Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 12078–12081, October 1996

Commentary

Interplay of tRNA-like structures from plant viral RNAs with partners of the translation and replication machineries

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The surprise was great in the early 1970s when it was shown that valine could be covalently attached to the 3′ terminus of the genomic RNA from turnip yellow mosaic virus (TYMV) by valyl-tRNA synthetase (1) and, soon after, that tyrosyl- and histidyl-tRNA synthetases can aminoacylate the RNAs from brome mosaic virus (BMV) and tobacco mosaic virus (2, 3). The surprise was even greater when it was realized that the sequences and secondary foldings of the anticipated "tRNA" domains deviated markedly from those of canonical tRNAs (reviewed in refs. 4–6; Fig. 1) and that tRNA mimicry was linked with the mandatory presence of pseudoknots, the new type of RNA fold discovered in the tRNA-like domain of TYMV (8) and now found in many other RNAs (9, 10). It was soon conjectured about possible physiological roles of these tRNA-like structures (e.g., refs. 4, 11, and 12). When it was proven that they do not participate in protein synthesis, an easy explanation was to consider tRNA-like structures as insignificant remnants of evolutionary processes, but a more positive viewpoint was to consider them as possible actors during the life cycles of the viruses. On the other hand, the peculiar structural features of tRNA-like structures makes them attractive natural tRNA variants useful for investigating the specificity rules underlying recognition of tRNAs by aminoacyl-tRNA synthetases (6, 13).

Aminoacylation Requested for Replication and Infectivity

Despite many attempts to find a function to viral tRNA-like structures and despite striking results suggesting their involvement in genomic RNA replication (e.g., refs. 14–18), no definitive mechanistic explanation of their necessity in the virus biology and no satisfying answer as to the reasons of their potential to be aminoacylated have emerged yet. The paper by Dreher *et al.* that appears in this issue of the *Proceedings* (19) is a milestone contribution that sheds novel light on the biological role of the tRNA-like domain of TYMV RNA. It brings also novel knowledge to the understanding of the tRNA identity rules.

In short, the reported experiments confirm that replication of TYMV RNA requires aminoacylation and demonstrate that the nature of the amino acid attached to the RNA is not crucial. This conclusion is supported by strong results that come from two complementary experimental approaches of reductionist and holistic type. In the first approach, the authors engineer *in vitro* the aminoacylation properties of the tRNA-like structure of TYMV RNA, and in the second approach, they study *in vivo* the biological implications of this engineering. By doing so, they show that genomes with switched but low methionine acceptance replicate poorly, as shown previously for genomes with poor valine acceptance (18) and, correlatively, that genomes with efficient methionylation activity replicate well, are infectious, and are stable in plants. The immediate consequence of these results is that the interaction of TYMV RNA with valyl-tRNA synthetase, the enzyme that naturally aminoacylates TYMV RNA, is not the indispensable event responsible for genome replication.

Are Elongation Factors the Link Between tRNA-Like Structures and RNA Replication?

If the nature of the amino acid is not crucial, the role of the tag on TYMV RNA must be to make its tRNA-like domain recognizable by a partner of the replication machinery. This recognition of the aminoacylated viral RNA, thus, would be the key event triggering replication, and productive interaction with a synthetase would be its necessary prerequisite.

The finding of Dreher *et al.* (19) with its functional implications rejuvenates the old proposal according to which replication of RNA genomes from plant viruses necessitates interaction of the RNA with a translation elongation factor (4). This view received good support from the observation that aminoacylated viral RNAs can interact *in vitro* with elongation factors EF-Tu and EF-1α (20, 21), and more strikingly, that EF-Tu is a subunit of the replicase of RNA bacteriophage Qβ (22). It was, however, moderated by the impossibility, up to now, of isolating or even detecting a plant elongation factor associated with a viral replicase (23, 24).

The above scenario for TYMV RNA replication should also account for other aminoacylatable plant viral RNAs. However, for replication of BMV RNA, other studies also by Dreher but with Rao and Hall (25) have shown that mutants of this RNA defective in tyrosylation can be replicated when inoculated to host protoplasts. This leads to a puzzling situation, suggesting the existence of idiosyncratic mechanisms for the replication of the plant viral RNAs.

However, for evolutionary reasons and because of the chemical rational underlying biological processes, we sustain the view that replication mechanisms within plants of viral RNA genomes terminating with tRNA-like folds should be of similar type. The contradiction between the TYMV and BMV functional data is resolved if the BMV RNA mutants defective in tyrosylation can be mischarged by another amino acid, in the same way as the TYMV RNA mutants defective in valylation that became methionine acceptors are mischarged (19). Results from our laboratory indicate that such a possibility is plausible, since the tRNA-like structure of BMV RNA has the potential to be histidinylated (unpublished data and ref. 26), like that of TYMV (27) (Fig. 1). This mimicry between amino acid-accepting stems of histidine tRNAs and pseudoknotted tRNA-like structures explains why all structures of this type found up to now in plant viruses are potential substrates of histidyl-tRNA synthetase.

As an alternative or additional possibility for reconciling the apparent contradiction, it can be proposed that the presence of the amino acid tag on the 3′ termini of the viral RNAs is not a necessity. The absolute necessity for replication would be the participation of a translation elongation factor that recognizes a tRNA-like region mimicking the surface of elongator tRNAs contacting such factors. In bacterial systems, such a surface is contained within the helix constituted by the 10 terminal base pairs of elongator tRNAs and their aminoacylated —$NCCA_{OH}$ 3′ single-stranded extension (28). By analogy, this surface would comprise the pseudoknotted region of the tRNA-like

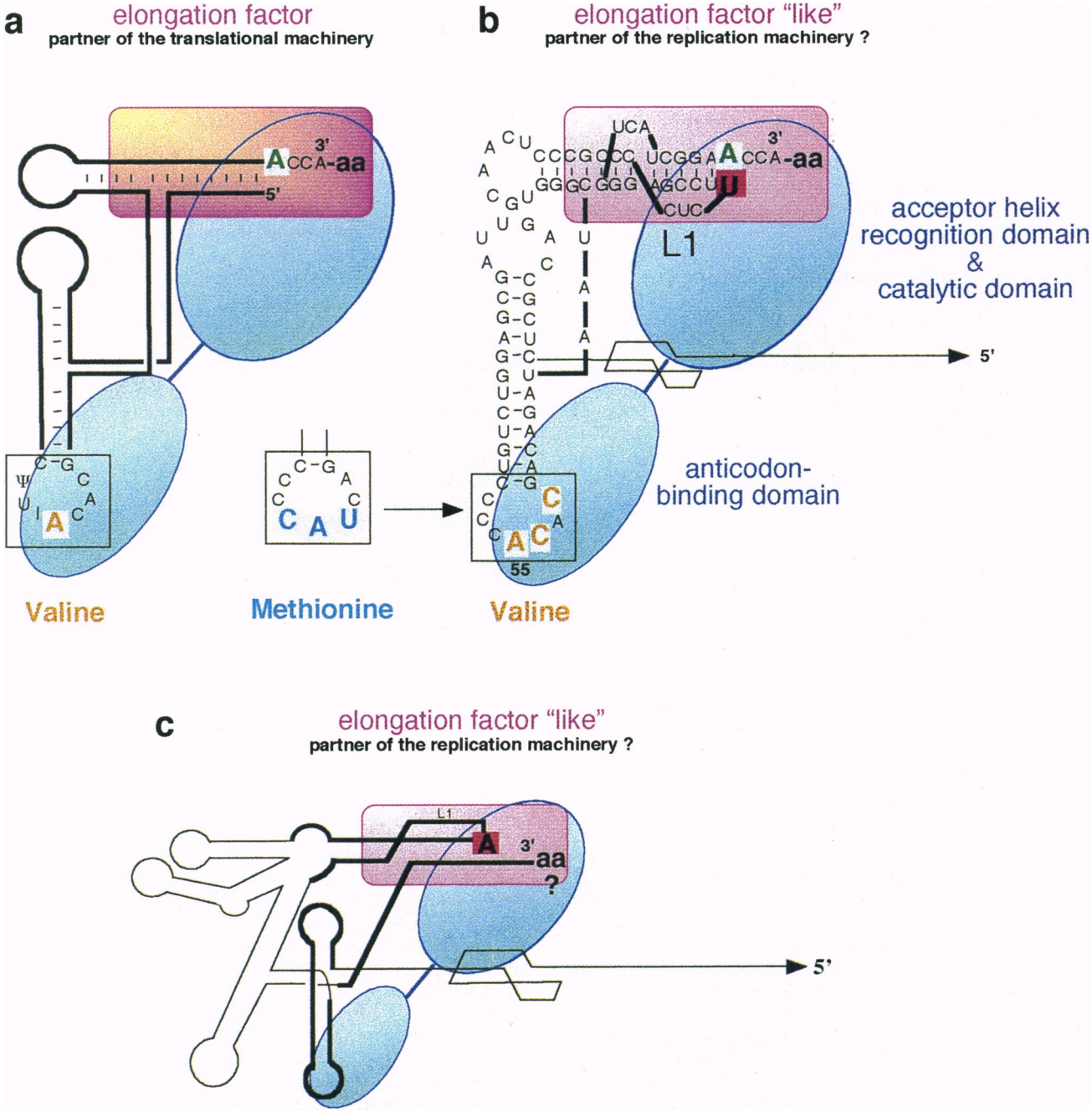

FIG. 1. Alternate architectural characteristics of tRNA and tRNA-like domains from plant viruses and interrelation of their functional properties. The figure compares the structural organization of canonical tRNA (*a*) and of the 3′ termini of TYMV (*b*) and BMV RNAs (*c*) encompassing tRNA-like domains. RNA folds are displayed to emphasize the L-shaped conformations of the tRNA and tRNA-like domains. Sequence data are given for the anticodon loop (that of yeast $tRNA^{Val}$) in *a* and the complete TYMV tRNA-like domain in *b*; the other structural elements of the RNAs are schematized by their ribophosphate backbones, with the tRNA and tRNA-like features emphasized in boldface type. Notice the elaborate folding of the 3′ end of BMV RNA (7), with the tRNA-like features interspersed by sequence elements not participating in the tRNA mimicry (*c*) and the presence of pseudoknots adjacent to the tRNA-like domains in both TYMV and BMV RNAs (*b* and *c*). The major valine identity nucleotide in the anticodon loop of $tRNA^{Val}$ (*a*) and valine determinants in the analogue of this loop in TYMV RNA (*b*) are colored in yellow. (*Inset*) Sequence of the mutated anticodon loop of TYMV RNA, with methionine identity nucleotides colored in blue. The discriminator A residue, next to the accepting CCA-end in *a* and *b*, is a determinant for both valine and methionine identities and is colored in green. The determinant responsible for histidine mischarging in *b* and *c* and belonging to stretch L1 of pseudoknots is in red. The schematic representation of synthetases (in light blue) and elongation factors (in magenta) in interaction with the RNAs illustrates the functional role these macromolecules have in protein synthesis or could have in replication of viral genomes. The coloring of elongation factor in *a*, slightly different from that in *b* and *c*, accounts for its likely alternate functional roles, well-defined in translation and more hypothetical in replication.

domains. For some elongation factors, like those in turnip, the added amino acid tag would reinforce the interaction of the tRNA-like domain with the factor; for others, like those in brome grasses, the affinity between the two partners would be such that aminoacylation can be dispensed. Finally, the possibility cannot be excluded that the actual protein in the replication machinery that will recognize the 3′ termini of viral RNAs is not an authentic translation elongation factor, as for the replicase of phage Qβ RNA, but a protein mimic of such a factor. If so, this could account for the negative results

obtained until now in the quest to find canonical elongation factors associated with replicases.

It is clear that at the present stage of our knowledge, no definitive answer can be given as to the actual nature of the protein factor that recognizes the aminoacylated (or aminoacylatable) viral RNAs in infected plant cells. The implications of Dreher's results on TYMV RNA, however, strongly encourage investigators to pursue their efforts to isolate the plant protein(s), either authentic elongation factors or their mimics, able to interact, even transiently, with aminoacylatable viral RNAs.

Identity Rules in tRNAs and tRNA-Like Molecules

Identity of tRNAs is given by a limited number of positive and negative molecular signals, the identity determinants and antideterminants, that ensure specific aminoacylation by the cognate synthetases and prevent false recognitions by noncognate enzymes (reviewed in refs. 13, 29, and 30). The origin of the chemical rules underlying tRNA aminoacylation identity is obviously ancient and most likely related to the emergence of the genetic code (31). This ancient origin is in line with the increasing number of observations showing the universal character of the identity rules. Moreover, the tRNA identity rules account for the specificity of translational control of *Escherichia coli* threonyl-tRNA synthetase biosynthesis in a mechanism where the synthetase recognizes a tRNA-like domain in the promoter region of its own message (32). The pending question, now, concerns the tRNA-like structures from plant viruses: does nature use the same chemical strategy to ensure their specific aminoacylation?

The work of Dreher *et al.* (19) demonstrating an aminoacylation identity switch of TYMV RNA from valine to methionine is of particular significance with regard to the above question and brings novel understanding on the interactions that synthetases can have with noncanonical RNA substrates. First at all, it adds methionyl-tRNA synthetase to the list of three synthetases (specific for valine, tyrosine, and histidine) recognizing tRNA-like structures from plant viruses. As anticipated from evolutionary considerations (see below), the Dreher's paper is a further support to the universality of tRNA identity rules. Indeed, changing in the TYMV tRNA-like domain, the loop containing the major valine identity elements (33), by an anticodon loop with the sequence of a plant $tRNA^{Met}$ (Fig. 1), triggers the identity switch. This exchange replaces a valine CAC anticodon by a methionine CAU anticodon and so replaces a valine determinant (C55) by a residue (U55) that mimics a major methionine determinant, as found in the anticodon loop of *E. coli* and yeast methionine tRNAs (34, 35). However, as often the case for identity switches in canonical tRNAs (13), the mutated viral RNA has acquired its new methionine identity with a catalytic efficiency (expressed as V_{max}/K_M) $\approx$60-fold lower than that of the normal tRNA substrate of the wheat germ methionyl-tRNA synthetase. This loss of catalytic efficiency could be progressively rescued by shortening the L1 strand of the pseudoknot. For a length of 1 nt, instead of 4 nt in the wild-type molecule, the methionylation of the TYMV RNA became kinetically identical to that of $tRNA^{Met}$. These remarkable results were obtained in buffer conditions designated by the authors as "physiological" (i.e., at rather high ionic strength) in contrast to "nonphysiological" conditions (i.e., at much lower ionic strength), which are less stringent and under which efficient mischarging is already possible for the RNA variant with the 4-nt L1 loop (19).

Altogether, these aminoacylation data call for several comments. First, the viral RNA, with its valine identity elements exchanged by methionine determinants, behaves as a noncognate molecule for the plant methionyl-tRNA synthetase, in a way reminiscent of what noncognate tRNAs in mischarging reactions do. Indeed, it has been known since the 1970s that tRNA mischarging is more sensitive to buffer conditions than aminoacylation by cognate synthetases (e.g., ref. 36). As found with canonical tRNAs, mischarging reactions are facilitated at low ionic strength and under solvent conditions relaxing the conformation of the tRNA. The reason for the more efficient charging of the noncognate tRNAs is thus their better structural adaptation on the synthetases due to their greater structural plasticity. This explanation holds as well for the aminoacylation of tRNA-like structures.

In view of their *in vivo* experiments, Dreher *et al.* decided to overcome the negative effects of the physiological buffer conditions by introducing additional mutations in loop L1 of the tRNA-like structure, guessing that shortening L1 would slightly destabilize its pseudoknotted domain and, in turn, facilitate adaptation of the engineered molecule on methionyl-tRNA synthetase. These expectations were admirably fulfilled.

Seen from another perspective, the possibility of switching the identity of the TYMV tRNA-like structure from valine to methionine illustrates well the logical concept that identity elements in RNAs must be presented properly and optimally to synthetases. In other words, the RNA structure is the architectural scaffolding that allows this optimal presentation. According to this view, different scaffoldings can fulfill the same role, which is indeed the case for the scaffoldings of tRNA-like structures, which are different from that of canonical tRNAs (Fig. 1). For each given aminoacylation system, evolution has tuned the RNA structures in such a way that the presentation of the identity nucleotides to the enzymes is optimal. This tuning can be achieved in different ways, by alterations of the relative position of conserved residues in the cloverleaf structure of tRNAs, by introduction of negative determinants, or by posttranscriptional modifications. Experimental verifications of these strategies used to optimize specificity have been done in a few instances for canonical tRNAs (13). The work of Dreher *et al.* (19) shows that the same chemical logic applies for tRNA-like structures.

The Role of Evolution

After the separate discussions on the role of the viral tRNA-like domains in replication of viral RNA genomes and on the nature of the chemical rules responsible for their recognition by certain aminoacyl-tRNA synthetases, the key enzymes in translation, the obvious question of why there is a relationship between tRNA-like domains and these two seemingly unrelated molecular processes comes to mind. We do not believe that this relationship is fortuitous, but we think that it has a deep biological significance.

As a preamble, one can notice that most contemporary macromolecular systems are too large and elaborate for having emerged as such during evolution. They have thus most probably derived from simplified systems that were built by the assembly of a limited number of structural blocks selected during the ancestral genetic tinkering period. This is most likely the case for many macromolecules involved in replication/transcription or translation events and was in particular discussed for aminoacyl-tRNA synthetases and tRNAs (31). On the other hand, since replication and translation are among the most basic processes in life, it is likely that components of their molecular machineries have coevolved. It is therefore not astonishing to find functional relationships between the two processes with coutilizations of structurally related macromolecular components. This view is supported by strong arguments from structural biology that has revealed in recent time many examples of mimicries between proteins of seemingly unrelated functions and shown that similar structural motifs, often found in primordial proteins, can serve for different functions [e.g., for the biosynthesis of asparagine and the aspartylation of tRNA in a metabolic enzyme and in aspartyl-

tRNA synthetase (37), or to provide mechanical movement and huge conformational changes in the motor domains of kinesin or myosin and in G proteins (38)].

Concerning the tRNA-like structures from the plant viruses, it is now well-established that they are substrates of a number of macromolecules of the translational machinery (4–6), and the paper by Dreher *et al.* (19) confirms unambigously, in the case of TYMV, that they are also partners of the replication machinery, in addition to the fact that these structures are integral part of the viral genomes. We conjecture that elongation factor-like proteins could be a link between the two processes, but that other and nonexclusive links could exist as well, such as telomeric functions of tRNA and tRNA-like structures (39–41).

The likelihood of a coevolution of basic life processes implies the presence in contemporary systems of cryptic remnants of the ancestral evolutionary events. Finding such remnants is not an easy task because the evolutionary history of the various phylae has erased many of the ancestral structural similarities. Search of molecular fossils is even rendered more laborious, because convergent evolution has selected alternate macromolecular structures for a same or similar function. This was the case for the alternate RNA structures interacting with aminoacyl-tRNA synthetases, and it also occurred for synthetases, which are divided in two structurally different classes (42). In these two cases, the most striking structural differences concern the primordial catalytic domain of the proteins (either with the classical parallel β-folded nucleotide binding domain in class I synthetases or the alternate antiparallel β-fold in class II enzymes) and the amino acid acceptor minihelix domain of the RNAs (either pseudoknotted in tRNA-like molecules or double-stranded in tRNAs). In translation, the RNA solution with pseudoknots was eliminated but was maintained in replication. Because of less stringent requirements in replication, the canonical tRNA solution was retained in some systems, as in retroviruses that use host-specific tRNAs as primers for replicating their RNA into cDNA copies (43). Likewise, common "protein" solutions, such as the "G protein fold" found in elongation factors, were retained in both translation and replication machineries.

Summarizing, we argue that ancestral biochemical life processes originated from the shuffling and assembly of a limited number of structural motifs, consequently used for different functional purposes. Memory of this early structural tinkering is conserved in modern systems but its existence is hidden by the "noise" created by the more recent evolutionary events. Because of their ancient origin and their conserved universality, replication and translation systems represent appropriate experimental fields to explore these ideas and to decipher hidden functional interrelations and the early evolutionary events at the origin of life. It is stimulating that these theoretical considerations find a good starting experimental support by the recent finding of Dreher *et al.* reported in this issue of the *Proceedings* (19).

I am grateful to Catherine Florentz for discussions and helpful comments on this manuscript. I also thank present and past members of my laboratory for valuable conceptual and experimental contributions in the tRNA-like field.

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