infected cells

To determine whether this novel double spliced transcript is biologically relevant, MMTV transcription was analyzed in MMTV producer cells. RNA was extracted from CrFK cells stably transfected with a complete, biologically active, MMTV provirus (2C9), from nontransfected CrFK and from mouse mammary tumor cells (GR) producing large amounts of MMTV (Salmons et al., 1985). RT-PCR using the same primers as above revealed two transcripts in GR and 2C9 cells but not in CrFK cells: the 2-kb product corresponding to the full-length *env* message and the 0.9-kb product resulting from the novel splicing event (Fig. 1B, lanes 3 and 4). In an attempt to identify the initiation site of the double spliced transcript, we performed an RT-PCR with a primer located in the U5 region of the LTR in combination with the same *env* reverse primer (8649R). Two predominant products of 2.3 kb and 1.1 kb were generated (Fig. 1B, lanes 6 and 8) and sequencing confirmed that the longer product represents the subgenomic *env* RNA transcript and the smaller fragment arises from a double spliced RNA species encoding an open reading frame. The intra-*env* splicing was identical to that in pCMVenv transfected cells.

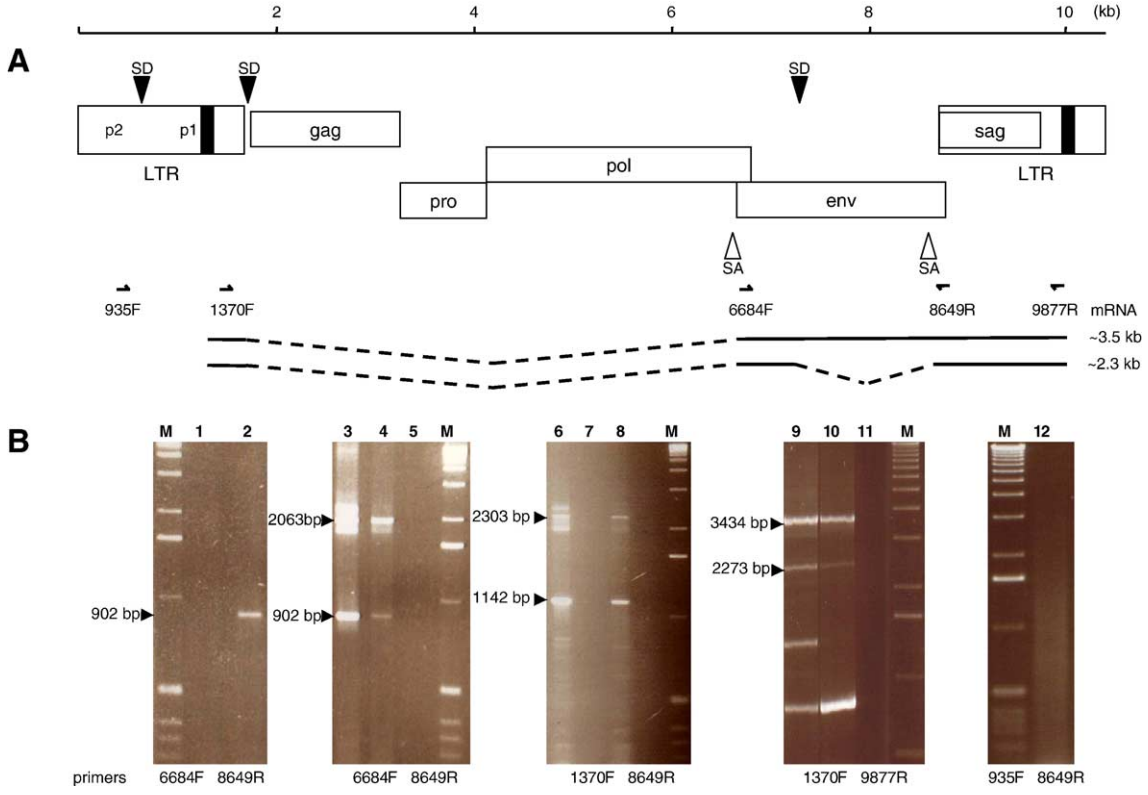

Fig. 1. (A) Schematic representation of the MMTV proviral genome is shown at the top. Open triangles indicate the locations of known splice acceptor sites, and closed triangles indicate the locations of the splice donor sites. The arrows represent the locations and orientations of the oligonucleotide primers used in RT-PCR. Using these primers a doubly spliced RNA species, in addition to env RNA, was identified. (B) RT-PCR was performed with primers 6684F and 8649R (lanes 1, 2, 3, 4, and 5); 1370F and 8649R (lanes 6, 7, and 8); 1370F and 9877R (lanes 9, 10, ans 11); 935F and 8649R (lane 12). RNA used for RT-PCR was extracted from CrFK cells (lane 1, 5, 7, and 11); CrFK cells transfected by pCMVenv expression vector (lane 2); GR cells (lanes 3, 6, 10, and 12) and 2C9 cells (4, 8, and 9). The sequencing of the product extracted from the agarose gel confirmed specificity of the reaction.