Journal of Computational Intelligence in Bioinformatics
ISSN: 0973-385X Volume 4 Number 2 (2011) pp. 115-126
© Research India Publications
http://www.ripublication.com/jcib.htm

Identification of Putative Promoter Motifs in Hepatitis C, Dengue, Japanese Encephalitis and Yellow Fever Virus

Mamta Singh[1] and Anindya Sundar Barman[2,*]

[1]Research Associate, ICAR Research Complex for NEH Region (Tripura Centre) Lembucherra, Tripura (W)-799210 India.
E-mail: mamta0131@gmail.com
[2]Department of Fish Genetics and Reproduction
College of Fisheries (Central Agriculture University) Lembucherra, Tripura (W)-799210 India.
**Corresponding Author E-mail: barman.anindya@gmail.com*

Abstract

Hepatitis C, dengue, Japanese encephalitis and yellow fever viruses are medically significant pathogenic virus; causing several infections in humans worldwide. Their genome size ranged from 9.5-11.0 kb approximately. In the large scale genomic era promoter prediction is crucial for gene discovery and annotation. Complete genome of above mentioned viruses were analyzed for the identification of putative promoter motifs in present study. All six genotype of hepatitis C, four type of dengue, one genotype of Japanese encephalitis and yellow fever virus were included in this study. Genotype 2 and 3 of HCV and type 4 dengue viruses did not encode the promoter regions in their genome. All other studied virus genotypes showed the presence of promoter region in the genome. Total 52 different types of promoter were identified in their genomes. All these identified promoter motifs were confirmed with experimentally proved existing data. Our study may help in designing efficient expression vector or target specific delivery system in the gene therapy and the live attenuated vaccine candidate through the site directed mutagenesis in the promoter region.

Keywords: HCV, Dengue, JEV, YFV, Promoters and flavivirus.

Introduction

The members of genus flavivirus of family flaviviridae cause hepatitis C, Dengue,

Japanese encephalitis and yellow fever. Virus included in present study, come under group of arbovirus transmitted mainly by tick or mosquito vectors except Hepatitis C virus. Hepatitis C virus (HCV) is now well established to be heterogeneous in nature, showing multiple genotypes and subtypes, with the basic structure and genome organization being conserved [1]. HCV is now established to be the major causative agent of post-transfusional Non-A, Non-B hepatitis (PTNANBH) [2]. There are six major genotypes of the hepatitis C virus, which are indicated numerically (e.g., genotype 1 to genotype 6). Transmitted by parenteral and nosocomial routes, infection with this virus is the leading cause of chronic liver disease. There being no vaccine and the current treatments successful only up to 11–30%, hepatitis C is rarely diagnosed until its chronic stages, when it can cause severe liver damage.

Dengue fever, dengue hemorrhagic fever and dengue shock syndrome are the prevalent mosquito borne viral infections worldwide. Dengue was circulated as quasi-species, which is categorized into four serotypes [3]. These four serotypes of dengue virus (DEV I-IV) cause dengue hemorrhagic fever and dengue shock syndrome [4, 5]. The infection caused by dengue viruses are widely recognized as a major public health concern, with more than one million cases of dengue hemorrhagic fever (DHF) per year with fatality rates 1 to 10%. The most susceptible to the disease are children and young adults.

Japanese encephalitis is an infection of the brain caused by a virus. Japanese encephalitis is caused by the mosquito-borne Japanese encephalitis virus. Domestic pigs and wild birds are reservoirs of the virus; transmission to humans may cause severe symptoms. One of the most important vectors of this disease is the mosquito *Culex tritaeniorhynchus*. Symptoms include headache, fever, neck stiffness, tremors, seizures, spastic paralysis, and coma. Mortality rate ranges widely from 0.3% to 60%. Various neurologic and psychiatric squeals are common. JE has increasingly been recognized throughout most countries of east and South East Asia, where it is the leading cause of viral encephalitis and approximately 30,000 to 50,000 cases are reported each year (http://www.cdc.gov/ncidod/dvbid/ jencephalitis/facts.htm).

Yellow fever virus is transmitted to humans through the bite of infected mosquitoes. Illness ranges in severity from a self-limited febrile illness to severe hepatitis and hemorrhagic fever. Yellow fever disease is diagnosed based on symptoms, physical findings, laboratory testing, and the possibility of exposure to infected mosquitoes. There is no specific treatment for yellow fever; care is based on symptoms. Up to 50% of severely affected persons without treatment will die from yellow fever. There are an estimated 200 000 cases of yellow fever, causing 30 000 deaths, worldwide each year. The virus is endemic in tropical areas of Africa and Latin America, with a combined population of over 900 million people (http://www.who.int/mediacentre/factsheets/fs100/en/)

Flavivirus genome is a single-stranded, positive sense RNA of 10-11 kb containing a single ORF and is the only viral mRNA produced during the virus replication cycle. The replication takes place in the perinuclear region of cytoplasm in the infected cells. Proteolytic processing of the single polyprotein by viral and cellular proteases produces three structural (capsid, premembrane and envelope) and seven nonstructural (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) viral proteins. The

open reading frame of *Flavivirus* genome is flanked by the 5` and 3`untranscribed region (UTR). The UTR form complex RNA structure containing functional domain that are believed to play a role in virus translation, replication or assembly. These generates lots of scientific interest since, genetic modification within these region are known to attenuate *Flavivirus* without altering their antigenic specificity making them potential vaccine candidate for live attenuated vaccine.

A wide range of algorithms has been developed to assist the identification of promoters in genomic sequence of many gene prediction methods. Promoter is a most important regulatory region that controls and regulates gene expression at the transcription level. It contains specific DNA sequences, response elements that are recognized by proteins known as transcription factors. These factors bind to the promoter sequences, recruiting RNA polymerase, the enzyme that synthesizes the RNA from coding region of the gene. However, a limited number of data is available on the promoter motifs in the genus *Flavivirus*. The present study was carried out to identify and analyze the putative promoter region present in *Flaviviruses* namely hepatitis c, dengue, Japanese encephalitis and yellow fever virus.

Methodology

Retrieval of genome sequence

The complete genome sequences of HCV, dengue, JEV and YFV were retrieved from biological databases such as National Centre for Biotechnology Information cited at http://www.ncbi.nlm.nih.gov/genomes/VIRUSES/viruses.html and the Universal Virus Database of the International Committee on Taxonomy of Viruses (ICTVdB) genome database cited at http://www.ncbi. nlm.nih.gov/ ICTVdb/.

Blast

The relatedness of sequences deposited in databases was evaluated by BLAST (Basic Local Alignment Search Tool) implemented via the NCBI website (www.ncbi.nlm.nih.gov/blast) against the complete training dataset which is extracted from Genbank database. All these identified promoters were verified and searched for homology in the database. The G+ C content of each genome was also predicted with the help of BioEdit.

Transcription start site

The transcription factor sites are over represented in the promoter region. It is natural to seek a prediction program based on putative TF site density. The PROMOTERSCAN program was used to identify the putative promoter in the genome of HCV, dengue virus, JEV and YFV. This program comprises three databases such as TF database; promoter database and non-promoter set constructed from protein and RNA gene sequences. The density of all putative TF site is calculated separately for promoter and non-promoter sequences scoring functions supplemented with a TATA matrix score [6].

Results

In the present study, complete genome sequences of four *Flavivirus* was analyzed i.e. 6 genotype of HCV (genotype 1, genotype 2, genotype 3, genotype 4, genotype 5 and genotype 6), 4 types of dengue virus (type1, type2, type3 and type4), one genotype of JEV and YFV. In present study the size of HCV genomes ranged from 9343-9711 bases, dengue virus genomes ranged from 10649-10735 bases. Size of JEV and YFV genomes were 10967 bases and 10862 bases respectively. The smallest genome size (9343 bases) is of HCV genotype 5 and the highest genome size (10967 bases) is of JEV. All the genotypes of HCV showed the higher GC content in their genome as compared to other studied viral genome. G+ C percentage of HCV genotype 1 was highest (58.23) and Dengue type 2 was lowest (45.82). The NCBI accession number, genome size and G+C contents of each studied virus are given Table 1.

Identification of important putative promoter in the complete genome of four medically significant *Flavivirus* was done. Total 52 different types of promoter were identified in the genomes and given (Table 2). All the studied virus genotypes showed the presence of promoter region in the genome except genotype 2 and 3 of HCV and type 4 dengue viruses. The identified putative promoter of *Flavivirus* was given (Table 3). *Flavivirus*, which did not have the promoter sequences were, utilized all the host machinery during the time of replication and multiplication. All the identified promoter motifs were confirmed with experimentally obtained existing data.

Table 1: List of medically important flavivirus used in the present study.

S. No.	Virus	Accession No.	Size (bp)	GC %
1	Hepatitis C genotype 1	NC 004102	9646	58.23
2	Hepatitis C genotype 2	NC 009823	9711	56.85
3	Hepatitis C genotype 3	NC 009824	9456	55.65
4	Hepatitis C genotype 4	NC 009825	9355	56.17
5	Hepatitis C genotype 5	NC 009826	9343	57.09
6	Hepatitis C genotype 6	NC 009827	9628	55.39
7	Dengue type 1	NC 001477	10735	46.55
8	Dengue type 2	NC 001474	10,723	45.82
9	Dengue type 3	NC 001475	10707	46.71
10	Dengue type 4	NC 002640	10649	47.12
11	Japanese encephalitis	NC 001437	10976	51.42
12	Yellow fever	NC 002031	10862	49.72

Table 2: Promoter sequences identified in present study.

S. No.	Promoter name	Transcription factor	Sequence	Size (bases)
1	AP1-SV40.2	AP-1	TGACTAA	7
2	AP-2 CS6	AP-2	YCSCCMNSS	10
3	AP-2 CS4	AP-2	YCSCCMNSS	10
4	APRT-CHO US	Unknown	GCCCCACC	8

5	ATF RS	ATF	TGACGT	6
6	CREB CS1	ATF/CREB	ACGTCA	6
7	beta-pol CS	Unknown	NTGACGTCA	10
8	CP1-MLP	CP1	AACCAAT	7
9	CRE.l	CREB	CGTCA	5
10	CRE.2	CREB	KWCGTCA	7
11	CREB CS2	CREB	ACGTCA	6
12	CRE somatostatin	CREB	TGACGTC	7
13	hsp70.5	CTF	GATTGG	6
14	CTF/NF-la	CTF/NF-1	AGCCAAT	7
15	E2F CS.l	E2F	TTTCGCGC	8
16	E4F1-Ela.3	E4F1	ACGTCAG	7
17	EARLY-SEQ1	Unknown	YYCCGCCC	8
19	EIIF-EIIaEl	EIIF	GCGCGAAA	8
20	EivF CS	EivF	GTKACGT	7
21	Element II rs-4	Unknown	TTTCGCG	7
22	GCF CS	GCF	SCGSSSC	7
23	INF.l	Unknown	AAGTGA	6
24	JCV repeated sequen	Unknown	GGGNGGRR	8
25	MBF-I CS	MBF-I	TGCRCRC	7
26	NF-GMa CS	NF-GMa	GRGRTTKCA	10
27	NFkB CS2	NF-kfi	RGGGRMTY	11
28	NFkB CS3	NFkfi	GGGRATYY	10
29	NFkB CS4	NFkfi	GGGRNTYY	9
30	NFkB-IL2Ra.1	NFkfi	GGGAATCTC	10
31	NFkB-TCR-beta	NFkfi	GGGAGATT	10
32	NF-S CS	NF-S	YGTCAGC	7
33	PEA1 CS	PEA1	TKAGTCA	7
34	PR-uterogl.3	PR	TGTTCACT	8
35	PuF RS	PuF	GGGTGGG	7
36	SDR RS	SDR	GRGSGGTG	8
37	SIF core RS	SIF	CCCGTC	6
38	Spl-IE-3.4	Spl	CCGCCC	6
39	Spl CS1	Spl	KGGGCGGR	10
40	Spl-HIV-1.3	Spl	GGGGAGTG	10
41	Spl-hsp70 (1)	Spl	GGCGGG	6
42	Spl-IE-3.3	Spl	CCCGCC	6
43	SP1-SV/6	Spl	GGGGCGGG	10
44	Spl-SV40.4	Spl	GGGGCGGG	8
45	CArG box CS	SRF	CCWWWWW	10
46	T-Ag-SV40.3	T-Ag	GGGGC	5
47	TATA-box-CS	TFIID	TATAWAW	7
48	Ad2MLP US.5	TFIID	TATAAAA	7

49	TATA-box.2	TFIID	TATAAA	6
50	UCE.2	Unknown	GGCCG	5
51	U2snR.2	(Spl)	ACGCCC	6
52	Y box (2)	Y	CTGATTGG	8

Table 3: Different promoters identified in studied flavivirus and their genomic location.

S. No.	Virus	Promoter	Range of location in genome	Strand
1	HCV 1	AP-2 CS6	276 to 526	Forward
		AP-2 CS4		
		ATF-RS		
		CREB CS1		
		CRE-somatostatin		
		CRE.2		
		E4F1-Ela.3		
		EivF CS		
		element II rs-4		
		GCF CS		
		hsp-70.5		
		NF-S CS		
		T-Ag-SV40.3		
2	HCV 2	No promoter regions		
3	HCV 3	No promoter regions		
4	HCV 4	AP-2 CS4	471-721	Forward
		AP-2 CS6		
		GCF CS		
		NFkB CS2		
		NFkB CS3		
		NFkB CS4		
		NFkB-IL2Ra.1		
		NFkB-TCR-beta		
		PuF RS		
		SIF core RS		
		T-Ag-SV40.3		
		PR-uterogl.3	3723 to 3973	Forward
		TATA-box.2		
		AP-2 CS6		
		GCF CS		
		UCE.2	5956 to 5706	Reverse
		AP-2 CS4		
		AP-2 CS6		
		GCF CS		

		U2SnR.2		
		Spl-IE-3.4		
		Spl-SV40.4		
		Spl-IE-3.3		
		Spl-hsp70 (1)		
5	HCV 5	U2snR.2	166-416	Forward
		AP-2 CS6		
		Element_II_rs-4		
		T-Ag-SV40.3		
		SDR RS		
		UCE.2		
		Spl-SV40.4		
		Spl-IE-3.4		
		ATF-RS		
		CREB CS1		
		CRE.1		
		CRE.2		
		GCF.RS		
		CArG Box CS		
		JCV repeated seq	6705-6955	Forward
		Nf-GMa CS		
		AP-2CS6		
		EARLY-SEQ1		
		UCE.2		
		Spl-hsp70 (1)		
		Spl-IE-3.3		
		Spl-SV40.4		
		Spl-IE-3.4		
		Spl-GC Box (1)		
		Spl CS1		
6	HCV6	T-Ag-SV.40.3	6602-6352	Reserve
		UCE.2		
		GCF-CS		
		TATA-box2		
		NFkB CS2		
		U2snr2		
		UCE.2		
		T-Ag-SV40.3		
		Spl-GC box (1)		
		INF1		
		Spl-IE 3.4		
		Spl-SV 40.4		
7	DV1	ATF RS	5206-4956	Reverse

		CREB CS1		
		MBF-1CS		
		CRE.2		
		E4F1-Ela.3		
		CRE.1		
8	DV2	AP-2CS6	5381-5631	Forward
		Spl-HIV-1.3		
		Hsp70.5		
		TATA-box-CS		
		Ad2MLP US.5		
		TATA-box2		
		CRE.2	7892-8142	Forward
		CRE CS2		
		AP-2 CS6		
		CP1-MLP		
		ATF RS		
		CREB CS1		
		Spl-hsp70 (1)		
		Spl-IE-3.3		
		JCV repeated sequence		
		T-Ag-SV40.3	9381-9631	Forward
		GCF RS		
		EIIF-EIIAE1		
		E2F CS1		
		Element II rs-4		
9	DV3	No promoter region		
10	DV4	ATF RS	4499-4749	Forward
		INF.1		
		Hsp70.5		
		Beta-pol CS		
		cAMP RE		
		CREB CS1		
		CREB CS2		
		CRE.2		
		E41-Ela.3		
		NF-S CS	4980-4730	Reverse
		ATF RS		
		CREB CS2		
		cAMP RE		
		CRE somatostatin		
		CRE.2		
		INF.1		
		AP-2 CS4		

		AP-2 CS6		
		hsp70.5		
		Beta-pol CS		
		CREB CS1		
11	JEV	CRE.1	4355-4605	Forward
		CTF/NF-1a		
		GCF CS		
		UCE.2		
		Spl-1E-3.4		
		AP-2 CS6		
		Spl-1E-3.3		
		Spl-hsp70(1)		
		APRT-CHO US	7846-8096	Forward
		Spl-IE-3.3		
		Spl CS1		
		Spl-HIV-1.3		
		Spl-hsp70(1)		
		Spl-IE-3.4		
		SP1-SV/6		
		Spl-SV40.4		
		JCV repeated sequence		
		EARLY-SEQ1		
		U2snR.2		
		UCE.2		
		T-Ag-SV40.3		
12	YFV	Y box (2)	10196-10446	Forward
		Ap1-sv40.2		
		PEA1 CS		
		JCV repeated sequence		
		TATA-box.2		
		hsp70.5		
		U2snR.2	2584-2334	Reverse
		Spl-SV40.4		
		Spl-IE-3.4		

Discussion

Accurate prediction of transcription factor binding sites is needed to unravel the function and regulation of genes discovered in genome sequencing projects. The numbers of experimentally confirmed reports are available on the identification and characterization of promoter in the virus genome. A eukaryotic promoter-specific activator protein (activators) stimulates the transcription. An acidic activator can directly interact with the transcription factor TFIIB and increase the stable assembly into a pre-initiation complex [7]. The transcription factor TFIID consisting of TATA-

binding protein (TBP) and TBP-associated factors (TAFs) plays a central role in both positive and negative regulation of transcription. The TAF N-terminal domain (TAND) of TAF1 has been shown to interact with TBP and to modulate the interaction of TBP with the TATA box, which is required for transcriptional initiation and activation of TATA-promoter operated genes [8]. The regulatory element utilizing TATT box has been reported in the genome of Epstein - Barr virus (EBV). The motif is present in promoters of lytic cycle genes and resembled a crucial host genome motif (TATA-box). Since the binding specificity of eukaryotic proteins recognizing TATA-box (TBP) was determined and no specific preference for interaction with TATT motif was found [9].

Adult T-cell leukemia (ATL) is a complex and multifaceted disease associated with human T-cell leukemia virus type 1 (HTLV-I) infection. Viral oncoprotein is considered a major contributor to cell cycle deregulation in HTLV-I transformed cells by either directly disrupting cellular factors or altering their transcription profile. Tax transactivates these cellular promoters by interacting with transcription factors such as CREB/ATF, NF-kappaB, and SRF [10].

Consensus patterns of baculovirus sequences upstream from the translational initiation sites have been analyzed and a web tool Local Alignment Promoter Predictor (LAPP) for the prediction of baculovirus promoter sequences has also been developed. Potential consensus sequences, i.e., TCATTGT, TCTTGTA, CTCGTAA, TCCATTT and TCATT plus TCGT in approximately 30 bp spacing context, have been found in baculovirus promoter regions, in addition to well characterized late and early promoter elements G/T/ATAAG and TATAA, which is accompanied about 30-bp downstream by a transcriptional initiation sequence CAGT or CATT [11].

The K1 gene of Kaposi's sarcoma-associated herpesvirus (KSHV) encodes a 46-kDa transmembrane glycoprotein that possesses transforming properties initiates signaling pathways in B cells and prevents apoptosis. K1 promoter demonstrated that purified Rta protein bound to the K1 at three locations independent of other DNA-binding factors [12]. Kaposi sarcoma-associated herpesvirus vIRF is a viral transcription factor that inhibits interferon signaling and transforms NIH 3T3 cells but does not bind interferon-stimulated response element (ISRE) DNA sequences [13].

The adenovirus E1A gene and bICP0 encode proteins that are potent activators of viral gene expression. They do not specifically bind DNA and both proteins interact with chromatin-remodeling enzymes. A functional similarity of E1A was tested initially to see if it could stimulate BHV-1 productive infection. E1A consistently stimulates BHV-1 productive infection, but not as efficiently as bICP0. The ability of E1A to bind Rb family members plays a role in stimulating productive infection, suggesting that E2F family members activate productive infection. E2F-4, but not E2F-1, E2F-2 or E2F-5, activates productive infection with similar efficiency as E1A [14].

The complete genome of White Spot Syndrome was analyzed *in silico* to identify the conserved promoter motifs. In the 5` upstream region contained the TATA box element is similar to the Drosophila RNA polymerase II core promoter sequences and utilization of the cellular transcription machinery for generating early transcripts [13].

Present study may help in the Delineation of the promoter that is fundamental for

understanding gene expression patterns, regulation networks, cell specificity and development. It is also important for designing efficient expression vector or to target specific delivery system in the gene therapy. These results might help in designing the live attenuated vaccine candidate through the site directed mutagenesis in the promoter region. In the large scale genomic era promoter prediction is crucial for gene discovery and annotation.

References

[1] van Regenmortel, M.H.V., Fauquet, C.M.F., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C. R. and Wickner, R.B., 2000, Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses, Academic Press, San Diego pp. 872–878.

[2] Choo, Q.L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W. and Houghton, M., 1989, Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science, 244(4902), pp. 359–362.

[3] Mongkolsapaya, J., Duangchinda, T., Dejnirattisai, W., Vasanawathana, S, dee Avirutnan, P., Jairungsri, A., Khemnu, N., Tangthawornchaikul, N., Chotiyarnwong, P., Sae-Jang, K., Koch, M., Jones, Y., McMichael, A., Xu, X., Malasit, P. and Screaton, G., 2006, T Cell Responses in Dengue Hemorrhagic Fever: Are Cross-Reactive T Cells Suboptimal? J Immunol., 176(6), pp. 3821-3829.

[4] Halstead, S.B., 1988, Pathogenesis of dengue: challenge to molecular biology. Science, 239, pp. 476–481.

[5] Gubler, D.J., 2002, Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol., 10, pp.100-103.

[6] Prestridge, D.S., 1995, Predicting Pol II promoter sequences using transcription factor binding sites. J. Mol. Biol., 249, pp. 923-932.

[7] Roberts, S.G., Ha, I., Maldonado, E., Reinberg, D. and Green, M.R., 1993, Interaction between an acidic activator and transcription factor TFIIB is required for transcriptional activation. Nature, 363(6431), pp. 741–744.

[8] Mal, T.K., Masutomi, Y., Zheng, L., Nakata, Y., Ohta, H., Nakatani, Y., Kokubo, T. and Ikura, M., 2004, Structural and functional characterization on the interaction of yeast TFIID subunit TAF1 with TATA-binding protein. J Mol Biol., 339, pp. 681–693.

[9] Wyrwicz, L.S, and Rychlewski. L., 2007, Identification of Herpes TATT-binding protein. Antiviral Res., 75, pp. 167-172.

[10] de la Fuente, C., Gupta, M.V., Klase, Z., Strouss, K., Cahan, P., McCaffery, T., Galante, A., Soteropoulos, P., Pumfery, A., Fujii, M. and Kashanchi, F., 2006, Involvement of HTLV-I Tax and CREB in aneuploidy: a bioinformatics approach. Retrovirology, 3, pp. 43.

[11] Xing, K., Deng, R., Wang, J., Feng, J., Huang, M. and Wang, X., 2005, Analysis and prediction of baculovirus promoter sequences. Virus Res., 113(1), pp.64–71.

[12] Bowser, B.S., Morris, S., Song, M.J., Sun, R. and Damania, B., 2006, Characterization of Kaposi's sarcoma-associated herpesvirus (KSHV) K1 promoter activation by Rta. Virology, 348, pp. 309–327.

[13] Jayachandra, S., Low, K.G., Thlick, A.E., Yu, J., Ling, P.D., Chang, Y. and Moore, P.S., 1999, Three unrelated viral transforming proteins (vIRF, EBNA2, and E1A) induce the MYC oncogene through the interferon-responsive PRF element by using different transcription coadaptors. Proc. Natl. Acad. Sci. USA, 96, pp. 11566-11571.

[14] Geiser, V. and Jones, C., 2003, Stimulation of bovine herpesvirus-1 productive infection by the adenovirus E1A gene and a cell cycle regulatory gene E2F-4. J. Gen. Virol., 84, pp. 929-938.