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Minireview

Basepairing with 18S ribosomal RNA in internal initiation of translation

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Abstract In concert with the translation initiation factors '*trans*-acting' factors function specifically during internal initiation on picornaviral mRNAs. Of these *trans*-acting factors, two have been identified as the La-protein and the polypyrimidine tract binding protein. Within the internal ribosomal entry site on the viral RNA, sequences are present that direct the ribosome to the initiation codon. We suggest that selection of the correct AUG initiation codon occurs through basepairing with a part of 18S ribosomal RNA.

Key words: Picornavirus; Translation; Internal initiation; Polypyrimidine tract; 18S ribosomal RNA; Basepairing

1. Internal initiation and eukaryotic initiation factors

Internal initiation of translation on the 5′ untranslated region (5′UTR) of picornavirus RNAs has been studied extensively (for reviews see [1,2]). Sequences within the 5′UTRs form an internal ribosomal entry site (IRES), enabling initiation of protein synthesis without the need for a cap-structure at the 5′ end of the RNA. The IRES directs eukaryotic initiation factors (eIFs) and the small ribosomal subunit to the 3′ part of the 600–1200 nucleotides long 5′UTR, in the case of encephalomyocarditis virus (EMCV) RNA close to the initiation codon [3]. This mechanism seems to be in contrast to the mechanism of translation initation on capped cellular mRNAs, in which cap-binding eIFs and 5′UTR unwinding factors create a stretch of single-stranded RNA to which the small ribosomal subunit binds [4]. However, several reports have indicated that the same initiation factors are needed for cap-dependent and internal initiation, including the cap-binding factors eIF-4E and eIF-4F [5,6]. eIF-4F consists of three subunits: eIF-4E, the cap-binding protein, eIF-4A, an ATP dependent RNA helicase, and p220. Although the function of p220 is unknown its intactness is needed for cap-dependent translation [6]. Various picornaviruses encode a protease whose activity leads to cleavage of the p220 subunit of eIF-4F, thereby inhibiting cap-dependent translation, and causing 'host shut-off' [7]. The need for intact p220 is the only difference found in eIF-dependence between cap-dependent amd cap-independent translation.

Besides all known eIFs additional proteins play a role in internal initiation. Binding of these proteins to the picornaviral 5′UTRs was detected by RNA retardation and UV cross-linking [1,8,9]. The identity of two such proteins has been established. The first one is a protein of 52 kDa which is identical to the La-protein, an RNA polymerase III termination factor [10]. The other one is the polypyrimidine tract binding protein (PTB) of 57 kDa [11]. PTB is involved in splicing of pre-mRNAs but seems to have no splicing activity itself [12]. Some other proteins have been detected to interact specifically with the picornaviral 5′UTRs [13,14] but they have not been identified yet. This minireview focuses on the role of basepairing between 18S rRNA and the 5′UTR of picornavirus mRNAs, and roles for PTB- and La-binding will be shortly discussed.

2. Interaction of picorna 5′UTRs and 18S rRNA

Interaction between the 3′ end of ribosomal RNA of the small ribosomal subunit and part of the picornavirus 5′UTRs has been proposed by a few groups [15–17]. Interestingly, the Shine-Dalgarno sequence in prokaryotic mRNA also basepairs with the 3′ end of ribosomal RNA. The possibility of basepairing between the 3′ end of 18S rRNA and the polypyrimidine (polyY) tract in the 5′UTR of foot-and-mouth disease virus (FMDV) was originally noted by Beck et al. [18]. On the basis of this assumed basepairing small mutations in the polypyrimidine tract were made and shown to have a negative effect on the translation efficiency conferred by the FMDV 5′UTR [8]. This suggested a role for the basepairing with 18S rRNA in the efficiency of internal initiation.

Pilipenko et al. showed that the spacing between the polyY tract and an AUG codon, conserved among all picornaviruses, was important for virus formation [15]. This conserved AUG will be denoted as AUG^{y} and is the initiation codon in the 5′UTRs of the aphtho-and cardioviruses. In the entero- and rhinovirus 5′UTRs AUG^{y} does not function as initiation codon and a sequence of 30–150 nucleotides is present between AUG^{y} and the downstream initiation codon. Deletion of AUG^{y} or changes in the distance between the polyY tract and AUG^{y} in poliovirus RNA resulted in viruses with a small-plaque phenotype [15]. Revertants from these small-plaque viruses were shown to have re-created the polypyrimidine tract and an AUG codon with the proper spacing. Basepairing with 18S rRNA was postulated for the entero- and rhinovirus 5′UTRs. We extended this search for 18S rRNA basepairing with the aphtho-, cardio-, and hepatitis A virus RNAs (Table 1).

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Abbreviations: AUG^{y}, AUG codon located approximately 20 nucleotides downstream of conserved polypyrimidine tract; eIF, eukaryotic initiation factor; EMC, encephalomyocarditisvirus; FMDV, foot-and-mouth disease virus; IRES, internal ribosomal entry site; HAV, hepatitis A virus; HRV, human rhinovirus; polyY, polypyrimidine tract; PTB, polypyrimidine tract binding protein; 5′UTR, 5′ untranslated region.

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Table 1
Basepairing between 18S rRNA and picornavirus RNAs

	3'		5'		BASE-PAIRING around polyY	AUG^{y}
	CUAGGAAGGCGUCCAAGUGGAUGCCUUU			18S rRNA		
	5'		3'			
	CGUGUUUCCUUU	22	GCUUAUGGUGA	P1 Mah	8	10
	···•••••••••	22	•••••••••••	P1 Sab	8	10
	···••••••••	22	•••••••••••	P1 Lan	8	10
	···••••••G•	22	•••••••••••	P2 Sab	7	10
	···••••••••	23	•••••••••••	P3 119	8	10
	···••••••••	22	••••••••••G	P3 23127	8	10
	···••••••••	22	•••••••••••	P3 37	8	10
E	···••••••••	22	•••••••••••	P3 Sab3	8	10
N	···••••••••	22	•••••••••••	Ent 70	8	10
T	···••••U•••	22	•••••••••••	Cox A9	8	10
E	···••••C•••	22	•••••••••••	Cox A21	8	10
R	···••••A•••	23	•••••••••••	Cox B1	7	10
O	···••••A•••	23	•••••••••••	Cox B32	7	10
	···••••A•••	23	•••••••••••	Cox B3	7	10
	···••••••••	23	•••••••••••	Cox B4	8	10
	···••••••••	23	A••••••••••	SVDV uk	8	10
	···••••••••	23	A••••••••••	SVDV	8	10
	CUG•••••••••	22	CA••••••A••	ECHO 22	6	9
R	···•••••••A	23	AU•••••••••	BEV	9	10
H	···••••AC••	21	•••••••••••	HRV 1b	7	10
I	···••••AC••	20	•••••••••••	HRV 2	7	10
N	···••••••G•	22	UU•••••••••	HRV 89	7	9
O	···••••UCA•	21	U••••••••C•	HRV 14	7	8
C	UGGUUUUCCUUU	21	UAAUAUGGCCA	EMC R	8	6
A	••••••••••••	21	···G••••U••	EMC D	8	5
R	••••••••••••	21	···G••••U••	EMC B	8	5
D	••••••••••••	21	···•••••U••	MENGO	8	6
I	••••••••••••	20	C•C•••••UU	TMEV da	8	6
O	••••••UC••	19	C•••••••UU	TMEV Gd7	8	5
	••••••AC••	20	C•••••••U	TMEV BeAn	7	5
	GCACC••UUC••	19	CUU••••AA••	FMDV A12 L_{ab}	8	8
	GCACC••U••C•	18	••U••••AAU•	FMDV A10 L_{ab}	8	7
	GCACC••U•••	20	AC•C•••AAU•	FMDV O1 L_{ab}	8	7
	GCACC••U•C••	19	CCC••••AAU•	FMDV C1 L_{ab}	8	7
	GCGCC••U•••	20	•UUC•••AA••	FMDV Asia1 L_{ab}	8	6
	GCACC••UUCC•	20	•UC••••AG••	FMDV SAT1 L_{ab}	8	8
A	GCACC••UUCC•	20	•UU••••AA••	FMDV SAT2 L_{ab}	8	8
P	GCACC••UUCC•	19	•UU••••AAG•	FMDV SAT3 L_{ab}	8	9
H	GCACC••U•C••	20	•U•A••••A•G	FMDV A24 L_{ab}	8	7
T	CAU••••••••	20	A••G••••AAU	FMDV A12 L_{b}	9	7
H	C•C••••••G••	20	A••G••••AAU	FMDV A10 L_{b}	9	7
O	CAC••••••AC	20	A••A••••AAC	FMDV O1 L_{b}	7	7
	CAC••••••AC	20	A••A••••AAU	FMDV C1 L_{b}	7	7
	CAC•G••U•••	20	A••A••••AAU	FMDV Asia L_{b}	7	7
	•AAAC•••UGC•	19	G••G••••UAU	FMDV SAT1 L_{b}	8	6
	•AAAC••UUG••	19	A••G••••AGU	FMDV SAT2 L_{b}	8	7
	G•U•CGGA•AGA	22	A••G••••AAU	FMDV SAT3 L_{b}	7	7
	CAC••C••U•••	20	A••A••••AAU	FMDV A24 L_{b}	7	7
	GGUUUUUCCUCA	16	AAUAAUGAAUA	HAV HM175	11	7
H	••••••••••••	16	••••••••••••	HAV MBB	11	7
E	••••••••••••	16	••••••••••••	HAV HAS15	11	7
P	••••••••••••	16	••••••••••••	HAV CR326	11	7
A	••••••••••••	16	••••••••••••	HAV La	11	7
T	••••••••••••	16	••AU••••••••	HAV KRM003	11	7
O	••••••••••••	16	•••U••••••••	HAV PA21	11	8
	••••••••••••	16	•••CU••••••	HAV KRM003	11	8
	3'		5'			
	CUAGGAAGGCGUCCAAGUGGAUGCCUUU			18S rRNA		

The sequence in the 3' end of human 18S rRNA (nucleotides 1837–1864) is indicated in the first and last line. The number of basepairs are indicated in the two columns on the right. The first column shows the number of basepairs around the UUUCC polypyrimidine tract, the second column shows the number of basepairs around AUG^{y}. The number of nucleotides between the polyY tract and AUG^{y} are given in the left part of Table 1. Basepairs are underlined. Identical nucleotides are indicated with •. The sequences were taken from published sequences (for details see [19]).

3. Basepairing around the polypyrimidine tract

The average number of potential basepairs between the 3' sequence of 18S rRNA and the polyY tract (UUUCC) plus surrounding sequences is 8. No obvious correlation exists between this number and the translational efficiency of the picornaviral 5'UTRs. However, the 5' untranslated regions of the picornaviruses that are translated very efficiently (cardio-and aphthoviruses) seem to bind PTB more efficiently than those picornaviral 5'UTRs that are inefficiently translated in reticulocyte lysate (entero- and rhinoviruses) (unpublished results). The function of PTB in internal initiation is unknown, but a role for PTB binding to the polypyrimidine tract could be to initiate the formation of the translation initiation complex.

The polyY tract does not seem to be the sole bindingsite for PTB, as other binding sites have been proven in the 5'UTRs of

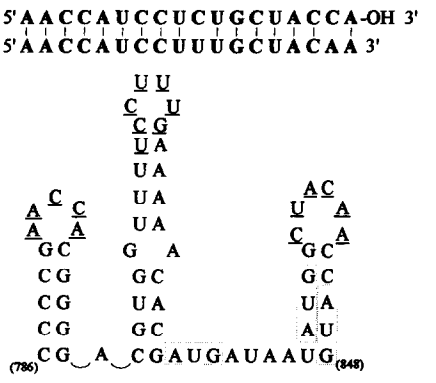


Fig. 1. Sequence comparison between initiator tRNA^{Met} and EMC RNA. The lower part of the figure shows the folding of the 3′ end of the 5′UTR of EMCV-D [31]. The nucleotides in the EMC 5′UTR that were aligned with the tRNA sequence are underlined. AUG codons are boxed, the initiation codon is situated at nucleotides 834–836. The upper part shows the alignment of the EMC sequence with nucleotides 59–76 of tRNA^{Met} [32].

EMC, poliovirus, and FMDV. Some of the SAT strains of FMDV show no apparent polyY upstream of the initiation codon for L_b. However, in spite of the absence of a pyrimidine stretch, this region of the IRES can still form 7 or 8 basepairs with 18S rRNA. The sequence variation in the polyY of SAT strains seems to exclude the binding of PTB to this sequence. PTB-binding to a different site within the IRES combined with a proper folding of the messenger could ensure formation of the initiation complex at the 3′ end of the IRES, as suggested above.

The group of hepatitis A viruses, also called the hepatoviruses, forms an exception: they show a greater extent of possible basepairing in this region: 11 out of 12. Cells can be persistently infected with HAV with no apparent damage to the cells. HAV is a very slow growing virus, in contrast to most other picornaviruses [20]. The extensive basepairing at the polyY tract may have a negative influence on translation initiation.

4. Spacing

The distance between the polypyrimidine tract and AUG^y is approximately 22 nucleotides. The spacing in the entero- and rhinoviruses (average 23) is slightly larger than in aphtho- and cardioviruses (average 20). In HAV RNA only a small number of nucleotides is present between the polypyrimidine tract and the initiation codon, compared to the other picornaviruses. In the HAV 5′UTR a second initiation codon is situated only six nucleotides downstream of the one depicted in Table 1. The spacing between polyY tract and this second initiation codon is 22 nucleotides, as found for the other picornaviruses. Both initiation codons are used by HAV [21].

5. Basepairing around AUG^y

La has been shown to play a role in initiation correction [10,22]. Especially at higher poliovirus RNA concentrations, La increased initiation in reticulocyte lysate at the authentic initiation codon and aberrant initiation was decreased. Table 1 shows that if AUG^y is not the initiation codon, as in the entero- and rhinovirus 5′UTRs, the number of potential basepairs between the 3′ end of 18S rRNA and the sequence around AUG^y is 10 basepairs. When AUG^y is the initiation codon (as in the aphtho- and cardiovirus 5′UTRs) this number of basepairs is about 7. AUG^y is not used as initiation codon in the entero- and rhinoviruses. The more extensive basepairing apparently masks AUG^y as initiation codon.

The selection of an AUG as initiation codon has been studied thoroughly by Kozak. She extrapolated a consensus sequence for a 'good' initiation codon: CCA/GCCAUGG [23]. Within this consensus sequence the purines at positions −3 and +4 (with the A of the AUG being position 1) are the most important for determining the efficiency by which this AUG is used as initiation codon. The entero- and rhino- AUG^y only have the purine at position +4, while the cardios have both crucial purines around their initiation codon. Most of the FMDV L_{ab} initiation codons lack both essential purines, while these codons are efficiently used to initiate protein synthesis. The Hepatitis A virus 5′UTRs contain the essential purine at −3, but translation initiation efficiency of these 5′UTRs is low. Therefore, the inability of AUG^y to function as initiation codon in the entero- and rhinoviruses, and not in the cardio- and aphthoviruses can only partially be explained by differences in the sequence around the AUG according to the Kozak rules.

Table 1 may explain some results in the literature that have been poorly understood. Two initiation codons (L_{ab} and L_b) are used on the FMDV 5′UTR. Table 1 shows that around AUG L_{ab}, the upstream AUG, the number of possible basepairs with 18S rRNA is higher than for EMC, mengo, and TMEV RNA. In the SAT serotypes of FMDV the sequence around AUG L_{ab} can form 8 or 9 basepairs with 18S rRNA, similar to the situation in polio- and rhino-RNA. This could prevent efficient initiation at this AUG, forcing a fraction of the initiation complexes to start at the next AUG. Around AUG L_b less basepairs can be formed, facilitating initiation at this AUG, as in EMC RNA. The SAT strains predominantly synthesize L_b [24]. In most of the FMDV serotypes basepairing at the two initiation codons is very similar. These AUGs are used as initiation codon with similar occurrence.

The coding region influences initiation on the EMC 5′UTR [25]. Changing the sequence immediately downstream of the initiation codon from GCCA to GGCG or GGGG resulted in a decrease of translation, a result that cannot be explained with Kozak's consensus rules. Of GCCA the G and A can basepair, as the sequence in 18S rRNA is CUUU (3′→5′) (see Table 1). Changing this part of the transcript to GGCG would result in 3 possible basepairs, while all four guanidines in a GGGG sequence could hybridize with the CUUU part of 18S rRNA. The number of basepairs of 18S rRNA with the region around the initiation codon would change from 6 to 8 or 9. These higher numbers of basepairs could prevent initiation, as for poliovirus AUG^y.

The sequence just downstream of the EMC initiation codon could also be involved in binding of eIF-2. Encephalomyocarditis virus (EMC) RNA does not encode a protease leading to p220 cleavage and therefore host shut-off has to be established in a different way. Efficient competition for translational components was suggested [26] and work on Mengovirus RNA (which is very similar to EMC RNA [27]) and results from our lab on EMC RNA have shown that eIF-2, the initiation factor

that delivers Met-tRNA$_i$ to the small ribosomal subunit, is the protein that is bound by these mRNAs. Sequences located approximately 100 nucleotides upstream of the initiation codon have been implicated to be part of an eIF-2 bindingsite [28]. A close look at the sequence of the initiator tRNA and the EMC sequence just upstream of and around the initiation codon in the EMC 5'UTR revealed a striking homology between sequences in some stemloops of the EMC 5'UTR and the 3' 17 nucleotides of the initiator tRNA (Fig. 1). Mutations in the sequence just downstream of the initiation codon may therefore influence binding of eIF-2. Similar stemloops and primary structure are not found in the 5'UTRs of, for example, poliovirus and FMDV. The idea of binding of eIF-2 binding to these sequences in the EMC 5'UTR does not conflict with results on minimal requirements of tRNA^{Asp}-binding by EF-Tu [29] and the structural similarities between eIF-2γ and EF-Tu [30]. The binding site for EF-Tu in tRNA also resides in the 3' terminal part of the molecule [29].

Translation of poliovirus RNA in reticulocyte lysate results in synthesis of the viral proteins, next to the synthesis of various proteins that are not derived from processing of the large polyprotein [33,34]. The aberrant proteins have been named Q, R, S, T, Y, and Z [34]. Translation initiation of these aberrant products is thought to occur within the coding region of the poliovirus RNA, especially in the 3' part. Of all AUGs present in the P2 and P3 region of the coding region, possibly leading to proteins of detectable size, three are preceded by a polyY tract comparable to the polyY-AUG^y situation in the 5'UTR. These polyY tracts are positioned approximately 20 nucleotides upstream of the AUG codons, and interestingly, the sequences around these three AUGs (at positions 7160, 6653, and 3629 [35]) can form 9 or 10 basepairs with 18S rRNA. Prevention by La of recognition of these AUGs as initiation codons, similar to the sitiuation for AUG^y in the 5'UTRs of the entero- and rhinoviruses would decrease aberrant initiation. An increase in synthesis of the correct proteins can be expected, as more eIFs and ribosomes become available for initiation at the correct AUG.

We have tried to align the sequences of the 5'UTRs of BiP, antennapaedia, and Cauliflower mosaic virus. The first two of these 5'UTRs are capable of internal initiation, although extensive secondary structures to form an IRES have not been shown within these 5'UTRs. The 5'UTR of the Cauliflower mosaic virus 35S RNA enables ribosomes to 'shunt' from the 5' end to the initiation codon [36]. For these three 5'UTRs it is hard to find a polyY with the proper distance to the initiation codon. The BiP 5'UTR can form basepairs with 18S rRNA similar to the cardioviruses; however, some G-nucleotides are present in the polypyrimidine tract. For the other two 5'UTRs no significant base-pairing could be found. The proposed basepairing with 18S rRNA seems to be a specific property of the picornavirus RNAs.

6. Experimental approach

The role of basepairing between 18S rRNA with a part of the picornaviral IRES could be studied by competition experiments in in vitro translation assays. Addition of primers containing the sequence anti-sense to the 3' end of 18S rRNA sequence or anti-sense to the IRES sequence could show the importance of the basepairing. Mutations in the sequences around AUG^y, resulting in more basepairs for the cardio- and aphtho-5'UTRs, or less basepairs in the entero- and rhino- 5'UTRs could also reveal the need for the interaction with 18S rRNA in internal initiation. This kind of study could also be carried out with the viral RNAs, to test the influence of the mutations on viability and phenotype of the viruses.

The influence of the addition of sense or antisense primers, or of the mutations in the 5' untranslated region could also influence binding of PTB to the RNA, and thereby the translation efficiency of the messenger. Changes in binding of PTB can easily be monitored by UV cross-linking of proteins.

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