VIROLOGY 192, 336–338 (1993)

Identification of pol-Related Gene Products of Human Foamy Virus

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Received June 5, 1992; accepted September 8, 1992

Human foamy virus pol gene fragments were molecularly cloned into a procaryotic expression vector. The expression pattern of the cloned fragments and nucleotide sequence analysis of the 5' pol gene region revealed that in HFV the protease (PR) is located in the pol open reading frame. Purified recombinant proteins were used to generate antibodies in rats. In immunoblot assay, using infected cells as antigen, a precursor protein with an apparent molecular mass (M_r) of 127K was identified by antibodies directed against the reverse transcriptase (RT), RNaseH, or integrase (IN) domains of *pol*. With concentrated virus as antigen, the RT and RNaseH antibodies recognized a protein of 80K, the IN antiserum recognized a protein of 40K, and the PR antiserum detected a protein of approximately 10K. © 1993 Academic Press, Inc.

Human foamy virus (HFV) belongs to the spumavirus subfamily of retroviruses (*1*). The HFV genome comprises the typical retroviral gag, pol, and env genes and accessory ORFs believed to encode for regulatory proteins (*2*). The viral genome has been molecularly cloned and sequenced (*3–5*) but there is little information about the HFV gene products. Since the debate on natural human infections with HFV is controversal (*6–8*) and the significance of HFV as a human pathogen is unresolved (*9–11*), the characterization of viral proteins is a main issue in developing criteria for the serodiagnosis of HFV infections. Furthermore, knowledge of the viral proteins is essential for a better understanding of viral replication and structure.

Previously, three viral glycoproteins with an apparent *Mr* of 170, 130, and 47K have been described, which probably represent *env*-related gene products (*12*). The study also revealed further viral proteins in the *Mr* range of 31–127K which were recognized by foamy virus-positive sera, but their functions remain obscure. Here we report the use of antisera raised against bacterial-expressed pol gene fragments to identify the HFV pol gene products.

Expression plasmids were constructed by inserting DNA fragments derived from restriction enzymatic digestion of the infectious molecular clone pHSRV (*13*) into the multiple cloning site of the bacterial expression vector pROS (*14*). The respective HFV genome organization and the DNA fragments cloned into pROS are shown in Fig. 1. The domains for the protease, reverse transcriptase, RNase H, and integrase were deduced by amino acid homology to other retroviruses.

The 500-bp *MspI/AccI* fragment comprising protease (PR) sequences was blunt-ended by Klenow polymerase and inserted into the *StuI* site of pROS, leading to pPR1. Similarly, pPR2 and pPR3 were generated by inserting the 1520-bp *MspI/NcoI* fragment and the 1000-bp *AccI* fragment into the *StuI* site and the *EcoRV* site of pROS, respectively. pRT1 was generated by cloning the 1020-bp *AccI/NcoI* RT fragment into *EcoRV* cut vector, and the 770-bp *NcoI/EcoRV* fragment comprising RNaseH sequences was inserted into the *StuI* site of pROS, giving rise to pRN1. Insertion of the 540-bp *EcoRV/AccI* and the 1060-bp *StyI* integrase (IN) fragments into *SmaI* and *EcoRV* cut vector led to pIN1 and pIN2, respectively. All recombinant DNA procedures were done according to established methods (*15*) using restriction enzymes and DNA modifying enzymes from Boehringer-Mannheim or GIBCO-BRL. The correct in-frame insertion of the fragments in all constructs was verified by DNA sequencing using a pROS-specific primer and the Pharmacia T7 sequencing system. Transformed *Escherichia coli* BMH 71-18 cultures (*16*) were induced with 1 m*M* isopropyl-β-D-thiogalactopyranoside, bacteria were sedimented after 4 hr, solubized in protein loading buffer, and applied to SDS–polyacrylamide gel electrophoresis (SDS–PAGE) according to Laemmli (*17*).

Since pROS contains 375 codons of the *E. coli* lac-Z gene 5' to the MCS and translational termination codons for each reading frame 3' to it, expression of any in frame DNA fragment leads to β-gal fusion proteins of predictable length (*14*). As shown in Fig. 2, the appar-

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0042-6822/93 $5.00

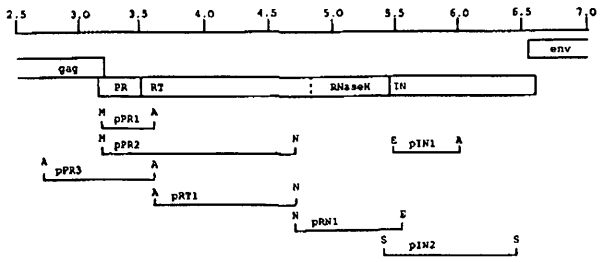

FIG. 1. HFV pol gene organization and DNA fragments expressed in the pROS vector. *Acc*I (A), *Eco*RV (E), *Msp*I (M), *Nco*I (N), *Sty*I (S). The scale represents kilobase pairs with respect to the HFV provirus.

ent *Mr* in SDS–PAGE of the recombinant proteins correlated well with the different lengths of the HFV gene fragments fused to the β-gal coding sequence.

Some confusion was caused by previous results on the location of the protease reading frame in foamy viruses. While PR in simian foamy viruses (SFV 1 and 3) is located in the pol reading frame (*18, 19*) it has been reported to be located in the gag reading frame for the related HFV (*2, 5*). If that were the case, one would expect pPR2 and pPR3 to give rise to shorter and longer fusion proteins, respectively, than shown in Fig. 2. To resolve this discrepancy, we have resequenced the region of the gag–pol junction of the full-length molecular clone pHSRV (*13*). In comparison to the previous report (*5*), we found the sequence from 3141 to 3160 to be CCGCTTCCGGCGGAGATCAAA and an additional T at nucleotide position 3643 (numbering of nucleotides is according to Ref. *5*). These nucleotide changes lead to the genomic organization shown in Fig. 1 which is consistent with the ones reported for SFV 1 and 3 (*18, 19*). Thus, all foamy viruses sequenced to date require a +1 ribosomal frame shift for expression of a putative gag–pol precursor, which is without precedent among exogenous animal retroviruses (*20*).

Fusion proteins were purified by two cycles of preparative SDS–PAGE as described in detail elsewhere (*21*). Proteins, more than 90% pure, as determined by Coomassie blue-stained SDS–PAGE were emulsified in adjuvant and used to immunize rats according to standard protocols (*21*).

Baby hamster kidney cells (BHK-21) were maintained and infected with HFV as described (*12*). Cells were lysed in detergent buffer (20 m*M* Tris–HCl, pH 7.4, 0.3 *M* NaCl, 1% sodium desoxycholate, 1% Triton X-100, 0.1% SDS, 1 m*M* PMSF). Lysates were clarified by brief ultracentrifugation, and the supernatant was resolved on a 12.5% SDS–PAGE prior to being electrophoretically transferred to nitrocellulose membrane in a one buffer/semidry system (*21*). Additionally, whole virus was prepared from cell free supernatant of infected cultures by ultracentrifugation (90 min at 100,000 *g*, 4°). The virus was lysed in detergent buffer, separated on a 12.5% SDS–PAGE with a protein concentration of approximately 30 μg/slot, and gels were processed for Western blot analysis.

Immunereaction was performed by blocking the strips with 3% bovine serum albumin in phosphate-buffered saline (PBS) and incubating with rat sera diluted 1:100 in PBS, 0.1% Tween 20. After extensive washing, the strips were incubated with peroxidase-labeled second antibody (Dako), washed again, and developed using 4-chloronaphtol (Sigma) as chromogen.

Rat RT1, RN1, and IN2 antisera detected a protein with an apparent M_r of 127K in lysates of infected cells (Fig. 3). PR1, RT1, and RN1 antisera recognized a protein of 80K and IN2 antiserum a 40K protein, which was also weakly stained by RN1 antiserum. The rat sera directed against pol gene fragments failed to identify a presumtive gag–pol precursor molecule in the M_r range of approximately 200k as did anti-gag directed antisera (data not shown). This may indicate either the low abundance and short half-life of this intermediate protein or a mechanism of foamy viral pol gene expression that is different from all other retroviruses.

When concentrated virus was used as antigen (Fig. 3), staining of the 127K molecule by the antisera was barely detectable, while the 40 and 80K proteins were recognized as in infected cell lysates. Furthermore, PR1 antiserum detected a protein of approximately 10K M_r. No bands appeared when lysates of uninfected cells were probed with the antisera (data not shown).

Our data suggest that the pol ORF of HFV is

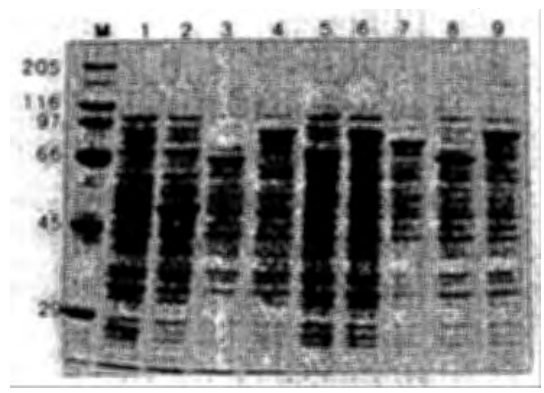

FIG. 2. Coomassie blue-stained 10% SDS–PAGE of BMH 71-18 (1) and IPTG-induced BMH 71-18 transformed with pROS (2), pPR1 (3), pPR2 (4), pPR3 (5), pRT1 (6), pRN1 (7), pIN1 (8), and pIN2 (9). Consistent with the sequence data fusion proteins are the apparent M_r of approximately 63K (3), 95K (4), 64K (5), 80K (6), 70K (7), 63K (8), and 80K (9).

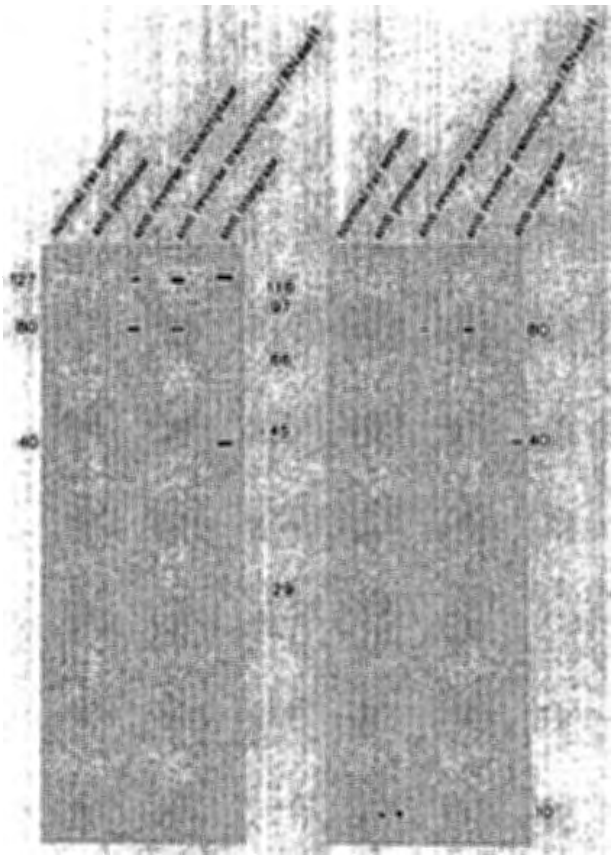

FIG. 3. Immunoblot of electrophoretically separated HFV proteins (12.5% SDS–PAGE) with rat antisera using HFV-infected BHK-21 cell lysate (left site) and concentrated virus (right site) as antigen. Blots were probed with sera as indicated. Migration of marker proteins (Sigma) is shown in the middle.

expressed as a 127K protein. The 127K protein is further cleaved into the 40K integrase, the 80K reverse transcriptase/RNaseH, and the protease of approximately 10K.

The exact termini of the cleaved pol proteins are not yet known and will have to be determined by sequencing of purified proteins. Since the expression plasmids were designed from the tentative domain structure of the pol gene, a weak staining of p80 by PR1 antiserum and of p40 by RN1 antiserum is likely due to overlapping of some RT and IN sequences in pPR1 and pRN1, respectively.

Comparison of RT amino acid sequences revealed HFV to show higher homology to murine leukemia virus (MLV) than to representatives of any other retroviral subgroup (*5, 22*). The pol reading frames of HFV and MLV are of comparable length, 3453 and 3597 bp, respectively (*4, 22, 23*). It is therefore of interest that MLV RT, IN, and PR have been identified as 80, 46, and 10K proteins, respectively, values very similar to those reported here for the respective HFV proteins (*24–26*).

In two previous reports the reverse transcriptase/RNaseH of SFV 1 was identified to be a 81K protein (*27, 28*). Since the pol proteins of HFV and SFV 1 are more than 85% homologous (*18*), our results confirm these earlier reports concerning foamy virus RT protein.

ACKNOWLEDGMENTS

Thanks are extended to Manuela Kranz and Sibylle Retzbach for expert technical assistance, Sylvia Ellinger for providing pROS, and Lee Dunster and Sandra Bräutigam for critical review of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 165) and the Japanese AIDS Foundation.

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