Effects of sequence elements in the potato virus X RNA 5' non-translated αβ-leader on its translation enhancing activity


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The 5' non-translated αβ-leader sequence of potato virus X RNA consists of two regions: the α sequence (41 nucleotides with no G) and the β sequence (42 nucleotides upstream from AUG). The αβ-leader has been shown to enhance strongly the expression of adjacent genes in chimeric mRNAs. This phenomenon has been postulated to be due to the unpaired conformation of the 5'-terminal 30 nucleotides and/or to the presence within the α region of the CCACC pentanucleotide complementary to the T-terminal conserved structure of 18S rRNA. Different derivatives of αβ-leader have been constructed for use in determining the contribution of separate elements of the αβ sequence to translational enhancement. It was found that deletion of the α sequence large fragment which was supposed to be unfolded did not reduce the Δαβ-leader enhancement activity. Moreover, translational enhancement was greater for this derivative. Deletion of the β sequence resulted in a considerable increase in activity of the α-leader showing that the β region was dispensable for translation. Disruption or 'masking' of CCACC led to inactivation of the αβ-leader as a translational enhancer. Thus, we identified the CCACC pentanucleotide as the primary motif responsible for the translation enhancing ability of αβ-leader.

Introduction

There is much evidence suggesting that non-translated sequences located between the cap structure and the initiation codon of mRNA have a role in regulation of translation (for reviews, see Kozak, 1990, 1991). The 5' non-translated leader sequences of several plant and animal viruses have been examined for their effect on translation efficiency of chimeric mRNAs (Jobling & Gehrke, 1987; Sleat et al., 1987, 1988; Gallie et al., 1987a, b; Carrington & Freed, 1990; Smirnyagina et al., 1991; Nicolaise et al., 1992; Berben-Bloemhevel et al., 1992). The 5' non-translated leader of tobacco mosaic virus (TMV) RNA (68 nucleotides upstream from AUG) contains no internal guanosine and forms part of the 70 nucleotide, unique, T1 RNase-resistant oligonucleotide called 'omega' (Ω). Wilson and co-workers demonstrated that Ω and its derivative Ω' (67 nucleotides upstream from AUG) enhance translation in vitro in 80S and 70S ribosome cell-free systems and in vivo (for review see Wilson et al., 1990; Sleat & Wilson, 1992).

Two nucleotide motifs, a reiterated eight base direct repeat ACACUUAC and a 25 base (CAA)6 region, have been found in the Ω-leader. A deletion analysis and replacement of the (CAA)6 region with poly(U) proved that functional redundancy existed within the Ω sequence (Gallie et al., 1988). A combination of one copy of the eight base repeat with the 25 base (CAA)6 region was identified as a core regulatory element in translational enhancement (Gallie & Walbot, 1992).

The genome of potato virus X (PVX), the type member of the potexvirus group (Koenig & Lesemann, 1978), consists of a ssRNA [6435 bases excluding poly(A)], which is capped (Sonenberg et al., 1978) and polyadenylated (Morozov et al., 1981; Skryabin et al., 1988; Huisman et al., 1988). The translational strategy of PVX RNA has been studied (Wodnar-Filipowicz et al., 1980; Adams et al., 1987; Morozov et al., 1990, 1991).

The 5' non-translated leader sequence of PVX RNA (83 nucleotides away from the cap structure) consists of two sub-sequences called α sequence (41 nucleotides with no G) and β sequence (42 nucleotides upstream from the first AUG) (Smirnyagina et al., 1991). We have previously demonstrated that αβ-leader strongly enhances the translation efficiency of different reporter genes in different 80S ribosome cell-free protein synthesizing systems and in protoplast transient expression assays (Smirnyagina et al., 1991; Zelenina et al., 1992). However αβ-leader was inefficient in a 70S ribosome cell-free system from Escherichia coli (our unpublished data).
Table 1. The nucleotide sequence of αβ-leader and its derivatives constructed for examination of their translation enhancing activity

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Leader name</th>
<th>Nucleotide sequence of the 5' leader*</th>
</tr>
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<tbody>
<tr>
<td>pLX</td>
<td>αβ</td>
<td>5' GAAAACUAAACCAUACCCACAAACAAAACCCACCAC 3'</td>
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<tr>
<td></td>
<td></td>
<td>α sequence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCCCAUUGGUUACACCGCGUUGGAAAAGCAAGUGCUCUUACAAUGG 3'</td>
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<td>β sequence</td>
</tr>
<tr>
<td>p948</td>
<td>αβ</td>
<td>5' GAAAACUAAACCAUACCCACAAACAAAACCCACAAACCAAACCCACCAC 3'</td>
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<tr>
<td></td>
<td></td>
<td>α sequence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCCCAUUGGUUACACCGCGUUGGAAAAGCAAGUGCUCUUACAAUGG 3'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β sequence</td>
</tr>
<tr>
<td>pLC</td>
<td>δαβ</td>
<td>5' GAAAACAAACCCACCAC 3'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>δα sequence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCCCAUUGGUUACACCGCGUUGGAAAAGCAAGUGCUCUUACAAUGG 3'</td>
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<td></td>
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<td>β sequence</td>
</tr>
<tr>
<td>p823</td>
<td>αβins</td>
<td>5' GAAAACUAAACCAUACCCACAAACAAAACCCACCAC 3'</td>
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<td></td>
<td></td>
<td>α sequence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCCCAUUGGUUACACCGCGUUGGAAAAGCAAGUGCUCUUACAAUGG 3'</td>
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<td>β sequence</td>
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<td>p843</td>
<td>δαβ</td>
<td>5' GAAAACAAACCCACCACCCCGCUUGGAAAAGCAAGUGCUCUUACAAUGG 3'</td>
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<td>δα sequence</td>
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<td>δβ sequence</td>
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<td>β</td>
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<tr>
<td>pXS</td>
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<td>5' GAAAACAAACCCACCACCAACCAAACAAAACCCACCAC 3'</td>
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<td>β sequence</td>
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<tr>
<td>p231</td>
<td>δα(CACC)β</td>
<td>5' GAAAACAAACCCACCACCAACCAAACAAAACCCACCAC 3'</td>
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<td>β sequence</td>
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</table>

* The initiation codon and the nucleotide in position +4 are included.

Based on computer prediction analysis, Smirnyagina et al. (1991) suggested that the 5' proximal region of αβ-leader was unstructured. This feature could confer high translation efficiencies of αβ-leader-carrying mRNAs as proposed for the TMV Ω-leader (Sleat et al., 1988). The second structural feature of αβ-leader was the presence of sequences apparently complementary to the 3'-terminal region of 18S rRNA (Smirnyagina et al., 1991; Tomashevskaya et al., 1992).

The idea that mRNA–rRNA interaction may facilitate the initiation of translation in eukaryotes has been proposed by Sargan et al. (1982). Eukaryotic mRNAs do not usually contain the clearly defined 'Shine-Dalgarno' sequence for mRNA–rRNA interaction. However, in several cases base-pairing has been proposed between the mRNA leader and the 3' end of 18S rRNA (Hagenbuchle et al., 1978; Wu et al., 1987; Yamaguchi et al., 1982; Yokoe et al., 1983; Pilipenko et al., 1992). The so-called translation initiation-promoting site (TPS), potentially forming complementary base-pairing with residues flanking a conserved hairpin structure on the 18S rRNA during formation of the 40S pre-initiation complex, has been revealed recently in mRNAs from highly expressed yeast genes (Thanaraj & Pandit, 1990). A part of TPS, namely 5' CCACC 3', regarded as the mandatory AUG-context sequence was revealed in αβ-leader. The TPS was separated from AUG by more than 40 nucleotides comprising the β-region, but it may come closer to the initiator AUG by formation of a hairpin structure involving the entire β sequence of αβ-leader (Smirnyagina et al., 1991).

Here we have examined the contribution of the structural elements of αβ-leader of PVX RNA to the translation enhancing activity by constructing chimeric mRNAs containing a common reporter gene [neomycin phosphotransferase I (NPTI)] fused with derivatives of αβ-leader carrying deletions, insertions or substitutions.

**Methods**

*Plasmid constructs.* All the plasmids used contained the T7 RNA polymerase promoter, followed by either αβ-leader or its derivatives and a NPTI reporter gene. The plasmid pLX with the αβ-leader sequence was constructed from pUC4K (Pharmacia) as a source of the NPTI gene (Smirnyagina et al., 1991). Detailed information concerning the structure of the derivatives of the αβ-leader preceding the NPTI gene in chimeric RNA transcripts from the plasmids described below is presented in Table 1 and Fig. 1.

To construct the plasmid p948, containing only the α region of the αβ-leader upstream from the NPTI gene, the DNA fragment was...
described for plasmid pXT12 (Smirnyagina et al., 1991). Plasmid p823 was constructed from pLX, which was Munl linearized, filled by Klenow fragment and ligated. As a result, p823 comprised the T7 RNA polymerase promoter followed by the NPTI gene under the control of the modified αβ-leader with a 24 nucleotide deletion in the x sequence (Δαβ-leader; Fig. 1, Table 1).

Plasmid p823 was constructed from pLX, which was Munl linearized, filled by Klenow fragment and ligated. As a result, p823 comprised the T7 RNA polymerase promoter followed by the NPTI gene under the control of the αβ-leader with a four nucleotide insertion (UUAA) in the β sequence (αβ-leader; Fig. 1, Table 1).

As a first step in p823 plasmid assembly, plasmid pKMS was constructed. To construct this plasmid, the NcoI/SalI-digested fragment from pLX was cloned into EcoRI/SalI-digested pTZ18R (Pharmacia) using a synthetic EcoRI/NcoI adaptor. The resulting short leader sequence (5' GGGAATTCTACATG) upstream from the NPTI gene was the same as in pTZ-G12 described previously (Zeleny et al., 1992). Plasmid p843 was assembled from SalI/EcoRI-digested pKMS (NPTI gene-carrying DNA fragment) and SalI/Munl-cut pLC and contained the Δz sequence, the first 8 bp from the β sequence and the five nucleotide spacer (Δαβ-leader; Fig. 1, Table 1).

To construct the plasmid pXS, which was enriched with CCACC sequences (TPS) instead of having the 24-nucleotide deletion, two complementary oligodeoxynucleotides (5' GGTATACGACTCAT- ATAGAAACACCACCCACAAACTCCACACGCCC 3' and 5' ATTGGGCGTGGTGGTTTTGGTTGTTTCTATATGTAGCTCTATACGTC- TGTTTCTATACGTC 3') were annealed and cloned into the Munl/KpnI-digested pLC, resulting in pXS [Δα(TPS)β-leader; Fig. 1, Table 1].

To obtain plasmid p230 with ‘destroyed’ TPS in the α sequence two complementary oligodeoxynucleotides (5' GGTAATAGCCTCAGC- TATAGAAACACCACCCACAAACTCCACACGCCC 3' and 5' ATTGGGCG- TAATAAATTTTCTATAGTATGCGTATTACGTCG 3') were annealed and cloned into Munl/KpnI-digested pLC [Δα(TPS)β-leader; Fig. 1, Table 1].

To construct the plasmid p231, in which the first G of the β sequence was replaced by A, two complementary oligonucleotides (5' GGTAATAGCCTCAGCATTAGAAACACCACCCACAAACTCCACACGCCC 3' and 5' ATTGGGCGTGGTGGTTTTGGTTGTTTCTATATGTAGCTCTATACGTC- TGTTTCTATACGTC 3') were annealed and cloned into Munl/KpnI-digested pLC and ligated [Δα(CACC)β-leader; Fig. 1, Table 1]. The nucleotide sequences of the αβ-leader and all of its derivatives were confirmed by dideoxynucleotide sequencing (Sanger et al., 1977).

In vitro transcription and translation. Plasmids pLX, p948 and p823 were linearized by EcoRI and plasmid p817 by SalI. Plasmids pLX, p843, pXS, p230 and p231 were linearized by PstI and protruding 3' ends were removed with T4 DNA polymerase before a transcription reaction with T7 RNA polymerase (40 mM-Tris-HCl pH 7.9, 15 mM-MgCl₂, 10 mM, 2 mM-spermidine, 2 mM-ATP/CTP/UTP/GTP, 1 unit/μl RNasin, 5 μg DNA template and 0.5 unit/μl T7 RNA polymerase at 37 °C for 1 h). The RNA transcripts were precipitated in 2 M-LiCl at 0 °C overnight, washed in 70% ethanol, resuspended in water and stored at -20 °C. This approximate RNA concentration was determined by electrophoresis in 15% agarose gel. The RNA concentration was quantified by SDS-PAGE and spectrophotometry.

Cell-free translation of RNAs was carried out in rabbit reticulocyte lysates as described earlier (Morozov et al., 1990; Smirnyagina et al., 1991). Radiolabelled translation products were analysed by SDS-PAGE and localized by autoradiography on the dried gel. Radioactive bands, corresponding to the NPTI protein, were excised from the dried gel and their radioactivity was determined.
Results

Translation efficiency of transcripts containing different derivatives of αβ-leader

Constructs containing the T7 polymerase promoter, different derivatives of the αβ-leader of PVX RNA and the common reporter NPTI gene were made (Fig. 1, Table 1). In a control construct (αβ) the full-length αβ-leader (83 nucleotides) plus the AUG codon of the PVX polymerase gene was attached to the 5'-truncated NPTI gene. Uncapped chimeric in vitro transcripts were translated in rabbit reticulocyte lysates. The efficiency of NPTI gene translation under the control of the αβ-leader was taken as 100%. In Fig. 2 the translation efficiency levels are presented as averages from five to 19 independent translation experiments. Fig. 3 represents the results of two individual gel electrophoresis runs illustrating some of the data averaged in Fig. 2.

(i) α construct
Complete deletion of β sequence from the αβ-leader resulted in about 50% stimulation of the translation efficiency of the transcripts (Fig. 2, Fig. 3b), i.e. the β region can be regarded as a dispensable part of the αβ sequence translational enhancer.

(ii) β construct
In this case the α region was deleted and the reporter gene was translated under the control of the β sequence only. It was found that deletion of the α sequence resulted in a considerable decrease in translational enhancing activity (Fig. 2, Fig. 3b).

(iii) Δαβ construct
Deletion of 24 nucleotides from the α sequence resulted in transcript Δαβ, which had lost most of the 5'-proximal region of the non-translated PVX RNA leader, i.e. that part which was suggested to be unfolded and consequently accessible to the ribosomes (Smirnyagina et al., 1991). Fig. 2, 3(a) and (b) show that contrary to our expectations the translation efficiency of Δαβ transcripts was not lower, but even higher than that of the control. It should be emphasized that TPS has been retained in the Δα part of Δαβ-leader.

(iv) ΔαΔβ construct
In this case the deletion of 24 nucleotides described for the Δαβ-leader was still retained, but 34 nucleotides were additionally deleted from the β sequence. Therefore, only 25 nucleotides of the intact αβ-leader were kept including the TPS in the α sequence. It is interesting that the translation-enhancing activity of ΔαΔβ-leader was more than twice as high as that of the control (intact αβ-leader) (Fig. 2).

(v) αβΔns construct
It was recently suggested (Smirnyagina et al., 1991) that the β sequence can be folded into a relatively stable hairpin structure (Fig. 4). It could be expected that destabilization or modification of this structure by inserting additional non-complementary nucleotides in an αβΔns transcript would change the conformation of the hairpin structure. Fig. 2 and Fig. 3(a) show that insertion of UUAA into the β sequence resulted in a decrease of the translation-enhancing activity of the modified leader, although it still contained an intact α part.

(vi) Δα(TPS–)β construct
Besides the 24-nucleotide deletion in the α region (as in Δαβ), in this construct the TP site was replaced by
PVX RNA has been shown to be translationally highly efficient and competitive (Smirnyagina et al., 1991), and the αβ-leader could act in cis in chimeric transcripts to enhance the translation of different foreign reporter genes (Smirnyagina et al., 1991; Zelenina et al., 1992; Pooggin & Skryabin, 1992).

In order to assess the motifs of αβ sequence involved in translation enhancement we compared the translational efficiencies of chimeric transcripts carrying different derivatives of the αβ-leader fused to the NPTI gene (Fig. 1). The accuracy of quantification of the relative translational enhancing activities of different 5′ non-translated leaders can be questioned since the level of reporter gene expression in any control transcript (mRNA carrying a certain 5′ non-translated sequence instead of the leader-enhancer) may vary considerably. Different non-physiological polylinker-derived leader sequences taken for controls may differ substantially in their ability to influence the translation of chimeric RNAs and are hardly comparable with each other (Zelenina et al., 1992). For this reason we took as a control (100% translational enhancement) the level of NPTI gene translation stimulated by a non-modified αβ-leader of PVX RNA.

As mentioned above, computer-based modelling of the secondary structure revealed that the Y-proximal 17 to 30 nucleotides of αβ-leader are not base-paired and may be accessible to 40S ribosomal subunits (Smirnyagina et al., 1991). To examine this possibility more closely 24 nucleotides were deleted from the α sequence thereby constructing the αβ-leader, which lost the region from the sixth to the 30th nucleotide within the α sequence (Fig. 1, Table 1). In other words, this derivative of the αβ-leader is devoid of practically the entire region of the αβ-leader that was supposed to be unfolded.

If the suggestion concerning the role of the non-structural 5′ region of the αβ-leader was correct, it could be expected that deletion of this region would have resulted in translation activity reduction. However, no translation reduction of the αβ-leader has been detected. On the contrary, Fig. 2 and Fig. 3 shows that the translational enhancement in this case was greater. We have no adequate explanation of this effect, but it should be noted that (i) the computer-based predictions can be regarded only as indirect evidence for the unfolded state of the 5′ end of the αβ sequence and (ii) the 24 nucleotide deletion removed a region of the α sequence containing at least three sketches of nucleotides (AAACC, CACC, AACC) complementary to the 3′ region of 18S rRNA, but keeping the TPS intact within the αβ-leader. It could be presumed that these blocks of complementary nucleotides within the α sequence might compete with TPS for the 3′-terminal region of 18S

**Discussion**

The mechanisms responsible for the translation-enhancing activity of the non-translated sequences between the cap structure and the AUG codon are not well defined. Moreover, it is possible that different mechanisms are responsible for translation-enhancing activity of different viral 5′ leaders.

(vii) Δα(TPS)β construct

Insertion of two additional entire TP sites (or three overlapping TP sites) into the α sequence resulted in a slight reduction of translation-enhancing activity as compared to Δαβ-leader (Fig. 2).

(viii) Δα(CACC)β construct

As can be seen from Table 1, the αβ-leader contains the first G between the α and β sequences located downstream of the TP site. Replacement of this G residue by A resulted in the formation of a CACC motif downstream from TPS in the Δα(CACC)β derivative of the αβ-leader (Table 1). Thus, the Δα(CACC)β-leader contained, in addition to TPS (CCACC), the CACC tetranucleotide both of them being complementary to the 3′ region of 18S rRNA. Fig. 2 shows that formation of the CACC in tandem to TPS caused rather little (if any) reduction in translation-enhancing activity of Δα(CACC)β-leader as compared with Δαβ-leader.

Fig. 4. The hypothetical secondary structure of part of the authentic αβ-leader of PVX RNA (a) and the modified sequence of the αβ derivative (b). The initiation AUG codon is marked with a box.

UUUAUU. It can be seen from Fig. 2 and Fig. 3 (b) that this modification of the αβ-leader resulted in a dramatic decrease of its translation-enhancing activity.

As mentioned above, computer-based modelling of the secondary structure revealed that the 5′-proximal 17 to 30 nucleotides of αβ-leader are not base-paired and may be accessible to 40S ribosomal subunits (Smirnyagina et al., 1991). To examine this possibility more closely 24 nucleotides were deleted from the α sequence thereby constructing the Δαβ-leader, which lost the region from the sixth to the 30th nucleotide within the α sequence (Fig. 1, Table 1). In other words, this derivative of the Δαβ-leader is devoid of practically the entire region of the Δαβ-leader that was supposed to be unfolded.

If the suggestion concerning the role of the non-structural 5′ region of the Δαβ-leader was correct, it could be expected that deletion of this region would have resulted in translation activity reduction. However, no translation reduction of the Δαβ construct has been detected. On the contrary, Fig. 2 and Fig. 3 shows that the translational enhancement in this case was greater. We have no adequate explanation of this effect, but it should be noted that (i) the computer-based predictions can be regarded only as indirect evidence for the unfolded state of the 5′ end of the αβ sequence and (ii) the 24 nucleotide deletion removed a region of the α sequence containing at least three sketches of nucleotides (AAACC, CACC, AACC) complementary to the 3′ region of 18S rRNA, but keeping the TPS intact within the Δαβ-leader. It could be presumed that these blocks of complementary nucleotides within the α sequence might compete with TPS for the 3′-terminal region of 18S.
rRNA, thereby reducing its overall translation initiation rate. Removing such competition might in some way accelerate the initiation of translation.

In the next series of experiments we examined the role of TPS in αβ-leader-induced translational enhancement. Analysis of translation efficiency of the Δα(Δααfl)β transcript (Fig. 2 and Fig. 3b) showed that removal of the TP site from the α sequence (replacement of CCCACC by UUUAUU) resulted in a very sharp reduction in NPTI gene expression. The decrease in translation in this case was comparable to that observed for the β transcript which was completely devoid of α region sequence including the TP site (Fig. 2 and Fig. 3b). Thus, both of the TPS-lacking derivatives [Δα(Δααfl)β and β] lost their translation-enhancing abilities, i.e. disruption of TPS leads to inactivation of αβ-leader as a translational enhancer.

The idea of an important contribution of TPS to translational enhancement efficiency is in agreement with the fact that all the TPS-carrying derivatives of the αβ-leader [α, Δαβ, ΔαΔβ, Δα(Δααfl)β, Δα(Δααfl)β] were characterized by high translation enhancing activity (Fig. 2). The only exception was the stretch of non-complementary nucleotides containing αβfl inserted into the hairpin structure of the β sequence (Fig. 4a). This insertion resulted in a profound reduction of NPTI gene translation of the αβfl transcript (Fig. 2). This effect may be explained by assuming that ‘masking’ of TPS by the conformationally changed β region occurs. We presume that the formation of a larger interior loop in close proximity to TPS (Fig. 4b) could have a negative effect on translational enhancement since it might hinder the proposed base pairing between TPS and 18S rRNA. These data taken together suggest that the TP site plays the most important role in the translation-enhancing activity of the PVX RNA αβ-leader which is apparently due to the interaction between the TPS and the 3′-proximal conserved hairpin structure of 18S rRNA.

In the Δα(Δααfl)β transcript two additional TP sites were generated within the α sequence (Fig. 1, Table 1). The efficiency of Δα(Δααfl)β transcript was somewhat lower than that of transcripts with Δαβ- and ΔαΔβ-leaders which could mean that amplification of TP sites was accompanied by a competition between individual TP sites for the ribosome upon translation initiation. Formation of the CACC motif downstream of TPS in Δα(Δααfl)β-leader did not cause any increase in the efficiency of the Δα(Δααfl)β transcript. Thus, the efficiency of the transcript with tandemly repeated TP sites was lower than or equal to that of a single TPS properly positioned within the αβ sequence.

As outlined above, the β sequence might merely perform the structural function of bringing together the initiator AUG codon and the TP site (Fig. 4a). The direct consequences of this function will be that (i) the β sequence by itself (after deletion of α region) will not enhance the translation of the reporter gene, (ii) deletion of the β sequence will not impair the αβ-leader as a translational enhancer and (iii) insertion of non-complementary nucleotides in the β sequence results in large interior loop formation (Fig. 4b) which could have a negative effect on translation enhancement preventing base pairing between TPS and 18S rRNA.

In agreement with these suggestions it was found that the translational efficiency of the β sequence-carrying transcript was remarkably low (Fig. 2 and Fig. 3b), i.e. the β sequence is not essential for efficient translation of PVX RNA. However, it is apparently indispensable for the infectivity (i.e. for the replication) of PVX RNA (D. Baulcombe, personal communication). In the case of the ΔαΔβ transcript the level of translational enhancement was even higher than that of the ΔαΔβ transcript (Fig. 2). Thus, the β sequence can be removed without affecting the translation efficiency of the NPTI gene. It should be emphasized that the TP site remained intact although it was spaced apart from the AUG codon by 15 nucleotides in the ΔαΔβ construct (Table 1). Recently, Zelenina et al. (1992) reported that αβ derivatives containing eight to 38 nucleotide vector-derived spacer sequences between the αβ sequence and AUG codon still retained considerable translation-enhancing activity, i.e. the TPS could be separated from AUG considerably, in contrast to the spacing required by a prokaryotic Shine-Dalgarno sequence and initiator codon (Kozak, 1991).

The role of consensus sequences for initiation of translation in eukaryotes has been well documented (Kozak, 1991). Table 1 shows that the authentic αβ-leader as well as the majority of its derivatives constructed in this work preceded the initiator AUG codon arranged in a nucleotide context of CAAAUGG which can be regarded as suboptimal in terms of Kozak (1981). But in two cases (α and ΔαΔβ) a strong initiator context (ACCAUGG) was introduced (Table 1). Comparison of translation data presented in Fig. 2 suggests that the nucleotide context of the AUG codon alone does not play a crucial role in controlling the translation efficiencies of the transcripts. The efficiency of transcripts with Δαβ- and Δα(Δααfl)β-leaders fused to AUG in suboptimal context was not lower than that of the α transcript in which AUG was in an optimal context (Fig. 2). Obviously, this evidence is hardly sufficient to discount the contribution of the AUG context to the translation efficiency of transcripts. Moreover, the fact that translation efficiency of a ΔαΔβ-carrying transcript was somewhat higher than that of Δαβ transcript (Fig. 2) could be partially due to the presence of a strong AUG. However, its contribution to the translational enhancement was only moderate compared to that of TPS. This
conclusion followed from comparison of the efficiencies of $\Delta\alpha/\beta$- and $\Delta\alpha(TPS)/\beta$-carrying transcripts (Fig. 2) which differed only in the presence or absence of TPS, respectively.

We suggest that the CCACC motif (TPS) plays the most important role in the translation-enhancing activity of the $\alpha\beta$-leader of PVX RNA, by promoting the selection of the translation initiation site by 4OS ribosomes based on TPS–rRNA interaction.

References


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