Hypothesis

Possible functional role of viral tRNA-like structures

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Although numerous hypotheses, reviewed here, have been proposed the functional role of viral tRNA-like structures remains unknown. We describe and discuss a new model based on observations made mainly on TYMV RNA. By interacting with the tRNA-like structure, valyl-tRNA synthetase would allow an efficient translation of the viral genome. The generalization for other viral RNAs is proposed.

tRNA-like structure *Aminoacyl-tRNA synthetase* *Translation* *Turnip yellow mosaic virus*

1. INTRODUCTION

The existence of a tRNA-like structure at the 3'-end of turnip yellow mosaic virus (TYMV) RNA was demonstrated in 1970 by Pinck et al. [1] and Yot et al. [2] and has been extended since then to many other viral plant RNAs [3] and to a few animal viral RNAs [4,5]. The tRNA character is mainly shown by the ability of the high-molecular-mass viral RNAs to be aminoacylated specifically by an aminoacyl-tRNA synthetase. For example, TYMV RNAs can be esterfied with valine and bromovirus RNAs with tyrosine in the presence of the corresponding aminoacyl-tRNA synthetase. Interestingly enough, for the two TYMV RNAs ($M_r \simeq 2\,000\,000$ and 260 000) it has been shown (i) that the valylation reaction occurs with similar kinetic constants to those found for the aminoacylation of canonical $tRNA^{Val}$ ($M_r \simeq 25\,000$) [6] and (ii) that the isolated 3'-fragment of the RNAs ($n = 159$) behaves identically to the intact viral RNA in the presence of the synthetase [7]. Besides interacting with aminoacyl-tRNA synthetases, these viral RNAs are also recognized by several other tRNA speific proteins, i.e., nucleotidyl-tRNA transferase [2,8], elongation factor EF-Tu and EF-1 [9,10].

Recently, the secondary structure of several tRNA-like 3'-ends have been determined experimentally [7,11,12]. Although they show poor resemblance at the level of secondary structure with the classical cloverleaf model of tRNAs, it was possible to propose three-dimensional models bearing some features of the L-shaped conformation of tRNA [13,14]. However, despite the important progress made recently in understanding the conformation of tRNA-like molecules, we still do not understand the functional necessity of these structures during the viral cycle.

2. PREVIOUSLY PROPOSED FUNCTIONS FOR VIRAL tRNA-LIKE STRUCTURES

Since their discovery, numerous functional possibilities have been proposed for viral tRNA-like structures (reviews [3,15,16]). Considering the involvement of tRNA-like structures in viral infectivity [3] and their potentiality to be efficiently aminoacylated in vitro [6] and in vivo [17–19], as well as to interact with several factors of the protein synthesis machinery, an obvious role would be the classical amino acid donor function in ribosome-dependent protein synthesis. This is not the case [3,16,20] as evidenced, for example, by negative protein synthesis assays conducted with purified tyrosylated 3'-fragments of brome mosaic virus RNAs [3] or with eggplant mosaic virus RNA [20]. This behaviour is in fact understandable because it seems unlikely that tRNA-like structures exhibiting various sizes [13,14], and consequently

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various overall tertiary conformations compared with classical tRNAs, could easily be accommodated in the ribosomal A and P sites. In agreement with this view is the absence in TYMV RNA of structural features found in the D-and-Tψ loops of elongator tRNAs [7]. Another reason is the weak binding of GTP in the ternary complex formed by elongation factor, GTP and aminoacyl-RNA [10] which would not allow an efficient positioning of charged viral RNA on ribosome.

Interaction of viral RNA with elongation factors has led several authors (e.g., [9,10]) to propose that tRNA-like structures are involved in viral RNA replication. In fact, the QB replicase includes the prokaryotic elongation factors EF-Tu and EF-Ts as subunits [21]. Thus by analogy, it was proposed that tRNA-like structures interacting with plant replicase could be active initiation sites for viral replication, provided these enzymes would contain an elongation factor as subunit, or more generally structural features of elongation factors. However, no experimental data have been reported to support this hypothesis.

The involvement of tRNA-like structures in some non-classical tRNA function cannot be eliminated. For example, there are structural analogies between the TYMV RNA 3'-end and *Staphylococcus epidermidis* $tRNA^{Gly}$ which is involved in ribosome-independent cell wall protein synthesis [7,22]. As with tRNAs priming oncornavirus reverse transcription [23], the viral tRNA-like structures could act as a primer in viral replication.

Although the very attractive hypotheses proposed so far cover numerous possibilities and are not mutually exclusive, it seems to us likely that the easiness with which tRNA-like structures are aminoacylated, or in a more general way their capacity to interact with aminoacyl-tRNA synthetases, has to be related to their putative biological function.

3. POSSIBLE INVOLVEMENT OF THE tRNA-LIKE STRUCTURE IN TYMV GENOMIC RNA TRANSLATION

We propose here an alternative biological function for the tRNA-like structure of TYMV, in agreement with the general frame recently proposed by Ames et al. [24] according to which structural features of tRNA can act as regulatory signals in genetic expression. The model which will be discussed here is based on the following observations:

(i) TYMV genomic RNA ($M_r \simeq 2\,000\,000$) can exist in a circular form as first evidenced by light scattering measurements [25]. This was later substantiated by the observation of base complementarities between the 3'- and 5'-end of the viral RNA [26]. In a systematic search we could compute several possible base-pairing schemes with stretches of 6 or more continuous base-pairs. The existence of a circular form is strengthened by the similar energies in double-stranded regions in the closed RNA and the folded tRNA-like structure (fig.1,2);

(ii) all base-pairing schemes present the same structural features, namely a disruption of the secondary structure of the free 3'-end of the RNA except for the anticodon arm;

(iii) the potential initiation codon located at the 5'-end of the RNA [26,28] is base-paired with a complementary sequence present in the tRNA-like structure (see fig.1A) suggesting that circular RNA is untranslatable.

In our model, summarized in fig.2, the tRNA-like structure present at the 3'-end of the viral RNA would be, via interaction with cellular valyl-tRNA synthetase, an enhancer of viral RNA translation.

Inside the infected cell the two forms of genomic TYMV RNA might exist in an equilibrium governed by intracellular local conditions. Valyl-tRNA synthetase, interacting either with the open or the circular RNA would shift this equilibrium toward the linear form in which the initiation codon AUG is accessible so that translation of the viral RNA is favoured. Interaction of valyl-tRNA synthetase with the structured tRNA-like end would maintain the linear form of TYMV RNA. Interaction of the synthetase with the circular form implies that recognition with the remaining anticodon stem is possible. Our recent studies on the interaction domain of yeast valyl-tRNA synthetase and the tRNA-like fragment support this last proposal.

Experiments based on the accessibility of the RNA to ribonucleases or chemicals show that the most important interaction sites with valyl-tRNA synthetase occur in the anticodon stem of the tRNA-like structure (to be published). These domains (inset of fig.2) are identical with those found in yeast $tRNA^{Val}$ when it interacts with its cognate aminoacyl-tRNA synthetase [29,30]. It is interesting to note that the anticodon stem and loop region of tRNA is involved in interaction with

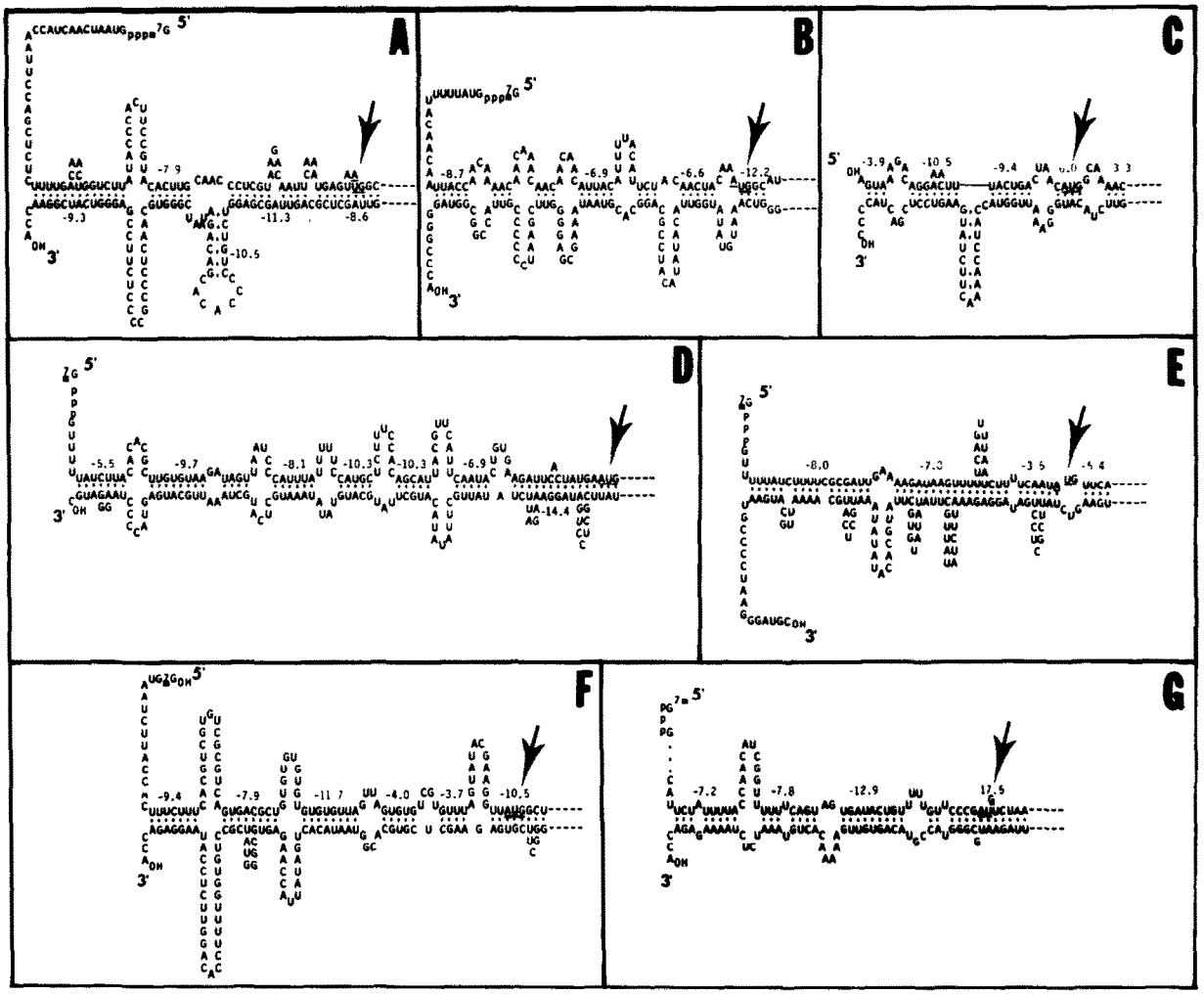

Fig.1. Probable base-pairings between the 5′- and 3′-end of several plant viral RNAs. Sequence data are from [26,36,37,39,48,50,51]. (A) Turnip yellow mosaic virus genomic RNA [26,48]; (B) tobacco mosaic virus genomic RNA [49]; (C) satelite tobacco necrosis virus RNA [39]; (D) alfalfa mosaic virus RNA1 [50]; (E) alfalfa mosaic virus RNA2 [51]; (F) cucumber mosaic virus RNA3 [37]; (G) brome mosaic virus RNA3 [36]. The proposed models were computed from diagonal plots and the free energies calculated according to Salser [47]. The potential initiation codons AUG are underlined and pointed.

aminoacyl-tRNA synthetase in many tRNA/synthetase systems (e.g., [29,32]). An 'unwinding' property of valyl-tRNA synthetase can be understood if one assumes that the first event in that process would involve a recognition with limited structural features of the tRNA-like structure, i.e., the anticodon stem region in the TYMV system. Partial recognition might be possible since it is generally accepted that tRNA-aminoacyl-tRNA synthetase recognition does not require all potential interaction sites on tRNA [33].

TYMV possesses a subgenomic RNA bearing at its 3′-terminus the same tRNA-like structure. Thus, the possibility exists of duplex formation between the 5′-end of genomic RNA and the 3′-end of subgenomic RNA [26]. Here also interaction with valyl-tRNA synthetase could disrupt or prevent such complexes by a similar mechanism.

It could be suggested that competition of valyl-tRNA synthetase with endogenous tRNAs could prevent an efficient interaction with TYMV RNA. This possibility can be rejected since (i) the intracellular concentrations of aminoacyl-tRNA synthetases are high; they were shown to be in the

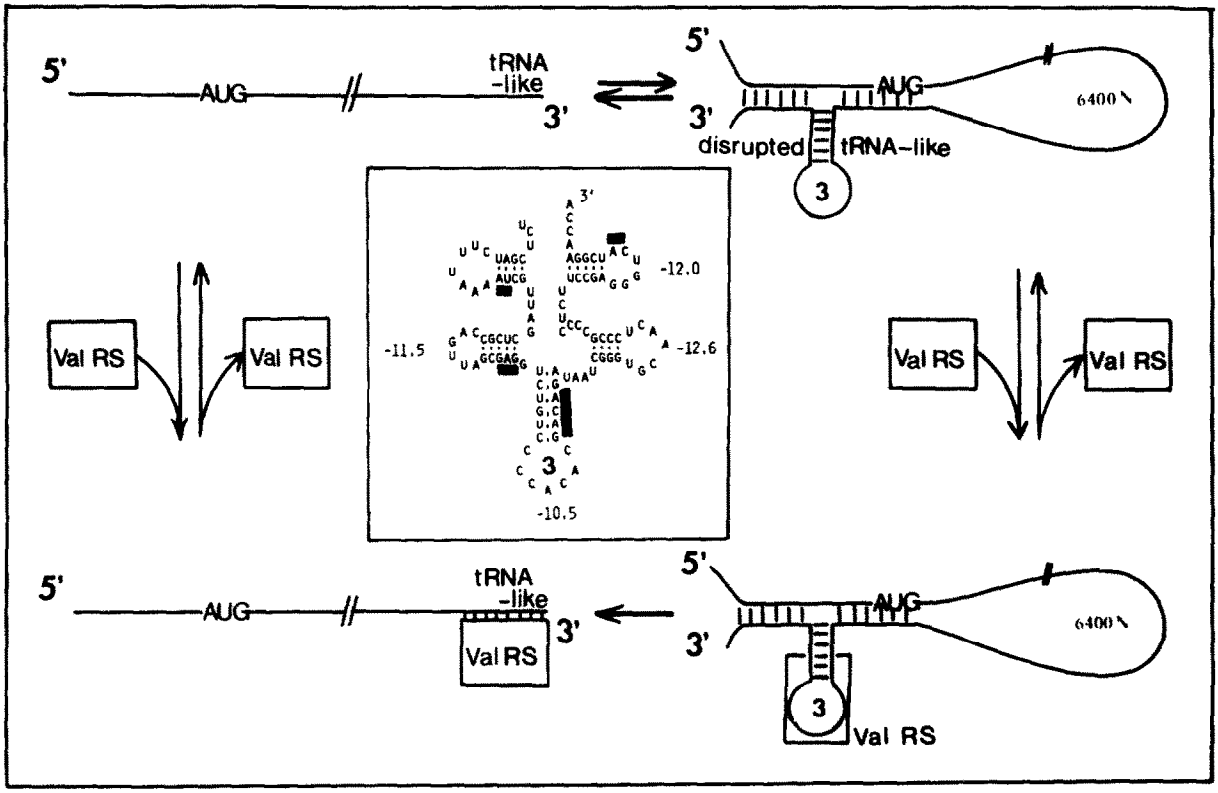

Fig.2. Model of alternative conformations of TYMV RNA involved in genome translation. The amount of open and closed forms, in which the active initiation codon AUG is free or buried, is shifted toward the open conformation by valyl-tRNA synthetase interacting with features of the tRNA-like structure. The sequence and the secondary structure of the 3'-terminal tRNA-like fragment [7] is given in the inset. Energies of the stems calculated according to [47] are given; heavy lines indicate contact points with valyl-tRNA synthetase. Loop 3 corresponds to the anticodon loop also schematized in the circular form of viral RNA.

micromolar range in several organisms (e.g., [34]) and (ii) all cellular aminoacyl-tRNA synthetases are not necessarily complexed with tRNA since charged tRNA molecules will be carried by elongation factors also present at high levels (e.g., [35]).

4. GENERALIZATION: REGULATORY FUNCTIONS FOR tRNA-LIKE STRUCTURES

The model discussed for TYMV can be extended to other RNA viruses. As seen in fig.1B–G, energetically favourable circular models can be proposed for many viral RNAs. Similarly to TYMV RNA, 5'/3' interactions between genomic and subgenomic RNA species might exist. In all cases the initiation codon AUG is buried in a higher order structure which makes such structures ineffective in translation. As will be discussed below, by interacting with tRNA-like structural features, aminoacyl-tRNA synthetase or other proteins, could promote the translation of those RNAs.

Concerning brome mosaic and cucumber mosaic viral RNAs, both aminoacylatable with tyrosine, circular forms with buried initiation codons were proposed [36,37]. The base-pairing between the two ends of the RNAs (fig.1F,G), however, is different from that of TYMV, so that no apparent structural domains of tRNA [11] capable of being recognized by the synthetase remain. Although it cannot be excluded that tyrosyl-tRNA synthetase could unwind such a structure, it seems more plausible that the synthetase could unwind such a structure, it seems more plausible that the synthetase acts as an inhibitor of circularization by interacting with the tRNA-like structure present at the 3'-end of linear RNA. This can also hold true for tobacco mosaic virus RNA (aminoacylatable by histidine) [38] and satellite tobacco necrosis virus

RNA (assumed to be aminoacylated by methionine) [39]. Both RNAs have a 3'-end which can be folded into a secondary structure model similar to that of TYMV [7,40] and strong base complementarities are present between their 5'- and 3'-ends (fig.1B,C).

The question about the biological significance of 5'/3' complementarities in viral RNAs might be raised. Gould and Symons [37] suggested that interaction between the 5'-end of cucumber mosaic virus RNA_3 and the 3'-end of RNA_4 would permit the coencapsidation of these two RNAs. One can also imagine that circularization of the high-molecular-mass RNA would allow a tight packaging inside the viral capsid. Similarly to messenger RNAs the TYMV RNA is highly susceptible to degradation [41], a property clearly evidenced for the tRNA-like 3'-end [7]; higher-order structure of the RNA would stabilize the molecule and enzymatic reparation of nicked RNA could even be facilitated in double-stranded regions.

In the framework of our model, the aminoacylation of the tRNA-like structure is not a necessary prerequisite for the function of these molecules. We note the quasi-absence of 3'-terminal adenosine in TYMV RNA [6]. Thus, this RNA is only valylatable after reconstitution with nucleotidyl-tRNA transferase [2]. Aminoacylation, however, might indirectly enhance the efficiency of the translation process. Indeed elongation factors interacting with charged viral RNA [9,10] could act as relay molecules for the synthetase in maintaining RNA in a translatable form, thus leaving the level of free synthetase high. On the other hand, some viral RNAs have been shown not to be aminoacylatable at all (review, [3,16]). However, the possibility cannot be excluded that these RNAs possess structural features able to exercise translational control, via interaction with aminoacyl-tRNA synthetases which does not involve the catalytic function of the enzyme. This could be the consequence, as already pointed out by Hall [3], of "the evolution of tRNA-like sequences towards structures gradually loosing the aminoacylating potentiality". Alternatively, other tRNA specific proteins recognizing the 3'-end of viral RNAs might play a similar role in translation as the synthetase. We note that coat protein from alfalfa mosaic virus can bind strongly to the 3'-ends of the viral RNAs [42]; interestingly enough these RNAs possess strong 5'/3' base complementarities (fig.1D,E).

In this context it is worth mentioning the recent proposal made by Ames and his colleagues [24] on the regulatory implications of a tRNA-like structure present in the leader mRNA of the histidine attenuator region in *Salmonella typhimurium*. This structure shows high sequence homologies with $tRNA^{His}$, especially for the anticodon and Tψ-stem and loop regions. As discussed by these authors any protein interacting with $tRNA^{His}$ (i.e., tRNA-modifying enzymes, aminoacyl-tRNA synthetases) might be able to interact with features of the tRNA-like structure. This could influence attenuator loop formation or mRNA stability, thus modulating transcription or translation or both [24]. The observation made for the histidine operon is not unique. In the *glyS* gene from *Escherichia coli* a 15-nucleotide long sequence was found similar to that in the anticodon region of $tRNA^{Gly}$ [43]. Furthermore a tRNA-like structure was described inside the *thrS* gene of *E. coli* [44]. For that example the hypothesis was put forward that threonyl-tRNA synthetase interacting with this cloverleaf would block translation of the synthetase mRNA [44]. Following these lines one could rationalize the presence of some unexpected tRNA-like structures assumed to be internally located in viral genomes (i.e., [4,5,45,46]).

Taking all these observations together one might wonder whether structures bearing conformational features of tRNAs do not represent a general class of signals designed by nature to regulate genome expression at the translational level. The fact that tRNA-like structures were first discovered in viral RNAs is understandable since these RNAs are simplified genomes in which such structures could early be experimentaly detected thanks to their aminoacylation ability.

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