Acta virologica **58**: 86 – 91, 2014 doi:10.4149/av_2014_01_86

The characteristics of synonymous codon usage in the initial and terminal translation regions of encephalomyocarditis virus

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Received July 2, 2013; accepted February 19, 2014

Summary. – The synonymous codon usage patterns in the initial and terminal translation regions (ITR, TTR) of the whole coding sequence of encephalomyocarditis virus (EMCV) were analyzed in relation to those in its natural hosts using the sequences accessible in databases. In general, some low-usage host codons were found over-represented in the ITR and TTR of the virus, while some high-usage host codons were found under-represented in the two viral regions. These relationships are thought to participate in the regulation of the speed of translation of viral proteins and in the suppression of ribosomal traffic jams, both aiming at the increase of virus yields.

Keywords: encephalomyocarditis virus; initial translation region; terminal translation region; synonymous codon usage

Introduction

The encephalomyocarditis virus (EMCV, the genus *Cardiovirus* of the family *Picornaviridae*) is an RNA virus with a worldwide distribution. This virus is a single-stranded positive-sense RNA virus with a genome of about 7.8kb, which consists of an open reading frame (ORF) flanked by two untranslated regions (UTR) at the 5'- and 3'-ends. The 5'-UTR comprises an internal ribosome entry site (IRES), from which viral protein translation is initiated in a cap-independent manner (De Pietri Tonelli *et al*., 2003; Denis *et al*., 2006). The 3'-UTR terminates with an heterogeneous poly (A) tail involved in the binding of the viral RNA-dependent RNA polymerase, whose encoding gene is located upstream of the 3'-UTR (Cui *et al*., 1993). The two ends flanking ORF of EMCV play an important role in the translation of the polyprotein (Davies and Kaufman, 1992; Oudshoorn *et al*., 1990). During the course of translation of the polyprotein of EMCV, the IRES controls binding of the 40S ribosomal subunit to initiate this translation (Jackson and Kaminski, 1995; Kaminski *et al*., 1994). The influence of the UTR on the codon usage pattern of the ITR of a particular gene has been reported by previous studies (Zhou *et al*., 2010, 2013b,c). Although evolutionary studies generally suggest that the viral genes with efficient expression represent high codon adaptation in host cell environment, the precise fitness of viral genome associated with translationally adapted codons remains a topic of active debate (Kudla *et al*., 2009; Stanssens *et al*., 1986). Tuller *et al*. (2010) reported that both ITR and TTR play a role in the control of the gene expression at the late stage of translation initiation (Tuller *et al*., 2010). In order to investigate the potential effects of the synonymous codon usage of the hosts on the translation of EMCV proteins, we employed the codon usage bias, which is included in the synonymous codon usage data of the hosts (pig, human and mouse), to analyze the codon usage bias of EMCV ITR and TTR in relation to the whole coding sequence of EMCV.

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Abbreviations: EMCV = encephalomyocarditis virus; IRES = internal ribosome entry site; ITR = initial translation region; RSCU = relative synonymous codon usage value; TTR = terminal translation region; UTR = untranslated region

Materials and Methods

The 16 ORFs of EMCV were downloaded from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.

nlm.nih.gov/Genbank/). The accession numbers are: AF356822, DQ464062, DQ464063, FJ604852, FJ604853, FJ897755, HM641897, DQ517424, EU780148, EU780149, DQ288856, X87335, AY296731, M37588, M22457, and X74312.

To investigate the synonymous codon usage bias of the ITR and TTR of the EMCV ORF, we employed multiple sequence alignments performed with the Clustal W (1.7) computer programs. To analyze the relationship of the codon usage between EMCV and its natural hosts, the synonymous codon usage frequencies of domestic pig, human and mouse were obtained from the codon usage database http://www.kazusa.or.jp/codon/ (Nakamura *et al*., 2000), and the synonymous codon usage data for EMCV and the three hosts were calculated as "relative synonymous codon usage value" (RSCU) by following formula (Sharp *et al*., 1986):

$$RSCU = \frac{g_{ij}}{\frac{n_i}{\sum_j g_{ij}}} \cdot n_i$$

where g_{ij} is the observed number of the the j^{th} codon for the i^{th} amino acid (which has n_i synonymous codons). The RSCU data were used to evaluate the difference of the synonymous codon usage between EMCV and its hosts.

In order to identify the usage bias of the 59 synonymous codons, it was arbitrary set that codons with RSCU values >1.6 were regarded as over-represented, while codons with RSCU values <0.6 were considered under-represented (Wong *et al*., 2010; Zhou *et al*., 2013a). Therefore, a synonymous codon with RSCU value <0.6 or >1.6 can be defined as a biased one in this study. In a comparison of the synonymous codon usage pattern between the virus and the hosts, if both RSCU values for a specific codon of the virus and that of the corresponding codon for host are in the same range (i.e. less than 0.6 or more than 1.6 or between 0.6 and 1.6), this pattern will be considered to be a similar codon usage pattern. In this study, a group of codons, whose RSCU values ranged from 0.6 to 1.6 needed to be defined again, namely, when both RSCU value of EMCV and that of the hosts for the same codon range from 0.6 to 1.0 or from 1.0 to 1.6, the usage pattern of the specific codon between the virus and the host is considered to be similar.

To calculate the codon usage preference (R) in synonymous codon usage between the target contexts and the whole coding sequence of EMCV, we developed a simple method based on a previous report (Zhou *et al*., 2011).

$$R = \ln\left(\frac{n_{240} / N_{240}}{n / N}\right)$$

where n_{240} is the sum of a synonymous codon in the 240 codons comprising the ITR or TTR of EMCV, N_{240} is the sum of the corresponding amino acids in the given region, n is the sum of this synonymous codon in the whole coding sequence, N is the sum of the corresponding amino acid in the whole coding sequence.

Results

The synonymous codon usage pattern between EMCV ORF and its hosts

As for the synonymous codon usage patterns of EMCV ORF, it is noted that some under-represented codons (UUA for Leu, UCG, AGU, AGC for Ser, CCG for Pro, ACG for Thr, GCG for Ala, and CGA for Arg) and only one over-represented codon (AGA for Arg) were found in this virus ORF (Table 1). It is interesting that the degree of codon usage bias for four amino acids (Phe, Tyr, Glu, and Cys), which are each encoded by two synonymous codons, is relative small. The phenomena might serve as a genetic marker of EMCV. In addition, we found that the codons with CpG, CpC, and GpG dinucleotides are not over-represented in EMCV ORF (Table 1). This feature might to some degree enable this virus to replicate successfully. As for the codon usage pattern of EMCV and the three hosts, 21 out of 59 codons are similarly used (Table 1). It is noted that the four amino acids (Phe, His, Asn, and Asp), which are each encoded by two synonymous codons, have a reversed tendency to be selected by EMCV and the three hosts (Table 1). Among the under-represented codons mentioned above, the synonymous codon usage patterns of UUA, UCG, CCG, ACG, and GCG in EMCV ORF are similar with the corresponding ones of the hosts (Table 1). This result may suggest that the translation selection of the hosts plays an important role in the formation of the synonymous codon usage of this virus.

The codon usage bias for the ITR and TTR of EMCV

The relationships between the synonymous codon usage data for pig, human and mouse and codon usage bias for the ITR and TTR of EMCV, respectively, were analyzed. It is interesting that the usage bias for some synonymous codons (all synonymous codons for Phe, Gln, Asn and Asp, UUA, CUU, CUC, and CUG for Leu, AUU for Ile, GUU, GUC and GUA for Val, UCA, UCG and AGC for Ser, CCC, CCA and CCG for Pro, ACC and ACG for Thr, GCC and GCG for Ala, CGU, CGC, CGA and AGA for Arg, GGU, GGA and GGG for Gly) was similar for both of the two target regions, since the codon usage preference (R) values of these codons in the two target regions are similar (both more than 0 or less than 0) (Table 2). Some codons (CUG for Leu, AGC for Ser, ACC for Thr, GCC for Ala, and CAG for Gln), which are rarely used by the three hosts, have a strong tendency to exist in the two target regions (Fig. 1-2; Tables 1 and 2). As

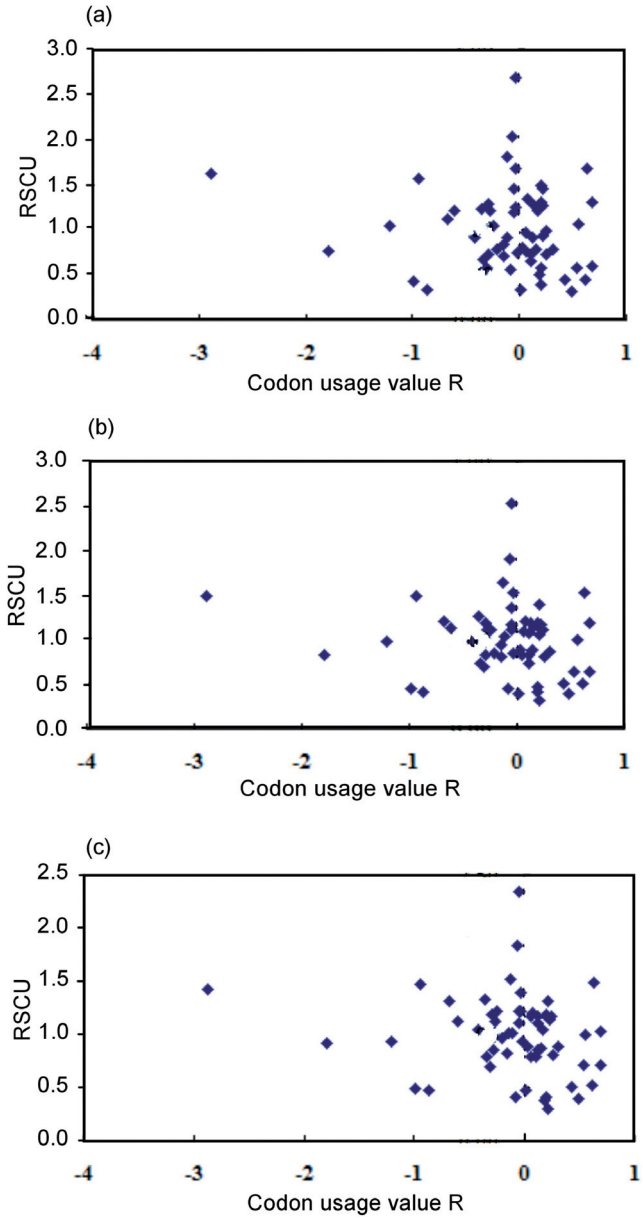

Fig. 1

The relationship between the synonymous codon usage (RSCU values) for the hosts and the codon usage preference (R values) for the EMCV ITR

(a) The relationship between the synonymous codon usage for pig and the codon usage for EMCV ITR; (b) the relationship between the synonymous codon usage for human and the codon usage for EMCV ITR; (c) the relationship between the synonymous codon usage for mouse and the codon usage for EMCV ITR. The R values indicate the difference of usage bias for synonymous codon between the regions of interest compared to the entire coding region of EMCV RNA.

Table 1. RSCU values for total sequences of EMCV and its natural hosts

Codons (amino acids)	EMCV	Pig	Mouse	Human
UUU(F)	1.11	0.79	0.88	0.87
UUC(F)	0.89	1.21	1.12	1.13
UUA(L)	0.45↓	0.32↓	0.40↓	0.39↓
UUG(L)	1.43	0.67	0.79	0.73
CUU(L)	0.96	0.65	0.79	0.73
CUC(L)	0.99	1.35	1.20	1.21
CUA(L)	0.77	0.33↓	0.48↓	0.40↓
CUG(L)	1.41	2.68↑	2.34↑	2.53↑
AUU(I)	1.56	0.91	1.02	1.03
AUC(I)	0.70	1.67↑	1.49	1.52
AUA(I)	0.73	0.42↓	0.49↓	0.44↓
GUU(V)	0.92	0.57↓	0.69	0.69
GUC(V)	0.95	1.07	1.00	1.00
GUA(V)	0.64	0.34↓	0.48↓	0.42↓
GUG(V)	1.49	2.03↑	1.84↑	1.90↑
UCU(S)	1.54	0.99	1.18	1.11
UCC(S)	1.32	1.50	1.31	1.39
UCA(S)	1.43	0.73	0.86	0.84
UCG(S)	0.54↓	0.39↓	0.30↓	0.33↓
AGU(S)	0.59↓	0.77	0.92	0.84
AGC(S)	0.58↓	1.62↑	1.43	1.50
CCU(P)	1.06	1.05	1.22	1.12
CCC(P)	1.11	1.46	1.21	1.35
CCA(P)	1.33	0.94	1.15	1.07
CCG(P)	0.50↓	0.56↓	0.41↓	0.46↓
ACU(T)	1.26	0.83	1.01	0.94
ACC(T)	1.28	1.68↑	1.40	1.52
ACA(T)	1.14	0.92	1.18	1.07
ACG(T)	0.32↓	0.57↓	0.41↓	0.46↓
GCU(A)	1.21	0.96	1.17	1.09
GCC(A)	1.44	1.80↑	1.52	1.64↑
GCA(A)	0.84	0.74	0.93	0.85
GCG(A)	0.52↓	0.50↓	0.38↓	0.42↓
UAU(Y)	1.01	0.73	0.86	0.84
UAC(Y)	0.99	1.27	1.14	1.16
CAU(H)	1.22	0.70	0.82	0.81
CAC(H)	0.78	1.30	1.18	1.19
CAA(Q)	0.83	0.44↓	0.52↓	0.51↓
CAG(Q)	1.17	1.56	1.48	1.49
AAU(N)	1.18	0.79	0.87	0.89
AAC(N)	0.82	1.21	1.13	1.11
AAA(K)	0.85	0.76	0.79	0.82
AAG(K)	1.15	1.24	1.21	1.18
GAU(D)	1.18	0.80	0.89	0.89
GAC(D)	0.82	1.20	1.11	1.11
GAA(E)	0.93	0.72	0.81	0.81
GAG(E)	1.07	1.28	1.19	1.19
UGU(C)	0.99	0.79	0.96	0.86
UGC(C)	1.01	1.21	1.04	1.14
CGU(R)	0.83	0.44↓	0.51↓	0.51↓
CGC(R)	0.69	1.31	1.02	1.20
CGA(R)	0.34↓	0.60↓	0.72	0.63
CGG(R)	0.62	1.29	1.11	1.20
AGA(R)	2.52↑	1.12	1.32	1.20
AGG(R)	1.00	1.23	1.33	1.26
GGU(G)	1.06	0.57↓	0.71	0.64
GGC(G)	0.91	1.46	1.31	1.40
GGA(G)	1.26	0.91	1.04	0.98
GGG(G)	0.77	1.05	0.94	0.98

(↑) = over-represented; (↓) = under-represented.

Table 2. The R values for the initiation translation region and the terminal translation region of EMCV

Codon (amino acids)	Initiation translation region	Terminal translation region
UUU(F)	0.31	0.21
UUC(F)	-0.61	-0.33
UUA(L)	0.49	0.23
UUG(L)	-0.34	0.06
CUU(L)	0.11	0.11
CUC(L)	0.08	0.20
CUA(L)	0.01	-0.25
CUG(L)	-0.04	-0.30
AUU(I)	-0.11	-0.41
AUC(I)	0.64	-0.52
AUA(I)	-0.98	0.75
GUU(V)	-0.31	-0.24
GUC(V)	0.56	0.04
GUA(V)	-0.87	-0.57
GUG(V)	-0.06	0.26
UCU(S)	0.25	-0.32
UCC(S)	0.21	-0.86
UCA(S)	0.12	0.37
UCG(S)	0.21	0.82
AGU(S)	-1.79	0.07
AGC(S)	-2.88	-0.37
CCU(P)	-0.25	0.15
CCC(P)	-0.04	-0.33
CCA(P)	0.21	0.31
CCG(P)	-0.08	-1.15
ACU(T)	-0.14	0.33
ACC(T)	-0.04	-0.02
ACA(T)	0.12	-0.53
ACG(T)	0.20	0.04
GCU(A)	0.06	-1.00
GCC(A)	-0.12	-0.16
GCA(A)	-0.03	0.53
GCG(A)	0.19	0.56
UAU(Y)	-0.28	0.12
UAC(Y)	0.22	-0.14
CAU(H)	-0.15	0.50
CAC(H)	0.20	None
CAA(Q)	0.62	0.42
CAG(Q)	-0.94	-0.46
AAU(N)	0.15	0.13
AAC(N)	-0.27	-0.23
AAA(K)	0.05	-0.04
AAG(K)	-0.04	0.03
GAU(D)	0.03	0.26
GAC(D)	-0.05	-0.56
GAA(E)	0.25	-0.25
GAG(E)	-0.29	0.18
UGU(C)	-0.21	0.55
UGC(C)	0.17	-1.27
CGU(R)	0.43	0.28
CGC(R)	0.68	0.14
CGA(R)	0.68	0.25
CGG(R)	0.12	-0.17
AGA(R)	-0.68	-0.24
AGG(R)	-0.35	0.16
GGU(G)	0.53	0.62
GGC(G)	0.22	-0.15
GGA(G)	-0.42	-0.69
GGG(G)	-1.21	-0.22

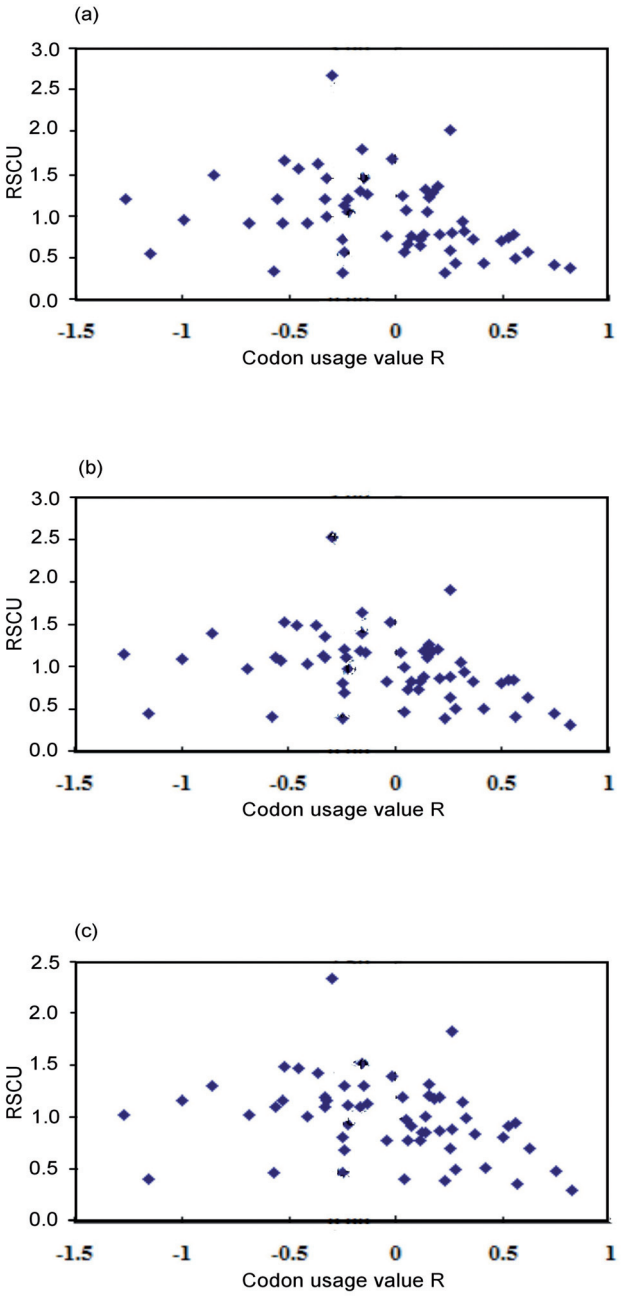

Fig. 2

The relationship between the synonymous codon usage (RSCU values) for the hosts and the codon usage preference (R values) for the EMCV ITR

(a) The relationship between the synonymous codon usage for pig and the codon usage for EMCV TTR; (b) the relationship between the synonymous codon usage for human and the codon usage for EMCV TTR; (c) the relationship between the synonymous codon usage for mouse and the codon usage for EMCV TTR. The R values indicate the difference of usage bias for synonymous codon between the regions of interest compared to the entire coding region of EMCV RNA.

for the under-represented codons of its hosts, UUA, CUA, UCG, ACG, GCG, CAA, CGU, CGA, and GGU have a strong tendency to exist in the EMCV ITR, and UUA, AUA, UCG, ACG, GCG, CAA, CGU, CGA, and GGU have a strong tendency to exist in the EMCV TTR (Tables 1 and 2). As for the over-represented codons of the hosts, CUG, GUG, ACC, and GCC have a slight tendency to exist in the EMCV ITR, and CUG, AGC, ACC and GCC have a slight tendency to exist in the EMCV TTR. These results suggest that the usage patterns of codons existing in the two target regions play a role in the translational regulation of EMCV ORF.

Discussion

Viruses are ubiquitous cellular parasites and have a strong tendency to replicate and evolve rapidly. In this study, we found that some synonymous codon usage patterns of EMCV are similar with those of the three hosts (human, pig and mouse). This genetic characteristic may assist this virus to adapt to the cellular environment of the host and replicate in the host (Bahir *et al*., 2009; Welch *et al*., 2009). More specifically, the adaptation refers to the usage of the highly abundant tRNAs within the host cell by the virus, which would be optimal when the codon usage of the virus matches with that of the host. In order to maximize the translation speed of viral protein synthesis, the viral production impairs the immune response inside virus-infected cells, otherwise a large amount of non-preferred codons highly selected in viral genes could lead to low yield of viral proteins (Dupas *et al*., 2003; Sanchez *et al*., 2003). It is clear that the property of high stacking energy can effectively impair viral genome replication and transcription. EMCV has no obvious tendency to select the codons with CpG, CpC, and GpG dinucleotide. This characteristic may help the virus to reduce stacking energy of ORF and perform viral genome replication and transcription quickly.

The codon usage pattern plays an important role in controlling the gene expression (Parmley and Huynen, 2009; Rocha, 2004; Rosano and Ceccarelli, 2009). The purpose of this study was to explore the potential influence of the synonymous codon usage pattern of the hosts on the EMCV ITR and TTR. The distribution of the low-usage codons in a message is a major factor impacting the translation rate and the distribution of ribosomes on that message, and low-usage codon clusters are much more effective in blocking ribosome movement on the message than the dispersed low-usage codons (Zhang *et al*., 1994). The rare codons close to the initiator may stall the ribosome and prevent the entry of new incoming ribosomes (Chen and Inouye, 1994). The low-usage codons of the hosts, which are highly selected by the EMCV ITR and TTR, might influence the ribosome scanning. Some previous reports pointed out that the synonymous codon usage of the translation initiation region in the gene can play an important role in regulating the translation elongation efficiency (Rocha, 2004; Varenne *et al*., 1984).

Acknowledgements. This study were supported by the grant (No. 12XB37) from the University's Scientific Fund for the middle-aged and young scientists supported by the Northwest University for Nationalities and the grant (No. 31160033) from the National Natural Science foundation of China and the grant (ZYZ2012067) from the Fundamental Research Funds for the Central Universities deriving from the Northwest University for Nationalities.

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