Volume 222, number 2, 286–288 FEB 05177 October 1987

The 3′-orf protein of human immunodeficiency virus 2 shows sequence homology with the *bel*3 gene of the human spumaretrovirus

Bernd Maurer and Rolf M. Flügel

German Cancer Research Center, Institute of Virus Research, PO Box 101949, 6900 Heidelberg, FRG

Received 17 July 1987; revised version received 17 August 1987

The primary amino acid sequence within a domain of 89 residues of the central part of the 3′-orf protein (p27 3′-orf) of human immunodeficiency virus (HIV-2) shares homology with the middle and carboxy-terminal portion of the *bel*3 gene product of human spumaretrovirus (HSRV). In addition, a limited region of the tat protein of HIV-2 but not HIV-1 shows a 28% degree of homology to the deduced protein sequence of the *bel*1 gene product of HSRV. Comparison between the viral sequences suggests that the 3′-orf and *bel*1 gene product of HSRV could serve similar functions to those in HIV-2.

3′-orf protein; Human foamy virus, Human immunodeficiency virus; *tat* gene; Sequence homology

1. INTRODUCTION

HIV is a retrovirus that causes AIDS. Studies in several laboratories have revealed that the HIV genome encodes viral *gag*, *pol* and *env* genes characteristic of all other retroviruses [1–4]. In addition, the HIV genomes have been reported to encode up to six other genes. The functions of these genes are of great interest and are being studied intensively to learn more about the mechanism of regulation of viral expression and, furthermore, to define those gene segments responsible for the cytopathogenicity of HIV. The six novel genes include *tat*, *art*, *sor* (orfA), 3′-orf (orfB), R and X [1–5].

Correspondence address: B. Maurer, German Cancer Research Center, Institute of Virus Research, PO Box 101949, 6900 Heidelberg, FRG

Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; HSRV, human spumaretrovirus; orf, open reading frame; bel, between env and LTR; tat, trans-acting transcription activator

HSRV, a member of the third subfamily of retroviruses, the spumaviruses, has been characterized recently [6]. Like HIV, HSRV harbors additional novel genes termed *bel*1, 2 and 3. The HSRV *bel* genes are located between the *env* gene and the 3′-LTR of the HSRV genome [6].

To gain more insight into the structural relatedness between HIV and HSRV and into possible functions of the *bel* genes, a search was conducted for sequence homology between the HSRV *bel* and the six HIV genes. This report describes sequence homologies between the *bel*3 gene product and the HIV-2 3′-orf protein and, in addition, a sequence homology between the *bel*1 gene and the tat protein of HIV-2.

2. EXPERIMENTAL

The deduced amino acid sequences of the HSRV *bel* genes were derived from the DNA sequence reported in [6]. The HIV-2 3′-orf protein and the tat protein sequence were taken from [5]. Analysis of the sequence data was performed using the BSA program devised by Dr S. Suhai at the EDV

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

department of the German Cancer Research Center, Heidelberg.

3. RESULTS AND DISCUSSION

A close visual inspection of the deduced protein sequence of the *bel3* gene product of the HSRV genome (fig.1A) showed that certain stretches of amino acid residues were clearly homologous to the 3′-orf protein of HIV-2. The region with the most striking homology to the 3′-orf sequence of HIV-2 is located within the central and carboxy-terminal portion (amino acid residues 79–167) of the bel3 protein sequence. The domain of homology extends over 89 amino acid residues to the end of the bel3 sequence and revealed a homology of 19.8% (fig.1B). When similar amino acid residues are taken into account, the homology reached 45.0%. As shown in fig.1, only two separate and single deletions have to be introduced into the bel3 sequence to achieve this degree of homology. When two gaps of three amino acid residues are introduced into the same comparison the degree of homology reaches 24.5%.

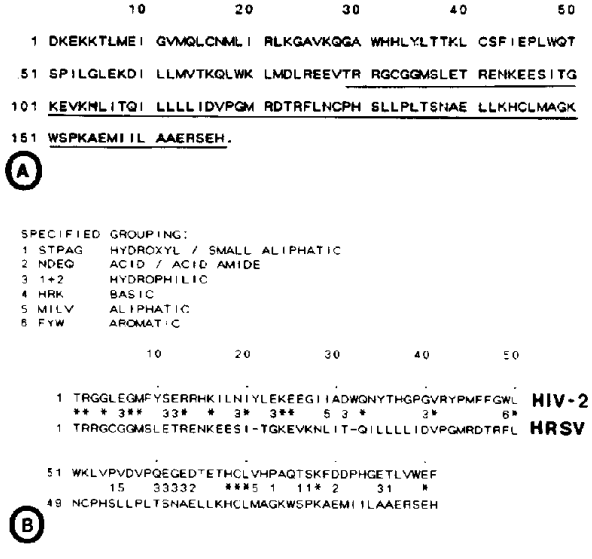

Fig.1. (A) The deduced amino acid sequence of the *bel3* gene product of HSRV taken from [6]. Underlining marks the protein of the bel3 sequence homologous to the 3′-orf of HIV-2. (B) Comparison of the amino acid sequence of the 3′-orf protein of HIV-2 (residue 1 corresponds to amino acid 125 of the 3′-orf [5] with the protein sequence of the bel3 of HSRV (residues 79–167). Asterisks denote identities; point deletions were introduced to maximize homology.

The significance of this homology with respect to function(s) of both viral proteins at the molecular level is not yet understood. Although the homology between 3′-orf proteins of HIV-1 and HIV-2 is 50.5% in this domain, the degree of homology between the 3′-orf of HIV-1 and the bel3 protein of HSRV is much less pronounced (12.6%). This result is independent of the various 3′-orf sequences that have been determined from various HIV isolates [7]. However, it was reported that one of the functions of the 3′-orf protein of HIV-1 is to down-regulate virus replication [8] and mutants in 3′-orf displayed altered cytopathic effects [9]. The relatively high sequence homology of the *bel3* gene product to the 3′-orf protein of HIV-2 suggests that these viral proteins serve similar functions during virus replication, particularly in view of the close overall relatedness between HIV-2 and HIV-1. It remains to be seen if this is true for the bel3 protein.

At first sight, HIV genes that are crucial for the virus life cycle, and particularly those involved directly or indirectly in virus replication, should be expected to be more conserved, e.g. the *pol* gene, than other non-essential genes. However, we have recently reported that the endonuclease/integrase region of HSRV has a degree of homology of 23.0% to that of HIV-1 [6]. When this domain was re-examined by using the corresponding region of HIV-2 that has been published recently [5], a homology of 26.9% was found. This result seems to indicate that HSRV and HIV-2 are phylogenetically closer than HSRV to HIV-1 in the central and 3′-part of their genomes. The relatively high protein sequence homology of the 3′-orf of HIV-2 to the HSRV bel3 is unexpected, since HIV-1 mutants in this region produced more virus and viral DNA indicating that orfB (or 3′-orf) is not crucial for HIV-1 replication [8,9].

To support this conclusion, other gene regions of HIV-2 and HSRV were inspected. It was found that a limited region of 50 amino acid residues that is located within the functionally important exon 2 of tat of HIV-2 was homologous to the bel1 region of HSRV. The degree of homology reached 28% when the portion of the bel1 protein sequence was taken that extends from residue 115 to 163 (fig.2). We consider this homology as significant, since it

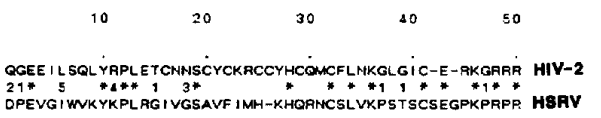

Fig.2. Comparisons of the amino acid sequence of the HIV-2 tat protein [5] (residues 10–58) with the bel1 protein sequence of HSRV [6] (residues 115–163). Symbols and numbers as in legend to fig.1.

has been found by using DNA recombinant techniques that only the second exon of the HIV-1 *tat* gene is responsible for the trans-acting activator function [10,11]. Thus, homology within exon 3 of the *tat* gene to the bel1 protein sequence might not be predicted.

In conclusion, protein sequence comparisons between the *bel*1 and *bel*3 gene products of HSRV to those of the tat and 3′-orf (orfB) proteins of HIV-2 show that they share sequence homologies. This indicates that these viral genes may have similar functions, although HIV-2 and HSRV belong to different subfamilies of retroviruses.

REFERENCES

[1] Ratner, L., Haseltine, W., Patarca, R., Livak, K.J., Starcich, B., Josephs, S.F., Doran, E.R., Rafalski, J.A., Whitehorn, E.A., Baumeister, K., Ivanoff, L., Petteway, S.R. jr, Pearson, M.L., Lautenberger, J.A., Papas, T.S., Ghrayeb, J., Chang, N.T., Gallo, R.C. and Wong-Staal, L. (1985) Nature 313, 277–284.

[2] Sanchez-Pescador, R., Power, M.D., Barr, P.J., Steimer, K.S., Stempien, M.M., Brown-Shimer, S.L., Gee, W.W., Renard, A., Randolph, A., Levy, J.A., Dina, D. and Luciw, P.A. (1985) Science 227, 484–492.

[3] Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S. and Alizon, M. (1985) Cell 40, 9–17.

[4] Muesing, M.A., Smith, D.H., Cabradilla, C.D., Benton, C.V., Lasky, L.A. and Capon, D.J. (1985) Nature 313, 450–458.

[5] Guyader, M., Emerman, M., Sonigo, P., Clavel, F., Montagnier, L. and Alizon, M. (1987) Nature 326, 662–669.

[6] Flügel, R.M., Rethwilm, A., Maurer, B. and Darai, G. (1987) EMBO J. 6, 2077–2084.

[7] Alizon, M., Wain-Hobson, S., Montagnier, L. and Sonigo, P. (1986) Cell 46, 63–74.

[8] Luciw, P.A., Cheng-Mayer, C. and Levy, J.A. (1987) Proc. Natl. Acad. Sci. USA 84, 1434–1438.

[9] Fisher, A.G., Ratner, L., Mitsuya, H., Marselle, L.M., Harper, M.E., Broder, S., Gallo, R.C. and Wong-Staal, F. (1986) Science 233, 655–659.

[10] Sodroski, J., Patarca, R., Rosen, C., Wong-Staal, F. and Haseltine, W. (1985) Science 229, 74–77.

[11] Arya, S.K., Guo, C., Josephs, S.F. and Wong-Staal, F. (1985) Science 229, 69–73.