JOURNAL OF VIROLOGY, Feb. 1996, p. 1213–1217 Vol. 70, No. 2
0022-538X/96/$04.00+0

NOTES

Replication Properties of dUTPase-Deficient Mutants of Caprine and Ovine Lentiviruses

PRISCILLA TURELLI,[1] GUDMUNDUR PÉTURSSON,[1]† FRANÇOIS GUIGUEN,[2] JEAN-FRANÇOIS MORNEX,[2] ROBERT VIGNE,[1] AND GILLES QUÉRAT[1]*

INSERM U372, Campus de Luminy, 13276 Marseille,[1] and Unité Associée INRA Ecole Vétérinaire de Lyon, 69280 Marcy L'Etoile,[2] France

Received 21 July 1995/Accepted 20 October 1995

The virion-associated dUTPase activities of caprine arthritis-encephalitis virus (CAEV) and visna virus were determined by using an assay which measures the actual ability of the dUTPase to prevent the dUTP misincorporations into cDNA during reverse transcription. We showed that the CAEV molecular clone from the Cork isolate was dUTPase defective as a result of a single amino acid substitution. Using this point mutant and deletion mutants of CAEV as well as a deletion mutant of visna virus, we demonstrated that dUTPase-deficient viruses replicate similarly to wild-type viruses in dividing cells but show delayed replication in nondividing primary macrophages.

Caprine arthritis-encephalitis virus (CAEV) (6) and the prototypic lentivirus visna virus (27) cause chronic inflammatory diseases in goats and sheep. The main target cells of these viruses in vivo are cells of the monocyte-macrophage lineage (18). Replication is restricted in monocytes and increases during maturation to macrophages (11, 17). In vitro, CAEV replicates in primary goat macrophages or in dividing fibroblastic-like goat synovial membrane (GSM) cells. Similarly, visna virus replicates in primary sheep macrophages and in dividing sheep choroid plexus cells.

Sequence comparison analyses have shown that a retroviral gene previously named pseudoprotease (15) is homologous to the dUTPase gene (16). In type D and B retroviruses, the dUTPase gene is located in the *pro* reading frame. In lentivirus genomes, the dUTPase gene is located in the *pol* reading frame between the RNase H and integrase (IN) coding regions. Retroviral dUTPases are packaged into the viral particles in an enzymatically active form (9). Primate lentiviruses are devoid of a dUTPase gene, and to date, dUTPase sequences have been identified only in feline immunodeficiency virus (FIV), equine infectious anemia virus (EIAV), CAEV, and visna virus.

dUTPase activity has also been demonstrated in some other viruses such as herpesviruses (4) and poxviruses (3) and in various prokaryotic and eukaryotic organisms, including *Escherichia coli* (5), *Saccharomyces cerevisiae* (8), and humans (39). This cellular enzyme hydrolyzes dUTP to dUMP and PP_i, the dUMP being used as a substrate for thymidylate synthase in a major biosynthesis pathway to TTP. Moreover, dUTPase helps to maintain a low dUTP/TTP ratio, thus minimizing the misincorporation of uracil into the DNA. Cellular dUTPase seems to be cell cycle regulated, with an elevated amount of activity in dividing cells and a low amount in terminally differentiated nondividing cells (12, 13, 19, 29, 31). The level of cellular dUTPase activity may parallel the size of the deoxynucleotide pool, which is high in dividing cells such as activated lymphoblasts and very low in nondividing cells such as macrophages (33).

dUTPase-deficient (DU^-) mutants have been constructed in the FIV (37) and EIAV (14, 34) backgrounds and have been shown to replicate like the parental viruses in continuous cell lines. However, DU^- EIAV exhibited delayed replication and up to 2-log-units-lower levels of production in primary equine macrophages derived either from blood (34) or bone marrow (14). Similarly, DU^- FIV demonstrated impaired replication in interleukin-2-stimulated peripheral blood mononuclear cells (37).

To test the role of the dUTPase gene of CAEV, we generated in-frame deletion mutants. Using the original clones of CAEV (22), we first constructed two new cloned fragments of CAEV which contain overlapping sequences and could be used without ligation to generate the virus in cotransfection experiments. Briefly, the PK-9Kb CAEV clone was constructed by inserting the large *Hin*dIII fragment into a long terminal repeat which had been made by PCR using C6 and CRT6 as primers (see Table 1 for primer sequences and positions). It consists of the nearly complete proviral DNA except for the first 22 bp at the 5′ end of the 5′ long terminal repeat and the small *Hin*dIII fragment (8662 to 9084). The second cloned fragment, called BS-3′LTR, contains the 3′ end of the proviral genome from position 8122 (*Xho*I) to position 150 in U5.

DU5 corresponds to a 37-amino-acid in-frame deletion between positions 3976 and 4086 generated by mild BAL 31 digestion beginning at the *Eco*RV site (position 4024). Amino acid sequence comparisons of dUTPases from various organisms show that five motifs are conserved (16). DU5 has a deletion of the central domain encompassing Tyr-74 (Fig. 1). This residue is thought to be the active site of the enzyme, since the *E. coli* Tyr counterpart is critical to the enzyme activity (36), and this Tyr (or Phe) amino acid is highly conserved in all dUTPases (16). DU4 (100-amino-acid deletion)

* Corresponding author. Mailing address: INSERM U372, Campus de Luminy, BP 178, 13276 Marseille Cedex 9, France. Phone: (33) 91 82 75 82. Fax: (33) 91 82 60 61. Electronic mail address: gquerat@inserm-u372.univ-mrs.fr.

† Permanent address: Institute for Experimental Pathology, University of Iceland, Keldur, 112 Reykjavik, Iceland.

TABLE 1. Oligonucleotide primers used

Name	Position	Sequence
CAEV		
C6	8840–8858	TATGTCGACGGACTAATAACAAGCTAGG
C22	3721–3741	GATAGGATAGGAGTGCATTGG
C33	4180–4200	ACGCATGAGGTTACCGGATTTGGGTCTACAGGAATG
CRT6	148–118	TATGTCGACTGGTATTGCACAGATTAAGG
CRT18	4348–4367	CCGCCTCTAATTACTGCAGG
Visna virus KV2	3833–3850 then 4070–4088	CAGGAGATAAGCATTCCGCTCATGCCTCTAATACATG

was deleted by recombinant PCR of the three central domains between positions 3886 and 4179, using C33 as mutagenic oligonucleotide primer. The mutated or unaltered PK-9Kb plasmids were cotransfected with BS-3′LTR into permissive primary GSM cells, using the Lipofectamine reagent (Gibco-BRL). Virus production in supernatants was monitored by reverse transcriptase (RT) activity and used to prepare virus stocks.

dUTPase activity. To study the enzymatic activity of the virion-associated dUTPase, we first used the standard triphosphatase assay which has been used by others on other viruses, including herpes simplex virus, EIAV, and FIV (34, 37, 40). In this assay, the dUTPase activity was determined by measuring the unhydrolyzed dUTP bound onto a DE81 filter paper (Whatman). dATP, dCTP, and TTP were introduced as control for unspecific phosphatase activity. Although we did find a slight dUTPase specific activity with a crude pellet of FIV taken as a positive control (up to 30% hydrolysis in 10 min for 1 μg of protein), we were not able to convincingly show such activity in any CAEV and visna virus preparations, using various purification procedures of viral particles.

Therefore, we devised a new assay which measures the actual amount of dUTP incorporated into the cDNA by the virion-associated RT. The packaged retroviral dUTPase should degrade the dUTP, thus preventing the misincorporation of dUTP into reverse-transcribed cDNA. The relative incorporation of dUTP and TTP into the synthetic template-primer poly(rA)-oligo(dT) was measured in the presence of wild-type or mutated dUTPase. The enzymatic conditions for the assay were those of the classical RT assay, and the source of viruses were either density gradient-purified viral particles or crude pellets from 1 ml of cell-free supernatants from infected cells. Briefly, the pelleted virions were resuspended in 20 μl of NTE (100 mM NaCl, 10 mM Tris-HCl [pH 7.5], 1 mM EDTA) containing 0.1% Triton X-100. Ten microliters of this disrupted virus was used in a 50-μl reaction mixture containing 50 mM Tris-HCl (pH 7.8), 20 mM $MgCl_2$, 2 mM dithiothreitol, 20 mM KCl, 0.25 optical density unit each of poly(rA) and oligo(dT) per ml, and either 2.5 μCi of [^{3}H]TTP (30 Ci/mmol; Amersham) or 1.5 μCi of [^{3}H]dUTP (19 Ci/mmol; Amersham).

Parental wild-type (WT_G) and mutated (DU4 and DU5) molecular clone-derived viruses and several biological isolates of CAEV and visna virus were gradient purified and tested for the incorporation of both dUTP and TTP in RT reactions. Crude pellets of the FIV Petaluma strain (32) and gradient-purified human immunodeficiency virus type 1 (HIV-1) NDK virions (30) were used as positive and negative controls, respectively. dUTP incorporation was normalized to that of TTP

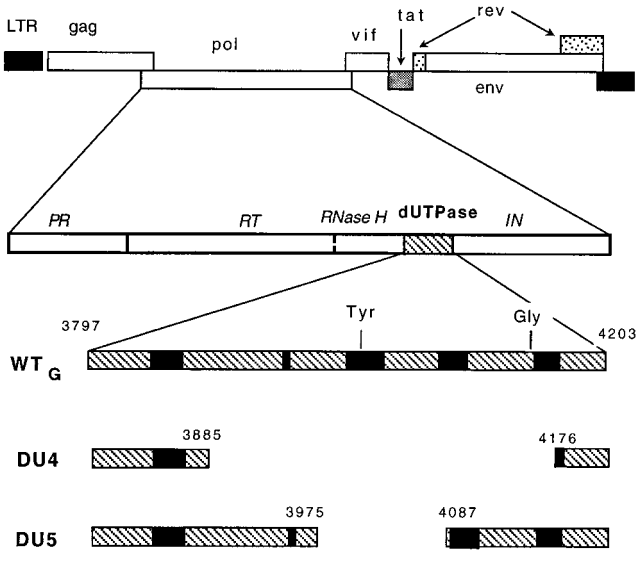

FIG. 1. Schematic representation of the CAEV mutant proviruses. Conserved (shaded areas) and variable (stripped areas) domains of the dUTPase are indicated. LTR, long terminal repeat.

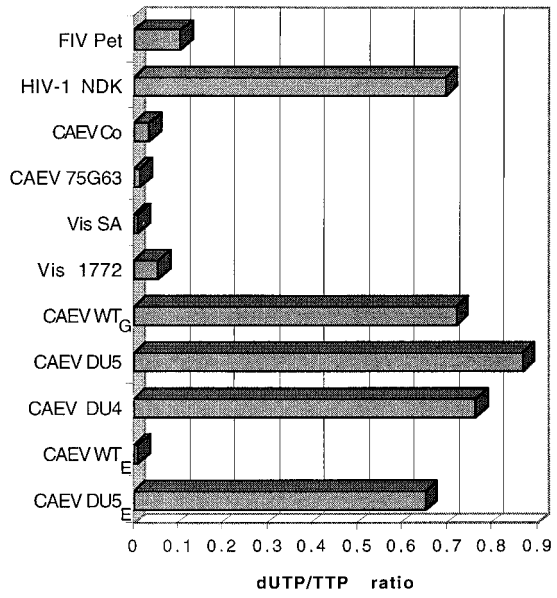

FIG. 2. dUTPase activities of biological isolates and molecular clone-derived visna virus and CAEV expressed as the ratio of dUTP to TTP incorporation into synthetic cDNA. All viruses were purified through isopycnic gradient centrifugation except for the molecular clone-derived visna virus strain KV1772 (Vis 1772) (1) and FIV strain Petaluma (FIV Pet) (32), which were pelleted from clarified supernatants of infected cells. Biological isolates were the Cork strain of CAEV (CAEV Co), (6), CAEV 75G63 (7), and a South African strain of visna virus (Vis SA) (24). HIV-1 NDK is a molecular clone-derived virus (30).

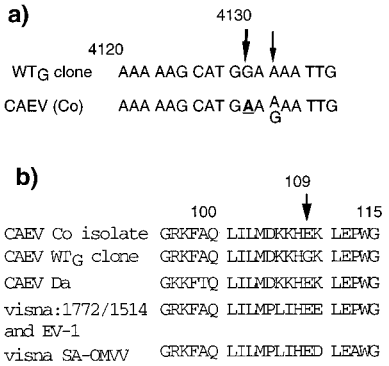

FIG. 3. Sequence of CAEV Cork dUTPase quasispecies. (a) Nucleotide sequence differences between the molecular clone and the biological isolate [CAEV Cork (Co)]. (b) Multiple alignment of the amino acid sequences (residues 95 to 115) of ungulate lentiviruses. Numbering assumes that the protease cleavage sites between the RNase H and dUTPase of CAEV and visna virus are homologous to that of FIV (10). Sequences are as follows: CAEV Cork (reference 25 and this work), a molecular clone of the Dahlberg strain of CAEV (CAEV Da) (38), the Icelandic visna virus isolates K1514 (28) and KV1772 (visna: 1772/1514) (1), a British visna virus strain (EV-1) (26), and a South African visna virus strain (SA-OMV) (23).

and expressed as the ratio of dUTP to TTP. As shown in Fig. 2, we found that a large amount of dUTP was incorporated into synthetic cDNA for HIV-1, which is known to be devoid of the dUTPase gene, for the CAEV dUTPase mutant DU4, for DU5, and also, unexpectedly, for the parental virus WT_G. Such a high level of incorporation of dUTP by DU^- viruses indicated that dUTP was an efficient substrate for CAEV and HIV-1 RTs. In contrast, all biological isolates of FIV, visna virus, and CAEV, including the Cork biological strain from which the infectious molecular clone was derived, exhibited a very low ratio of dUTP incorporation to TTP incorporation. This result argues that the dUTP was specifically and efficiently degraded by the dUTPase activity present in the purified viral particles. As a control of substrate specificity of the visna virus and CAEV dUTPases, the incorporation of dGTP was measured in a poly(rC)-oligo(dG) primer-template assay. The dGTP incorporation mimicked the TTP incorporation, with ratios of dGTP to TTP ranging from 0.75 to 1.35 irrespective of the dUTPase status of the viruses (data not shown). These results demonstrate that the molecular clone-derived virus WT_G is DU^- in contrast to the biological isolate from which it was derived, and strongly suggest that WT_G resulted from the molecular cloning of a minor variant.

Restoration of dUTPase activity. Since no obvious defects in the sequence of the dUTPase gene of the WT_G clone could explain the defect in activity, we decided to sequence quasispecies of the dUTPase gene from the parental biological Cork isolate. RT-PCR products (fragment C22-CRT18) from the genomic RNA were used as templates for sequencing. Some changes were present in some but not all quasispecies. At position 4132, an A-to-G change in approximately 30% of the sequences led to a Lys-to-Glu (amino acid 110) change; at position 3802, in 10% of the sequences, a T-to-C transition led to a Phe-to-Leu change. However, one nucleotide change was found in all sequenced quasispecies from the Cork isolate: at position 4130, a G (in the WT_G clone) to A (in the biological Cork strain) change led to a Gly-to-Glu (amino acid 109) change (Fig. 3a). Additionally, multiple alignments of ungulate lentivirus dUTPase sequences (Fig. 3b) showed that all other dUTPases from small ruminant lentiviruses exhibit a glutamate residue at that position. This result strongly suggests that the Gly-to-Glu change observed in all Cork quasispecies should be a prime candidate for the mutation responsible for the lack of dUTPase activity.

The Gly (G) residue was therefore replaced by Glu (E) in the WT_G and $DU5_G$ context by site-directed PCR mutagenesis. Glu-restored wild-type (WT_E) and mutant ($DU5_E$) viruses were transfected into GSM cells, and their dUTPase activities were measured. As shown in Fig. 2, the WT_E dUTPase activity was restored to a level (ratio of dUTP to TTP of 0.012) comparable to that of the visna virus and CAEV biological isolates. As expected, $DU5_E$ virus was still DU^-, since the DU5 deletion encompasses Tyr-74, which is thought to be involved in the catalytic function (36). Therefore, a single amino acid change was responsible for the dUTPase deficiency of the molecular clone-derived WT_G virus.

Growth properties of DU^+ and DU^- CAEV and visna virus. The question arose as to whether the deletion of the CAEV dUTPase gene could affect viral replication in cell cultures. First, the infectious titers of the WT_E, WT_G, $DU5_E$, and $DU5_G$ stocks were quantified on GSM cells, using inocula with equal amounts of RT activity. We observed no significant differences in virus titers in relation to the dUTPase status, suggesting that virion infectivity was not affected by the dUTPase mutation.

Equal amounts of viruses (titrated by RT activity) were used to infect either GSM cells, monocyte-derived adherent goat macrophages (MDM), or milk goat macrophages. MDM were prepared from heparinized blood by centrifugation on a Ficoll-Paque gradient (Pharmacia). To allow maturation of the monocytes, mononuclear cells were either precultured in Teflon bags (30×10^6 peripheral blood mononuclear cells) for 7 to 10 days and then plated onto culture plates or directly cultured in hydrophobic Petriperm plates (Heraeus), onto which mature macrophages eventually adhere after day 5. Milk macrophages were allowed to adhere onto plastic culture plates for 2 to 3 h and then washed vigorously with phosphate-buffered saline. These cells exhibited typical morphologies of macrophages and stained positively with an α-naphthyl acetate esterase kit (Sigma).

In GSM cells infected at a multiplicity of infection of 0.01, the replication kinetics of the DU^+ virus WT_E, the DU^- viruses WT_G and $DU5_E$, and the double mutant $DU5_G$ were similar, with a peak of virus production at day 6 postinfection (Fig. 4A).

In contrast, in milk macrophages infected at a multiplicity of infection of 0.01, the DU^+ WT_E virus exhibited a marked advantage for replication (Fig. 4B). Production of the WT_E virus peaked at day 7 postinfection with twice as much RT activity as for the DU^- viruses. Virus production of these DU^- viruses eventually reached the same level but with a lag of 5 days, indicating that the rate of replication was affected by the dUTPase deficiency. Similar results were obtained with MDM (data not shown). It was noteworthy that all DU^- viruses, irrespective of the type of mutation, either single point or deletion, replicate with nearly identical kinetics. Phenotypic (dUTPase assays) and genotypic (RT-PCR assays) characterization of the viruses in the supernatant were performed to ensure absence of contaminants (data not shown). Increasing the multiplicity of infection from 0.01 to 0.2 resulted in a decrease of the time lag between the time of infection and the peak of virus production but did not alter the general conclusion. Altogether, our data indicate that the replication of DU^- CAEV is similar to that of wild-type CAEV in dividing GSM cells but is delayed in primary goat macrophages.

We also examined the growth properties of DU^- visna virus in cell cultures. An in-frame deletion of the dUTPase gene was constructed in the molecular clone of visna virus strain

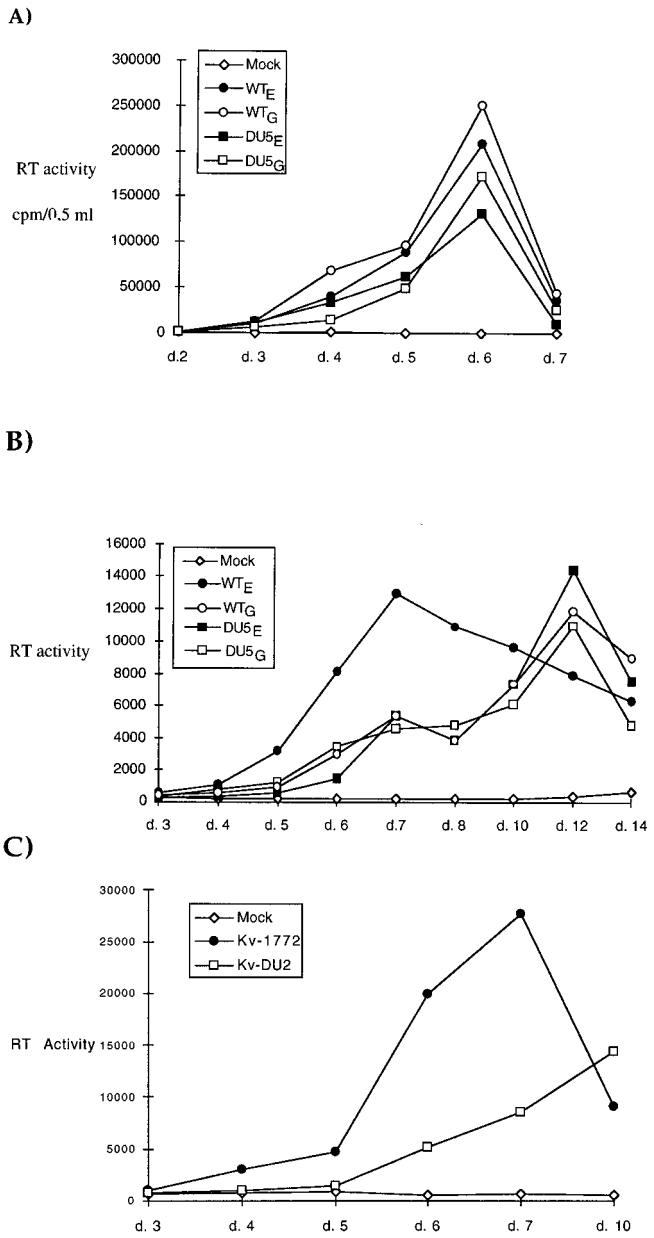

FIG. 4. Replication kinetics of DU^+ and DU^- viruses. Production of CAEV DU^+ virus WT_E was compared with those of single-point mutant WT_G, deletion mutant $DU5_E$, and combined mutant $DU5_G$ in dividing GSM cells (A) and in milk macrophages (B). (C) Replication kinetics of wild-type (KV1772) and mutated (KVDU2) visna virus in goat monocyte-derived macrophages infected with supernatant from transfected GSM cells containing 1,250 cpm of RT activity. d., day.

KV1772 (1), which is known to be infectious and neuropathogenic in vivo. KVDU2 corresponds to a 73-nucleotide deletion (positions 3850 to 4070) made by PCR-based site-directed mutagenesis using KV2 as the mutagenic primer. KV1772 and KVDU2 mutant proviruses were transfected into GSM cells, and their virion-associated dUTPase activities were measured by using crude pellets from GSM supernatants. Ratios of dUTP to TTP incorporated into the synthetic cDNA were 0.03 for KV1772 and 0.31 for KVDU2, indicating that the deletion did specifically abrogate the dUTPase activity.

Supernatants with equal amounts of RT activity were used to infect fresh cells from various origins. As for CAEV, replication profiles of the DU^- and the DU^+ visna viruses were similar in dividing GSM cells (data not shown) but clearly different in MDM (Fig. 4C). The replication of the DU^- mutant was slower than that of the wild-type virus, leading to a delay for the peak of virus production. Similar results were obtained when milk macrophages were substituted for MDM. Therefore, the absence of an active dUTPase resulted in a decrease in replication of small ruminant lentiviruses in nondividing primary macrophages.

In this report, we analyze the growth properties of three in-frame DU^- mutants of CAEV and visna virus in replicating goat synovial membrane cells. All viruses were equally competent for replication. This result could be explained by the existence of a high deoxynucleotide pool and/or a possible transcomplementation by the cellular dUTPase in dividing cells. However, transcomplementation of a deficient retroviral dUTPase by the cellular activity has not yet been demonstrated and may not play a significant role if the deoxynucleotide pool is already very high, as expected for rapidly dividing cells. A high intracellular concentration of TTP will make the misincorporation of dUTP into the proviral cDNA unlikely. Therefore, the replication of DU^- viruses was studied in terminally differentiated primary goat macrophages, in which the deoxynucleotide pool is more likely to be very low and the ratio of dUTP to TTP is likely to be high. In macrophages, DU^- visna virus and CAEV replicated with slower kinetics than the DU^+ virus.

We have demonstrated that the CAEV Cork molecular clone was in fact mutated in the dUTPase gene. A single amino acid change, Glu-109 to Gly, upstream of the fifth conserved domain was responsible for this deficiency. Interestingly, this residue (Glu-109) lies outside the five conserved motifs of dUTPases (16) in a region not previously thought to participate in the enzymatic activity. If the crystal structure of the *E. coli* enzyme (5) could be transposable to that of CAEV, then Glu-109 might be situated at the beginning of the eighth β-sheet strand, which makes extensive contact with the neighboring subunit. The Glu-109 residue might either stabilize the putative multimer or participate in the catalytic domain of the neighboring subunit.

In addition, we devised a new assay which measures the actual amount of dUTP incorporated into the cDNA by the virion-associated RT in the presence of native or mutated dUTPase. Using this assay, we demonstrated that visna virus and CAEV RTs utilized dUTP nearly as well as TTP. Surprisingly, that was also true for HIV-1, which is devoid of virally encoded enzymatic activity able to prevent dUTP incorporation into its genome. The presence of uracil residues in DNA might adversely affect gene expression because the binding of transcription factors to their target could be impaired if some thymidines have been replaced by uracil in the enhancer sequences (20, 35). This might well explain the decrease in production of the DU^- viruses and the induced delay in replication. It is tempting to speculate that uracil *N*-glycosylase, a DNA repair enzyme involved in the removal of uracil residues in DNA, which has been described to bind to the HIV-1 *vpr* gene product (2), can compensate for the lack of dUTPase in HIV.

The dUTPase of herpes simplex virus type 1 has been reported to exert an antimutator function (21). A similar function has been hypothesized for retroviruses (9). Incorporation of dUTP in place of TTP in the first or second round of DNA synthesis is not mutagenic per se unless the excision-repair system associated with the uracil *N*-glycosylase activity is inherently error prone. However, if uracil has been incorporated

into the minus DNA strand, then there could be some G misincorporations in the second-strand synthesis because of the relative stability of U · G base pairs. The frequency of A-to-G substitution would be highly dependent on the deoxynucleoside triphosphate pool size and would probably require both high dUTP/TTP and high dGTP/dATP ratios. In the context of a DU^- virus, accumulation of A-to-G mutations in the genome would eventually lead to replication-defective provirus. Compensation of dUTPase-dependent A-to-G substitution by the G-to-A hypermutation, as observed in CAEV (38), could help to explain the survival of the dUTPase mutant viruses. The antimutator function of dUTPase should be addressed by analyzing the nucleotide sequences of recovered viruses from experimentally infected animals.

We are indebted to Pierre Russo for providing samples of goat blood and milk. We are grateful to Josephine Sire for interesting and lively discussions and for critical reading of the manuscript and to Denise Naniche for carefully reading the manuscript.

This research was supported by the Institut National de la Santé et de la Recherche Médicale and by l'Agence Nationale de Recherche sur le SIDA.

REFERENCES

1. **Andrésson, O. S., J. E. Elser, G. J. Tobin, J. D. Greenwood, M. A. Gonda, G. Georgsson, V. Andresdottir, E. Benedikdottir, H. M. Carlsdottir, E. O. Mantyla, B. Rafnar, P. A. Palsson, J. W. Casey, and G. Petursson.** 1993. Nucleotide sequence and biological properties of a pathogenic proviral molecular clone of neurovirulent visna virus. Virology **193:**89–105.
2. **BouHamdan, M., S. Benichou, F. Rey, J.-M. Navarro, I. Agostini, B. Spire, J. Camonis, G. Slupphaug, R. Vigne, R. Benarous, and J. Sire.** 1996. Human immunodeficiency virus type 1 Vpr protein binds to the uracil DNA glycosylase DNA repair enzyme. J. Virol. **70:**697–704.
3. **Broyles, S. S.** 1993. Vaccinia virus encodes a functional dUTPase. Virology **195:**863–865.
4. **Caradonna, S. J., and Y.-C. Cheng.** 1981. Induction of uracil-DNA glycosylase and dUTP nucleotidohydrolase activity in herpes simplex virus-infected human cells. J. Biol. Chem. **256:**9834–9837.
5. **Cerdergren-Zeppezauer, E. S., G. Larsson, P. O. Nyman, Z. Dauter, and K. S. Wilson.** 1992. Crystal structure of a dUTPase. Nature (London) **355:** 440–443.
6. **Cork, L. C., W. J. Hadlow, J. R. Gorham, R. C. Pyper, and T. B. Crawford.** 1974. Infectious leukoencephalomyelitis of goats (CAEV). J. Infect. Dis. **129:**134–141.
7. **Crawford, T. B., D. S. Adams, W. P. Cheevers, and L. C. Cork.** 1980. Chronic arthritis in goats caused by retrovirus. Science **207:**997–999.
8. **Doignon, F., N. Biteau, M. Aigle, and M. Crouzet.** 1993. The complete sequence of a 6794 bp segment located on the right arm of chromosome II of *Saccharomyces cerevisiae*. Finding a putative dUTPase in a yeast. Yeast **9:** 1131–1137.
9. **Elder, J. H., D. L. Lerner, C. S. Hasselkus-Light, D. J. Fontenot, E. Hunter, P. A. Luciw, R. C. Montelaro, and T. R. Phillipps.** 1992. Distinct subsets of retroviruses encode dUTPase. J. Virol. **66:**1791–1794.
10. **Elder, J. H., M. Schnölzer, C. S. Hasselkus-Light, M. Henson, D. A. Lerner, T. R. Phillips, P. C. Wagaman, and S. B. H. Kent.** 1993. Identification of proteolytic processing sites within the Gag and Pol polyproteins of feline immunodeficiency virus. J. Virol. **67:**1869–1876.
11. **Gendelman, H. E., O. Narayan, S. Kennedy-Stoskopf, P. G. E. Kennedy, Z. Ghotbi, J. E. Clements, J. Stanley, and G. Pezeshkpour.** 1986. Tropism of sheep lentivirus for monocytes: susceptibility to infection and virus gene expression increase during maturation of monocytes to macrophages. J. Virol. **58:**67–74.
12. **Hokari, S., M. Hasegawa, Y. Sakagishi, and G. Kikushi.** 1987. Deoxyuridine triphosphate nucleotidohydrolase activity and its correlation with multiplication of erythroid cells in rat spleen. Biochem. Int. **14:**851–857.
13. **Hokari, S., Y. Sakagishi, and K. Tsukada.** 1982. Enhanced activity of deoxyuridine 5′-triphosphatase in regenerating rat liver. Biochem. Biophys. Res. Commun. **108:**95–101.
14. **Lichtenstein, D. L., K. E. Rushlow, R. F. Cook, M. L. Raabe, C. J. Swardson, G. J. Kociba, C. J. Issel, and R. C. Montelaro.** 1995. Replication in vitro and in vivo of an equine infectious anemia virus mutant deficient in dUTPase activity. J. Virol. **69:**2881–2888.
15. **McClure, M. A., M. S. Johnson, and R. F. Doolittle.** 1987. Relocation of a protease-like gene segment between two retroviruses. Proc. Natl. Acad. Sci. USA **84:**2693–2697.
16. **McGeoch, D. J.** 1990. Protein sequence comparisons show that the 'pseudoproteases' encoded by poxviruses and certain retroviruses belong to the deoxyuridine triphosphatase family. Nucleic Acids Res. **18:**4105–4110.
17. **Narayan, O., S. Kennedy-Stockoff, D. Sheffer, D. E. Griffin, and J. E. Clements.** 1983. Activation of caprine arthritis-encephalitis virus expression during maturation of monocytes to macrophages. Infect. Immun. **41:**67–73.
18. **Narayan, O., J. S. Wolinsky, J. E. Clements, J. D. Strandberg, D. E. Griffin, and L. C. Cork.** 1982. Slow virus replication: the role of macrophages in the persistence and expression of visna viruses of sheep and goats. J. Gen. Virol. **59:**345–356.
19. **Pardo, E. G., and C. Gutierrez.** 1990. Cell cycle- and differentiation stage-dependent variation of dUTPase activity in higher plant cells. Exp. Cell Res. **186:**90–98.
20. **Pu, W., and K. Struhl.** 1992. Uracil interference, a rapid and general method for defining protein-DNA interactions involving the 5-methyl group of thymines: the GCN4-DNA complex. Nucleic Acids Res. **20:**771–775.
21. **Pyles, R. B., and R. L. Thompson.** 1994. Mutations in accessory DNA replicating functions alter the relative mutation frequency of herpes simplex virus type 1 strains in cultured murine cells. J. Virol. **68:**4514–4524.
22. **Pyper, J. M., J. E. Clements, M. A. Gonda, and O. Narayan.** 1986. Sequence homology between cloned caprine arthritis encephalitis virus and visna virus: two neutropic lentiviruses. J. Virol. **58:**665–670.
23. **Quérat, G., G. Audoly, P. Sonigo, and R. Vigne.** 1990. Nucleotide sequence analysis of SA-OMVV, a visna-related ovine lentivirus: phylogenetic history of lentiviruses. Virology **175:**434–447.
24. **Quérat, G., V. Barban, N. Sauze, R. Vigne, A. Payne, D. York, E. M. de Villiers, and D. W. Verwoerd.** 1987. Characteristics of a novel lentivirus derived from South African sheep with pulmonary adenocarcinoma (Jaagsiekte). Virology **158:**158–167.
25. **Saltarelli, M., G. Quérat, D. A. Konings, R. Vigne, and J. E. Clements.** 1990. Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generates infectious virus. Virology **179:**347–364.
26. **Sargan, D. R., I. D. Bennett, C. Cousens, D. J. Roy, B. A. Blacklaws, R. G. Dalziel, N. J. Watt, and I. McConnell.** 1991. Nucleotide sequence of EV1, a British isolate of maedi-visna virus. J. Gen. Virol. **72:**1893–1903.
27. **Sigurdsson, B., and P. Palsson.** 1958. Visna of sheep. A slow demyelinating infection. Br. J. Pathol. **39:**519–528.
28. **Sonigo, P., M. Alizon, K. Staskus, D. Klatzmann, S. Cole, O. Danos, E. Retzel, P. Tiollais, A. Haase, and S. Wain-Hobson.** 1985. Nucleotide sequence of the visna lentivirus: relationship to the AIDS virus. Cell **42:** 369–382.
29. **Spector, R., and B. Boose.** 1983. Development and regional distribution of deoxyuridine 5′-triphosphatase in rabbit brain. J. Neurochem. **41:**1192–1195.
30. **Spire, B., J. Sire, V. Zachar, F. Rey, F. Barré-Sinoussi, F. Galibert, A. Hampe, and J. C. Chermann.** 1989. Nucleotide sequence of the HIV1-NDK: a highly cytopathic strain. Gene **81:**275–284.
31. **Strahler, J. R., X. X. Zhu, N. Nora, Y. K. Wang, P. C. Andrews, N. A. Roseman, J. V. Neel, L. Turka, and S. M. Hanash.** 1993. Maturation stage and proliferation-dependent expression of dUTPase in human T cells. Proc. Natl. Acad. Sci. USA **90:**4991–4995.
32. **Talbott, R. L., E. E. Sparger, K. M. Lovelace, W. M. Fitch, N. C. Pedersen, P. A. Luciw, and J. H. Elder.** 1989. Nucleotide sequence and genomic organization of feline immunodeficiency virus. Proc. Natl. Acad. Sci. USA **86:**5743–5747.
33. **Terai, C., and D. A. Carson.** 1991. Pyrimidine nucleotide and nucleic acid synthesis in human monocytes and macrophages. Exp. Cell Res. **193:**375–381.
34. **Threadgill, D. S., W. K. Steagall, M. T. Flaherty, F. J. Fuller, S. T. Perry, K. E. Rushlow, S. F. LeGrice, and S. L. Payne.** 1993. Characterization of equine infectious anemia virus dUTPase: growth properties of a dUTPase-deficient mutant. J. Virol. **67:**2592–2600.
35. **Verri, A., P. Mazzarello, G. Biamonti, S. Spadari, and F. Focher.** 1990. The specific binding of nuclear protein(s) to the cAMP responsive element (CRE) sequence (TGACGTCA) is reduced by the misincorporation of U and increased by the deamination of C. Nucleic Acids Res. **18:**5775–5780.
36. **Vertessy, B. G., and M. Zeppezauer.** 1994. Identification of tyrosine as an active site residue involved in the catalytic mechanism of E. coli dUTPase. Biochem. Soc. Trans. **22**(2)**:**233S.
37. **Wagaman, P. C., C. S. Hasselkus-Light, M. Henson, D. L. Lerner, T. R. Phillips, and J. H. Elder.** 1993. Molecular cloning and characterization of deoxyuridine triphosphatase from feline immunodeficiency virus (FIV). Virology **196:**451–457.
38. **Wain-Hobson, S., P. Sonigo, M. Guyader, A. Gazit, and M. Henry.** 1995. Erratic G (to) A hypermutation within a complete caprine arthritis-encephalitis virus (CAEV) provirus. Virology **209:**297–303.
39. **Williams, M. V., and Y.-C. Cheng.** 1979. Human deoxyuridine triphosphatase nucleotidohydrolase. J. Biol. Chem. **254:**2897–2901.
40. **Williams, M. V., and D. S. Parris.** 1987. Characterization of a herpes simplex virus type 2 deoxyuridine triphosphate nucleotidohydrolase and mapping of a gene conferring type specificity for the enzyme. Virology **156:**282–292.