Acta Biochimica Polonica

Vol. 44 No. 4/1997

827–838

QUARTERLY

This paper is dedicated to Professor Włodzimierz Ostrowski

Review

An enigma: the role of viral RNA aminoacylation

Anne-Lise Haenni and François Chapeville✉

Institut Jacques Monod, 2 Place Jussieu Tour 43, 75251 Paris Cedex 05, France

Key words: plant RNA viruses, aminoacylation, viral RNA replication, pseudoknots

The first demonstration on the aminoacylation capacity of the RNA genome of a plant virus appeared more than 25 years ago. Shortly thereafter, aminoacylation of the RNA genome of a number of other plant viruses was observed. This led to considerable work on the tRNA-like region of these viral RNAs, and to the first demonstration of the presence of pseudoknots in their folding pattern. In spite of the vast amount of efforts put into trying to understand the reason for the aminoacylation capacity of certain viral RNA genomes, as yet no clear general conclusion emerges. It rather looks as though the reason for aminoacylation may be different for different viruses, and that aminoacylation may operate at different levels in the virus life cycle. Given that certain RNA viruses possess structures which resemble that of tRNAs at their 5′- or 3′-termini, it is most likely that convergent evolution may have dominated the appearance of such structures in the virus world.

Viruses, because of the small size of their genome as compared to the genome of cells, have readily lent themselves to exquisite genetic and molecular dissection. As a consequence, they have been at the origin of the demonstration of a large number of previously unexpected properties. Whereas most of these characteristics were subsequently also encountered in cell systems, a few others still remain the appanage of the virus world. One of these striking and as yet unexplained traits is the capacity of the 3′ end of the RNA genome of certain viruses of the plant kingdom to be aminoacylated.

The first evidence that such a reaction could occur was provided in 1970 for turnip yellow mosaic tymovirus (TYMV) whose RNA genome can be valylated *in vitro* at its 3′ end by valyl-tRNA synthetase [1]. It was followed by extensive studies on i) the aminoacylation capacity and interaction with other enzymes observed with the RNA genome of several plant viruses, ii) the region and folding of the viral genome involved in

✉Corresponding author: François Chapeville: Institut Jacques Monod, 2 Place Jussieu-Tour 43, 75251 Paris Cedex 05, France, tel: 33-1-44.27.50.80; fax: 33-1-44.27.35.80; E-mail: haenni@ijm.jussieu.fr

Abbreviations: BBMV, broad bean mottle bromovirus; BMV, brome mosaic bromovirus; BSMV, barley stripe mosaic hordeivirus; CCA-enzyme, tRNA-nucleotidyl transferase; CcTMV, cowpea strain of TMV; CMV, cucumber mosaic cucumovirus; ELV, erysimum latent tymovirus; HeSV, *Helicoverpa armigera* stunt tetravirus; ss, single-stranded; TAV, tomato aspermy cucumovirus; TMV, tobacco mosaic tobamovirus; TRV, tobacco rattle tobravirus; TYMV, turnip yellow mosaic tymovirus.

such interactions, and iii) the possible role of these aminoacylatable regions in virus replication (reviewed in [2–5]). The aminoacylatable region of these viral RNAs has come to be designated the tRNA-like structure.

The aim of the present review is to summarize a few salient features of the aminoacylation capacity of viral genomes, and try to place these features in the light of molecular evolution.

Table 1 presents a list of virus groups and type members whose RNA genome can be aminoacylated *in vitro*, and the amino acid that can be bound to the 3′ end of the viral RNA by the corresponding aminoacyl-tRNA synthetase. Within each group, the RNA of most members for which such experiments were performed can be aminoacylated with an amino acid that appears to be specific of the group. Exceptions to these observations are discussed in the text.

Table 1. *In vitro* aminoacylation capacity of the genome of plant RNA viruses

Virus group	Type member	Amino acid bound
Tymovirus	turnip yellow mosaic virus (TYMV)	valine
Tobamovirus	tobacco mosaic virus (TMV)	histidine
Bromovirus	brome mosaic virus (BMV)	tyrosine
Cucumovirus	cucumber mosaic virus (CCMV)	tyrosine
Hordeivirus	barley stripe mosaic virus (BSMV)	tyrosine

ENZYMATIC AND STRUCTURAL APPROACHES TO STUDY tRNA-LIKE STRUCTURES

Tymoviruses

The viruses of this group contain a (+) monopartite single-stranded (ss) RNA genome. The RNA bears a cap structure at its 5′ end, and a tRNA-like structure at its 3′ end that covalently binds valine in the presence of valyl-tRNA synthetase. The 3′-terminal sequence of the viral RNA terminates with two cytidylic acid residues. It was demonstrated that the tRNA-nucleotidyl transferase (CCA-enzyme) must first bind an adenylic acid residue at the terminus of the RNA before valylation can proceed [6]. The kinetic parameters leading to valylation of TYMV RNA are very similar to those leading to valylation of $tRNA^{Val}$ [7].

In addition to the CCA-enzyme and the valyl-tRNA synthetase, a number of other enzymes originally believed to be specific of tRNAs, also interact with the tRNA-like structure of TYMV RNA. The peptidyl-tRNA hydrolase cleaves the ester linkage between the viral RNA and acetylvaline produced by chemical acetylation of Val-RNA of TYMV [6]. RNase P (the enzyme responsible for the maturation of precursor tRNAs) as well as its catalytic RNA component, cleave the viral RNA immediately upstream or immediately downstream of U19 (Fig. 1A) in a position stereochemically equivalent to the position at which it cleaves precursor tRNAs [8, 9]. The elongation factor of *Escherichia coli*, EF-T or of wheat germ, EF1α, forms a ternary complex with the viral Val-RNA and GTP in the same conditions as those leading to the formation of Val-tRNA·GTP·EF-Tu [10–12].

When TYMV RNA was injected into *Xenopus laevis* oocytes, valylation was as efficient as valylation of *E. coli* $tRNA^{Val}$ similarly injected into oocytes [13]. Valylation also occurs in infected Chinese cabbage leaves [14]. Since within the virus particle, the RNA is not valylated and is even devoid of a 3′ terminal adenylic acid residue, these experiments suggest that esterification *in vivo* is transient, or that it concerns only a fraction of the viral RNA population that would be subverted for as yet unidentified functions.

The minimum size of the viral RNA required for interaction with the valyl-tRNA synthetase indicated (Table 2) that 3′-coter-

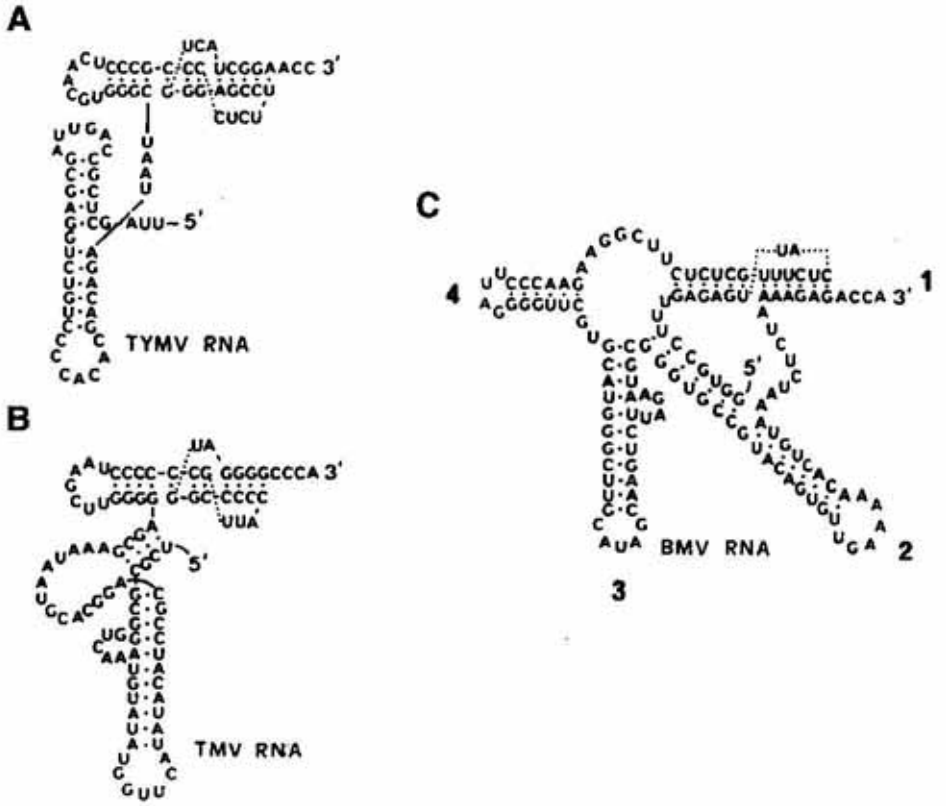

Figure 1. Schematic representation of folding of the tRNA-like regions of plant viral RNAs in their 'L'-shaped configuration.

A: TYMV RNA; B: TMV RNA; C: BMV RNA. In BMV RNA, the numbers in bold refer to various parts of the tRNA-like region.

minal fragments as short as 83 to 86 nucleotides are sufficient [15], although longer fragments are required for optimal valylation [16]. This led to careful chemical and enzymatic probing of this region of the RNA, and to the proposal of a possible folding pattern of the tRNA-like structure (Fig. 1A) [17].

The main characteristics of this folding pattern is that the aminoacyl acceptor domain is formed exclusively by the 3′ half of the tRNA-like structure, and is independent of the remaining part of this structure. This situation is totally different from that found in canonical tRNAs, in which both the 3′ and the 5′ part of the molecule participate in formation of the aminoacyl acceptor arm. Moreover, in the viral RNA, the aminoacyl acceptor arm forms a pseudoknot. By definition, pseudoknots arise from base-pairing between nucleotides within a loop and complementary nucleotides located outside of this loop. The pseudoknot results in coaxial stacking of two stem regions connected by two loops. The minimum length of the viral RNA required for interaction with the CCA-enzyme is about 50 nucleotides (Table 2), which corresponds to the aminoacyl acceptor arm [15]. Since the minimum length of the viral RNA required for recognition by EF-Tu is 47 nucleotides [11], it seems conceivable that the CCA-enzyme and EF-Tu share structural similarities.

With one exception, the RNAs of all the tymoviruses for which such experiments were performed, are capable of accepting valine. However, genome-sized RNA of erysimum latent tymovirus (ELV) is not valylated *in vitro* [18]. This might be correlated to the fact that the 3′ region of ELV RNA seems to possess only part of the tRNA-like structure found in the other tymoviral RNAs, and lacks the valine anticodon loop [19].

Tobamoviruses

The viruses belonging to this genus contain a (+) monopartite ssRNA genome that is capped at its 5′ end. The genome of tobacco

mosaic tobamovirus (TMV) can be histidinylated *in vitro* at its 3′-CCA end [20]. The minimum size of the viral RNA that can be aminoacylated is about 95 nucleotides (Table 2) [21]. This agrees with the proposed folding of the tRNA-like structure based on chemical and enzymatic probing studies and on phylogenetic evidence (Fig. 1B) [22]. In TMV RNA also, the aminoacyl acceptor arm is independent of the remainder of the tRNA-like structure, and results from the formation of a pseudoknot very similar to that existing in TYMV RNA.

In TMV RNA, 55 nucleotides are sufficient for interaction with the CCA-enzyme (Table 2), strongly suggesting once again that the aminoacyl acceptor arm is sufficient for this interaction [21]. *E. coli* EF-Tu also interacts with His-RNA of TMV [10]. On the other hand, RNAse P is incapable of cleaving TMV RNA [23]. This may be due to specific requirements of RNAse P at the level of the aminoacyl acceptor arm (reviewed in [5]).

Table 2. Minimum length requirements of viral RNAs (expressed in nucleotides)

Virus	CCA-enzyme	Aminoacyl-tRNA synthetase	EF-Tu
TYMV	50	86	55
TMV	55	95	nd*
BMV	132	134	nd
BBMV	nd	118	nd

*nd: not determined.

The genome of most tobamoviruses tested accepts histidine. However, the efficiency of aminoacylation is highly variable from one tobamovirus or from one tobamovirus strain to another [24]. This variation may be caused by structural variations in the anticodon loop (reviewed in [4]). One striking exception concerning the histidinylation of tobamovirus RNAs lies in the observation that the cowpea strain of TMV (CcTMV) also known as sunhemp mosaic tobamovirus, accepts valine but not histidine [25]. A close examination of the tRNA-like regions of TYMV, CcTMV and TMV reveals that the overall tRNA-like region of TYMV and CcTMV shows considerable similarity, suggesting that some of these common regions may be involved in recognition by the valyl-tRNA synthetase. On the other hand, structural similarities between the tRNA-like regions of CcTMV and TMV which accept different amino acids although the viruses belong to the same genus, are essentially confined to the aminoacyl acceptor arm [26]. This might imply that other enzymes, such as the replication complex, require some of these common nucleotides. It may also be worth recalling that a large unpaired loop of 25 nucleotides present between the aminoacyl acceptor arm and the anticodon arm in TMV RNA is absent from CcTMV as also from TYMV RNA. It has been proposed that the CcTMV strain might have emerged by recombination between the coding body of a tobamovirus (such as the *Vulgare* strain) and the 3′-non-coding tRNA-like region of a tymovirus [27].

Bromoviruses, cucumoviruses and hordeiviruses

The viruses belonging to these groups contain a (+) tripartite ssRNA genome. They also contain a subgenomic RNA (RNA 4) that derives from RNA 3 and codes for the capsid. The 5′ ends are capped. The 3′ ends of the four RNAs present extensive intraviral homology as well as interviral sequence similarity [28, 29]. The RNAs of brome mosaic bromovirus (BMV) and cucumber mosaic cucumovirus (CMV), the type-members of the bromovirus and cucumovirus groups, respectively, as well as of the RNAs of most of the other viruses tested within these groups, can be tyrosylated *in vitro* (Table 1) [30, 31]. The minimum length of BMV RNA and of broad bean mottle bromovirus (BBMV) RNA required for tyrosylation is 134 and 118 nucleotides from the 3′ end, respectively (Table 2). Adenylation of BMV RNA by the CCA-enzyme requires 132 nucleotides [32]. These results accompanied by careful enzymatic and chemical probing [33] led to the model presented in Fig. 1C for the tRNA-like structure of BMV. This structure which also contains a pseudoknot is, however, totally different and far more complex that the tRNA-like structure contained in the tymovirus and the

tobamovirus RNAs. The difference in length of the RNA required for tyrosylation between BMV and BBMV is due to the absence in the tRNA-like region of the BBMV RNAs of hairpin 4, present in the tRNA-like regions of all the other bromovirus and cucumovirus RNAs examined. Indeed, hairpin 4 can be deleted from BMV RNA without deleterious effects on the tyrosylation activity [34]. On the other hand, the RNAs of tomato aspermy cucumovirus (TAV) whose stem in hairpin 4 is particularly long (12 instead of the usual 6 base pairs), cannot be aminoacylated although it can be adenylated [35]. In the model presented for BMV, it is clear that the 5′ region of the tRNA-like structure participates in formation of the aminoacyl acceptor arm (Fig. 1C). This probably explains why the minimum length of the tRNA-like structure required for tyrosylation and adenylation is about the same. It has been shown that the RNAs of BMV and barley stripe mosaic hordeivirus (BSMV) are tyrosylated in barley protoplasts [36]. Furthermore, Tyr-RNA of BMV interacts with the wheat germ elongation factor EF1 and GTP [37]. On the other hand, whereas RNase P of *Escherichia coli* cleaves the tRNA-like domain of CMV RNA, it is inactive towards BMV RNA [23]. Again, this must probably be ascribed to specific requirements of RNase P for cleavage (reviewed in [5]).

Tobraviruses

Tobraviruses present an interesting case. Viruses of this group contain a (+) bipartite ssRNA genome. The RNAs are capped at their 5′ end, and are identical for over 400 nucleotides at their 3′ end. The tobacco rattle tobravirus (TRV) RNAs cannot be aminoacylated. Chemical and enzymatic probing experiments with TRV RNA [38] as well as sequence comparisons of tobraviral RNAs have led to the conclusion that the RNAs contain an aminoacyl acceptor domain similar to that present in tymovirus and tobamovirus RNAs. However, the tRNA-like structure of tobravirus RNAs appear not to possess an anticodon domain. This probably accounts for the fact that TRV RNA cannot be aminoacylated, but can be adenylated. This is similar to the situation already mentioned above for ELV.

ELEMENTS OF tRNA-LIKE STRUCTURES REQUIRED FOR RECOGNITION BY AMINOACYL-tRNA SYNTHETASES

By performing nuclease digestion and chemical modification studies on tRNA-like structures complexed to their cognate aminoacyl-tRNA synthetase, essential recognition elements were identified.

Foremost is the observation that mutations in the pseudoknot and the two stem regions in the amino acid acceptor arm greatly decrease the tyrosylation capacity of BMV RNA [39, 40]. The observation that hairpin 4 can be completely ablated without deleterious effects on tyrosylation [34] is in agreement with the fact that the RNAs of CCMV and BSMV, although deprived of this hairpin, are readily tyrosylated *in vitro*. Nucleotides upstream of the minimum core are also protected in the complex, suggesting that they are most likely involved in the formation of an anticodon-like arm [41].

Similar studies performed on the TYMV tRNA-like structure and Val-tRNA synthetase demonstrated the importance of the central position of the anticodon for efficient valylation [42, 43]. Interestingly, mutants that cannot be valylated still bind to valyl-tRNA synthetase [42], suggesting a kinetic mechanism rather than one related to affinity-based discrimination.

In certain experimental conditions, TYMV RNA can be quite efficiently mischarged with histidine by the yeast histidyl-tRNA synthetase [44], as can also a viral transcript containing a mutation in the central position of the anticodon. Consequently, the identity nucleotides for valine and histidine do not overlap. The main histidine determinants lie within the amino acid acceptor arm, since a minihelix of 42 nucleotides corresonding to this arm can be histidinylated [44].

POSSIBLE FUNCTIONS OF tRNA-LIKE STRUCTURES

It was early on believed that aminoacylation may participate in viral RNA replication, by homology with the RNA bacteriophage Qβ for which bacterial elongation factors are part of the replication complex, and because elongation factors interact with the aminoacylated plant virus tRNA-like regions. However, elongation factors appear not to be part of the TYMV [12] or the BMV [45] replication complex. To examine whether aminoacylation is required for replication of viruses, mutations were introduced in the tRNA-like regions with the aim of dissociating aminoacylation from replication.

In the case of BMV, certain mutations that greatly decreased tyrosylation had little effect on replication of the RNA *in vitro*, suggesting that different parts of the same tRNA-like region are recognized by the tyrosyl-tRNA synthetase and the replication complex [39]. The importance of tyrosylation in BMV RNA replication *in vivo* was investigated by co-inoculation into barley protoplasts of wild-type RNAs 1 and 2, and a mutant RNA 3 defective in tyrosylation. The results once again suggested that tyrosylation is dispensable for RNA replication [46]. However, this type of experiment does not make it possible to examine tyrosylation *in vivo* so as to compare it to *in vivo* replication. A fragment of 134 nucleotides from the 3′ end of the viral RNA serves as template for the replicase [47]. On the other hand, disruption of the pseudoknot as well as specific mutations within the tRNA-like region of BMV RNA strongly hinder replication of the viral RNA [48].

The situation concerning the requirement of aminoacylation for TYMV RNA replication is still somewhat confusing. As opposed to what had been postulated previously, namely that valylation [49] or histidinylation [50] *in vitro* could not be dissociated from RNA replication *in vivo*, it has recently been shown that certain mutations introduced into a chimeric construct can lead to highly infectious viruses. Such mutants cannot be valylated *in vitro*, but remain substrate of the CCA-enzyme [51]. These results tend to suggest that, as in the case of BMV RNA, aminoacylation is not required for RNA replication. It has been shown that fragments of 100 and 38 nucleotides from the 3′ end of TYMV RNA compete with the viral RNA for replication *in vitro*, and can themselves be replicated [52, 53]. Likewise, transgenic rapeseed plants expressing high levels of a chimeric gene containing the 3′-terminal 100 nucleotides from TYMV RNA are partially protected against infection by TYMV RNA or virions [54].

Transgenic tobacco plants that express the antisense RNA corresponding to the coat protein and the 3′ non-coding region of TMV are partially protected against infection by TMV. Since transgenic plants with antisense RNA devoid of the 3′ non coding region are not protected, the tRNA-like region is most likely responsible for the protection effect observed [55].

In addition to a role in viral RNA replication, other functions have been proposed for the viral tRNA-like structures. It has been suggested [56] that these structures could be molecular fossils of an RNA world. They would correspond to primitive telomeres ensuring that the 3′ end of the viral genomes are not lost during several cycles of RNA replication. Indeed, BMV mutants in which base changes were introduced in the 3′-CCA terminus and which presented neither tyrosylation nor replication activity *in vitro*, were capable of replication *in vivo*. This most likely results from the action of the CCA-enzyme which would act as a telomerase [57]. In the case of TYMV, the valyl-tRNA synthetase could act as a telomerase, since valylation protects the viral RNA against nucleases [58].

Another possible function of tRNA-like structures could reside in protection of the RNA against degradation. Indeed, if the TMV tRNA-like structure is tagged to the 3′ end of unrelated mRNAs, the resulting chimeric constructs present greater stability in host cells [59, 60].

Finally, since in certain cases the 5′ and the 3′ regions of viral RNAs can potentially base-pair, it is possible that tRNA-like structures could regulate translation of viral proteins.

In such a scenario, accessibility of the 3′ end of the viral RNA might be regulated by the aminoacyl-tRNA synthetase [61, 62].

VIRAL RNA AMINOACYLATION REMAINS AN ENIGMA

Within the last 25 years, we have come a long way in analyzing the viral tRNA-like structures and in dissecting them so as to gain insight into the function of their various elements. From several points of view, the discovery of tRNA-like structures has been paramount in unravelling certain features of RNAs. The most striking resides in the first biochemical demonstration of the existence of pseudoknots. These secondary/tertiary folding patterns are now known to occur in most RNAs, be they viral or cellular RNAs (reviewed in [4]). Another important outcome of studies on viral tRNA-like structures was the demonstration for the first time that a natural RNA requires no modified nucleotides for efficient aminoacylation or interaction with several tRNA-specific enzymes. These observations have since been substantiated by using *in vitro* transcripts of tRNAs that are as efficient in aminoacylation as are the corresponding normal tRNAs (reviewed in [63]). Nevertheless, it should be emphasized that lack of modified nucleotides leads to structural relaxation of tRNAs [64], and this may to a certain extent explain the histidinylation capacity of TYMV RNA.

On the other hand, the tRNA-like structures remain an enigma. RNA viruses of only a few families possess tRNA-like structures at the 3′ end of their genome, and aminoacylation appears to be confined to valine, histidine and tyrosine. What has dictated this choice? Within a virus family such as the cucumoviruses, the RNAs of most members are tyrosylated. The same applies to tymoviruses whose RNAs are generally valylated. Yet, notable exceptions exist, such as TAV whose RNA can be adenylated but not tyrosylated, and ELV whose RNA cannot be valylated. If aminoacylation were indeed primordial for the life cycle of the virus, then why would such exceptions be tolerated? Since exceptions do exist, why is it that certain determinants of aminoacylation have been maintained in other viruses? Should one be led to suppose that interaction with the CCA-enzyme is the pivotal feature to be preserved, and that for unknown reasons the aminoacylation capacity was not lost during the course of evolution?

It is also somewhat surprising that the aminoacylation capacity of viral RNA genomes has been confined to plant viruses. Indeed, attempts to aminoacylate the genome of RNA bacteriophages have been unsuccessful, in spite of the fact that these RNAs terminate with the sequence –CCA, and that protein elongation factors are involved in phage replication. There is as yet no definitive demonstration that the RNA genome of animal viruses can be aminoacylated.

It is worth glancing at other viruses whose RNA genomes present features reminiscent of tRNAs. One such example is the presence of a $tRNA^{Asp}$ sequence at the 5′ termini of defective interfering RNAs of Sindbis virus [65]. Another example is to be found in the two genomic RNAs of the insect *Helicoverpa armigera* stunt tetravirus (HeSV). The 3′ termini of both ssRNAs of (+) polarity of HeSV have a well-conserved structure related to $tRNA^{Val}$ [66]. As opposed to the plant viral tRNA-like structures, in HeSV, the 3′ regions are formed without a pseudoknot. They possess a valine anticodon, and hence resemble $tRNA^{Val}$ more than do the plant viral tRNA-like structures. Recent experimental evidence suggests that the tRNA-like region of HaSV RNAs, can be valylated *in vitro* (discussed in [67]). Yet, another somewhat more distantly related observation, is the presence within the 5′-terminal regions of retroviral RNAs of a sequence complementary to part of the host tRNA primer.

Because of these diverse forms of tRNA mimicry, one can expect to find, within tRNA-like regions, transcription signals known to be present in tRNAs. Indeed, the 5′ termini of the genomic RNAs of bromoviruses contain motifs that resemble the internal control regions of tRNAs [68, 69] and mimic promoters of RNA polymerase III. It was demonstrated that these motifs are indeed required for replication of BMV RNA [70] as

they are for tRNAs. Such motifs may have been retained in tRNAs and in certain viral RNAs, and could derive from an ancestral genomic RNA.

Finally, in view of the different folding patterns adopted by tRNAs and tRNA-like structures in viral RNAs, it seems likely that convergent evolution might have been the driving force directing molecules with such very different secondary structures to adopt similar conformations required for their interaction with common enzymes.

Professor W. Ostrowski's help to establish and maintain links between the authors and Academic Institutions of Poland is gratefully acknowledged.

The Institut Jacques Monod is an "Institut Mixte, CNRS-Université Paris 7".

REFERENCES

1. Pinck, M., Yot, P., Chapeville, F. & Duranton, H. (1970) Enzymatic binding of valine to the 3′ end of TYMV-RNA. *Nature* **226**, 954–956.
2. Hall, T.C. (1979) Transfer RNA-like structures in viral genomes. *Int. Rev. Cytol.* **60**, 1–26.
3. Haenni, A.-L., Joshi, S. & Chapeville, F. (1982) tRNA-like structures in the genomes of RNA viruses. *Prog. Nucleic Acid Res. Mol. Biol.* **27**, 85–104.
4. Mans, R.M.W., Pleij, C.W.A. & Bosch, L. (1991) tRNA-like structures. Structure, function and evolutionary significance. *Eur. J. Biochem.* **201**, 303–324.
5. Florentz, C. & Giegé, R. (1995) tRNA-like structures in plant viral RNAs; in *tRNA: Structure, Biosynthesis and Function* (Söll, D., RajBhandary, U., eds.) pp. 141–163, American Society for Microbiology, Washington.
6. Yot, P., Pinck, M., Haenni, A.-L., Duranton, H.M. & Chapeville, F. (1970) Valine-specific tRNA-like structure in turnip yellow mosaic virus RNA. *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1345–1352.
7. Giegé, R., Briand, J.-P., Mengual, R., Ebel, J.-P. & Hirth, L. (1978) Valylation of the two RNA components of turnip-yellow mosaic virus and specificity of the aminoacylation reaction. *Eur. J. Biochem.* **84**, 251–256.
8. Guerrier-Takada, C., Van Belkum, A., Pleij, C.W.A. & Altman, S. (1988) Novel reactions of RNase P with a tRNA-like structure in turnip yellow mosaic virus RNA. *Cell* **53**, 267–272.
9. Green, C.J., Vold, B.S., Morch, M.D., Joshi, R.L. & Haenni, A.-L. (1988) Ionic conditions for the cleavage of the tRNA-like structure of turnip yellow mosaic virus by the catalytic RNA of RNase P. *J. Biol. Chem.* **263**, 11617–11620.
10. Litvak, S., Tarrago, A., Tarrago-Litvak, L. & Allende, J.E. (1973) Elongation factor-viral genome interaction dependent on the aminoacylation of TYMV and TMV RNAs. *Nature New Biol.* **241**, 88–90.
11. Joshi, R.L., Faulhammer, H., Chapeville, F., Sprinzl, M. & Haenni, A.-L. (1984) Aminoacyl RNA domain of turnip yellow mosaic virus Val-RNA interacting with elongation factor Tu. *Nucleic Acids Res.* **12**, 7467–7478.
12. Joshi, R.L., Ravel, J.M. & Haenni, A.-L. (1986) Interaction of turnip yellow mosaic virus Val-RNA with eukaryotic elongation factor EF-1α. Search for a function. *EMBO J.* **5**, 1143–1148.
13. Joshi, S., Haenni, A.L., Hubert, E., Huez, G. & Marbaix, G. (1978) *In vivo* aminoacylation and 'processing' of turnip yellow mosaic virus RNA in *Xenopus laevis* oocytes. *Nature* **275**, 339–341.
14. Joshi, S., Chapeville, F. & Haenni, A.L. (1982) Turnip yellow mosaic virus RNA is aminoacylated *in vivo* in Chinese cabbage leaves. *EMBO J.* **1**, 935–938.
15. Joshi, S., Chapeville, F. & Haenni, A.-L. (1982) Length requirements for tRNA-specific enzymes and cleavage specificity at the 3′-end of turnip yellow mosaic virus RNA. *Nucleic Acids Res.* **10**, 1947–1962.
16. Dreher, T.W., Florentz, C. & Giegé, R. (1988) Valylation of tRNA-like transcripts from cloned cDNA of turnip yellow mosaic virus

RNA demonstrate that the L-shaped region at the 3′ end of the viral RNA is not sufficient for optimal aminoacylation. *Biochimie* **70**, 1719–1727.

17. Rietveld, K., Van Poelgeest, R., Pleij, C.W.A., Van Boom, J.H. & Bosch, L. (1982) The tRNA-like structure at the 3′ terminus of turnip yellow mosaic virus RNA. Differences and similarities with canonical tRNA. *Nucleic Acids Res.* **10**, 1929–1946.

18. Van Belkum, A., Bingkun, J., Rietveld, K., Pleij, C.W.A. & Bosch, L. (1987) Structural similarities among valine-accepting tRNA-like structures in tymoviral RNAs and elongator tRNAs. *Biochemistry* **26**, 1144–1151.

19. Srifah, P., Keese, P., Weiller, G. & Gibbs, A. (1992) Comparisons of the genomic sequences of erysimum latent virus and other tymoviruses: A search for the molecular basis of their host specificities. *J. Gen. Virol.* **73**, 1437–1447.

20. Öberg, B. & Philipson, L. (1972) Binding of histidine to tobacco mosaic virus RNA. *Biochem. Biophys. Res. Commun.* **48**, 927–932.

21. Joshi, R.L., Chapeville, F. & Haenni, A.-L. (1985) Conformational requirements of tobacco mosaic virus RNA for aminoacylation and adenylation. *Nucleic Acids Res.* **13**, 347–354.

22. Rietveld, K., Linschooten, K., Pleij, C.W.A. & Bosch, L. (1984) The three-dimensional folding of the tRNA-like structure of tobacco mosaic virus RNA. A new building principle applied twice. *EMBO J.* **3**, 2613–2619.

23. Mans, R.M.W., Guerrier-Takada, C., Altman, S. & Pleij, C.W.A. (1990) Interaction of RNase P from *Escherichia coli* with pseudoknotted structures in viral RNAs. *Nucleic Acids Res.* **18**, 3479–3487.

24. Carriquiry, E. & Litvak, S. (1974) Further studies on the enzymatic aminoacylation of TMV-RNA by histidine. *FEBS Lett.* **38**, 287–291.

25. Beachy, R.N., Zaitlin, M., Bruening, G. & Israel, H.W. (1976) A genetic map for the cowpea strain of TMV. *Virology* **73**, 498–507.

26. Joshi, R.L., Joshi, S., Chapeville, F. & Haenni, A.L. (1983)Primary and secondary structures of the tRNA-like regions of the genomes of plant RNA viruses; in *Endocytobiology II* (Schenk, H.E.A., Schwemmler, W., eds.) pp. 57–68, Walter de Gruyter & Co., Berlin, New York.

27. Boyer, J.-C., Morch, M.-D. & Haenni, A.-L. (1989) Can plant RNA viruses exchange genetic material? in *Evolutionary Tinckering in Gene Expression* (Grunberg-Manago, M, Clark, B.F.C., Zachau, H.G., eds.) vol. 169, pp. 175– 192, Plenum Press, New York, London.

28. Ahlquist, P., Dasgupta, R. & Kaesberg, P. (1981) Near identity of the 3′ RNA secondary structure in bromoviruses and cucumber mosaic virus. *Cell* **23**, 183–189.

29. Gustafson, G., Armour, S.L., Gamboa, G.C., Burgett, S.G. & Shepherd, J.W. (1989) Nucleotide sequence of barley stripe mosaic virus RNAa: RNAa encodes a single polypeptide with homology to corresponding proteins of other viruses. *Virology* **170**, 370–377.

30. Hall, T.C., Shih, D.S. & Kaesberg, P. (1972) Enzyme-mediated binding of tyrosine to brome-mosaic-virus ribonucleic acid. *Biochem. J.* **129**, 969–976.

31. Kohl, R.J. & Hall, T.C. (1974) Aminoacylation of RNA from several viruses: Amino acid specificity and differential activity of plant, yeast and bacterial synthetases. *J. Gen. Virol.* **25**, 257–261.

32. Joshi, R.L., Joshi, S., Chapeville, F. & Haenni, A.L. (1983) tRNA-like structures of plant viral RNAs: Conformational requirements for adenylation and aminoacylation. *EMBO J.* **2**, 1123–1127.

33. Rietveld, K., Pleij, C.W.A. & Bosch, L. (1983) Three-dimensional models of the tRNA-like 3′-termini of some plant viral RNAs. *EMBO J.* **2**, 1079–1085.

34. Bujarski, J.J., Dreher, T.W. & Hall, T.C. (1985) Deletions in the 3′-terminal tRNA-like structure of brome mosaic virus RNA differentially affect aminoacylation and replication *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5636–5640.

35. Joshi, R.L. & Haenni, A.-L. (1986) Search for tRNA-like properties in tomato aspermy virus RNA. *FEBS Lett.* **194**, 157–160.

36. Loesch-Fries, L.S. & Hall, T.C. (1982) *In vivo* aminoacylation of brome mosaic and barley stripe mosaic virus RNAs. *Nature* **298**, 771–773.

37. Bastin, M. & Hall, T.C. (1976) Interaction of elongation factor 1 with aminoacylated brome mosaic virus and tRNA's. *J. Virol.* **20**, 117–122.

38. Van Belkum, A., Cornelissen, B., Linthorst, H., Bol, J., Pleij, C. & Bosch, L. (1987) tRNA-like properties of tobacco rattle virus RNA. *Nucleic Acids Res.* **15**, 2837–2850.

39. Dreher, T.W., Bujarski, J.J. & Hall, T.C. (1984) Mutant viral RNAs synthesized *in vitro* show altered aminoacylation and replicase template activities. *Nature* **311**, 171– 175.

40. Dreher, T.W. & Hall, T.C. (1988) Mutational analysis of the tRNA mimicry of brome mosaic virus RNA. Sequence and structural requirements for aminoacylation and 3'-adenylation. *J. Mol. Biol.* **201**, 41–55.

41. Perret, V., Florentz, T., Dreher, T. & Giegé, R. (1989) Structural analogies between the 3' tRNA-like structure of brome mosaic virus RNA and yeast $tRNA^{Tyr}$ revealed by protection studies with yeast tyrosyl-tRNA synthetase. *Eur. J. Biochem.* **185**, 331–339.

42. Florentz, C., Dreher, T.W., Rudinger, J. & Giegé, R. (1991) Specific valylation identity of turnip yellow mosaic virus RNA by yeast valyl-tRNA synthetase is directed by the anticodon in a kinetic rather than affinity-based discrimination. *Eur. J. Biochem.* **195**, 229–234.

43. Dreher, T.W., Tsai, C.-H., Florentz, C. & Giegé, R. (1992) Specific valylation of turnip yellow mosaic virus RNA by wheat germ valyl-tRNA synthetase determined by three anticodon loop nucleotides. *Biochemistry* **31**, 9183–9189.

44. Rudinger, J., Florentz, C., Dreher, T. & Giegé, R. (1992) Efficient mischarging of a viral tRNA-like structure and aminoacylation of a minihelix containing a pseudoknot: Histidinylation of turnip yellow mosaic virus RNA. *Nucleic Acids Res.* **20**, 1865–1870.

45. Quadt, R., Kao, C.C., Browning, K.S., Hershberger, R.P. & Ahlquist, P. (1993) Characterization of a host protein associated with brome mosaic virus RNA-dependent RNA polymerase. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1498–1502.

46. Dreher, T.W., Rao, A.L.N. & Hall, T.C. (1989) Replication *in vivo* of mutant brome mosaic virus RNAs defective in aminoacylation. *J. Mol. Biol.* **206**, 425–438.

47. Miller, W.A., Bujarski, J.J., Dreher, T.W. & Hall, T.C. (1986) Minus-strand initiation by brome mosaic virus replicase within the 3' tRNA-like structure of native and modified RNA templates. *J. Mol. Biol.* **187**, 537–546.

48. Dreher, T.W. & Hall, T.C. (1988) Mutational analysis of the sequence and structural requirements in brome mosaic virus RNA for minus strand promoter activity. *J. Mol. Biol.* **201**, 31–40.

49. Tsai, C.-H. & Dreher, T.W. (1991) Turnip yellow mosaic virus RNAs with anticodon loop substitutions that result in decreased valylation fail to replicate efficiently. *J. Virol.* **65**, 3060–3067.

50. Dreher, T.W., Tsai, C.-H. & Skuzeski, J.M. (1996) Aminoacylation identity switch of turnip yellow mosaic virus RNA from valine to methionine results in an infectious virus. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 12212–12216.

51. Goodwin, J.B., Skuzeski, J.M. & Dreher, T.W. (1997) Characterization of chimeric turnip yellow mosaic virus genomes that are infectious in the absence of aminoacylation. *Virology* **230**, 113–124.

52. Morch, M.D., Joshi, R.L., Denial, T.M. & Haenni, A.L. (1987) A new 'sense' RNA approach to block viral replication *in vitro*. *Nucleic Acids Res.* **15**, 4123–4130.

53. Gargouri-Bouzid, R., David, C. & Haenni, A.-L. (1991) The 3'-promoter region involved in RNA synthesis directed by the turnip yellow mosaic virus genome in vitro. *FEBS Lett.* **294**, 56–58.

54. Zaccomer, B., Cellier, F., Boyer, J.-C., Haenni, A.-L. & Tepfer, M. (1993) Transgenic plants that express genes including the 3′ untranslated region of the turnip yellow mosaic virus (TYMV) genome are partially protected against TYMV infection. *Gene* **136**, 87–94.

55. Powell, P.A., Stark, D.M., Sanders, P.R. & Beachy, R.N. (1989) Protection against tobacco mosaic virus in transgenic plants that express tobacco mosaic virus antisense RNA. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6949–6952.

56. Weiner, A.M. & Maizels, N. (1987) tRNA-like structures tag the 3′-ends of genomic RNA molecules for replication: Implications for the origin of protein synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 7383–7387.

57. Rao, A.L.N., Dreher, T.W., Marsh, L.E. & Hall, T.C. (1989) Telomeric function of the tRNA-like structure of brome mosaic virus RNA. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5335–5339.

58. Tsai, C.-H. & Dreher, T.W. (1992) Second-site suppressor mutations assist in studying the function of the 3′ noncoding region of turnip yellow mosaic virus RNA. *J. Virol.* **66**, 5190–5199.

59. Gallie, D.R. & Walbot, V. (1990) RNA pseudoknot domain of tobacco mosaic virus can functionally substitute for a poly(A) tail in plant and animal cells. *Genes Dev.* **4**, 1149–1157.

60. Gallie, D.R., Feder, J.N., Schimke, R.T. & Walbot, V. (1991) Functional analysis of the tobacco mosaic virus tRNA-like structure in cytoplasmic gene regulation. *Nucleic Acids Res.* **19**, 5031–5036.

61. Briand, J.P., Keith, G. & Guilley, H. (1978) Nucleotide sequence at the 5′-extremity of turnip yellow mosaic virus genome RNA. *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3168–3172.

62. Dasgupta, R., Ahlquist, P. & Kaesberg, P. (1980) Sequence of the 3′ untranslated region of brome mosaic virus coat protein messenger RNA. *Virology* **104**, 339–346.

63. Schulman, L.H. (1991) Recognition of tRNAs by aminoacyl-tRNA synthetases. *Prog. Nucleic Acids Res. Mol. Biol.* **41**, 23–87.

64. Perret, V., Garcia, A., Grosjean, H., Ebel, J.-P., Florentz, C. & Giegé, R. (1990) Relaxation of transfer RNA specificity by removal of modified nucleotides. *Nature* **344**, 787–789.

65. Monroe, S.S. & Schlesinger, S. (1983) RNAs from two independently isolated defective interfering particles of Sindbis virus contain a cellular tRNA sequence at their 5′ ends. *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3279–3283.

66. Gordon, K.H.J., Johnson, K.N. & Hanzlik, T.N. (1995) The large genomic RNA of *Helicoverpa armigera* stunt tetravirus encodes the viral RNA polymerase and has a novel 3′-terminal tRNA-like structure. *Virology* **208**, 84–98.

67. Hanzlik, T.N. & Gordon, K.H.J. (1997). The *Tetraviridae*. *Adv. Virus Res.* **48**, 101– 168.

68. Marsh, L.E. & Hall, T.C. (1987) Evidence implicating a tRNA heritage for the promoters of positive-strand RNA synthesis in brome mosaic virus and related viruses. *Cold Spring Harbor Symp. Quant. Biol.* **52**, 331–341.

69. Marsh, L.E., Pogue, G.P. & Hall, T.C. (1989). Similarities among plant (+) and (–) RNA termini imply a common ancestry with promoters of eukaryotic tRNAs. *Virology* **172**, 415–427.

70. Pogue, G.P., Marsh, L.E., Connell, J.P. & Hall, T.C. (1992) Requirement for ICR-like sequences in the replication of brome mosaic virus genomic RNA. *Virology* **188**, 742–753.