The EMBO Journal Vol.1 No.8 pp.935–938, 1982

Turnip yellow mosaic virus RNA is aminoacylated *in vivo* in Chinese cabbage leaves

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Communicated by A.L.Haenni
Received on 25 June 1982

Turnip yellow mosaic virus (TYMV) contains a tRNA-like structure as an integral part of its genome. This structure is located at the extreme 3′ end of the viral RNA and is the acceptor of valine after 3′-terminal adenylation. It is known that *in vitro* (with bacterial, yeast, or plant systems) and *in vivo* (upon microinjection into *Xenopus laevis* oocytes) a series of tRNA-specific enzymes can recognize this structure in the viral RNA. We report that TYMV RNA is valylated and consequently adenylated *in vivo* in its natural host, Chinese cabbage leaves. This suggests that the acylated form of the viral RNA could play an important role in the life-cycle of the virus.

Key words: in vivo aminoacylation/tRNA-like structure/turnip yellow mosaic virus RNA

Introduction

Several plant and animal RNA viruses possess a tRNA-like structure as an integral part of their genome. In the case of plant viruses the tRNA-like structure is located at the extreme 3′ end of the RNA; viruses with a multi-partite genome have conserved this structure in all of their split genomes (for a review, see Haenni *et al.*, 1982).

The tRNA-like structure of TYMV RNA is one of the most widely studied. It is recognized *in vitro* by a series of tRNA-specific enzymes such as the tRNA-nucleotidyltransferase (Yot *et al.*, 1970; Litvak *et al.*, 1970), valyl-tRNA synthetase from different origins (Pinck *et al.*, 1970; Yot *et al.*, 1970; Kohl and Hall, 1974), a tRNA cytosine-methyltransferase (Haenni *et al.*, 1975), extracts containing 'RNase P' (Prochiantz and Haenni, 1973), the peptidyl-tRNA hydrolase (Yot *et al.*, 1970), and the elongation factors eEF-1 (Litvak *et al.*, 1973) and EF-T (Haenni *et al.*, 1974). The kinetic constants (K_m and V_{max}) for the aminoacylation of TYMV RNA using the yeast enzyme are comparable to those for yeast $tRNA^{Val}$ (Giégé *et al.*, 1978).

The 3′-terminal region of TYMV RNA has been sequenced (Prochiantz, 1976; Briand *et al.*, 1977; Silberklang *et al.*, 1977); its tRNA-like structure is entirely encompassed within the non-coding region of the viral RNA, it is devoid of modified bases and interestingly CAC – a valine anticodon – can be adequately positioned in a possible 'anticodon' loop. Experiments have recently been performed using chemical modification methods and specific RNases to determine more precisely the secondary structure of the tRNA-like region in TYMV RNA (Rietveld *et al.*, 1982; Florentz *et al.*, 1982). Surprisingly, the tRNA-like fragment contains relatively few sequence similarities with $tRNA^{Val}$ (Haenni and Chapeville, 1980), and even its secondary structure bears little resemblance to the classic clover-leaf model of a tRNA (Briand *et al.*, 1977; Silberklang *et al.*, 1977; Rietveld *et al.*, 1982; Florentz *et al.*, 1982). However, upon tertiary folding, the 80–86 nucleotides from the 3′ end of the viral RNA can be superposed (Rietveld *et al.*, 1982) onto the 'L'-shaped structure of $tRNA^{Phe}$ (Kim *et al.*, 1974; Ladner *et al.*, 1975). This coincides well with the predicted length for the tRNA-like structure of TYMV RNA (Joshi *et al.*, 1982): the minimum length of the 3′-terminal fragment required for valylation *in vitro* is 86 nucleotides.

Since tRNA-like structures are highly conserved, they must play an important role in virus development. A step in understanding how this structure is involved in the life-cycle of the virus, would be to determine whether the viral RNA is aminoacylated *in vivo*. After microinjection into *Xenopus laevis* oocytes, we have previously shown that TYMV RNA is adenylated, aminoacylated, and 'processed' *in vivo*, releasing a 4–5S Val-RNA fragment (Joshi *et al.*, 1978). In this paper we present evidence that TYMV RNA is adenylated and aminoacylated in its natural host, Chinese cabbage leaves.

Results

To examine whether TYMV RNA is aminoacylated *in vivo*, it is necessary to distinguish between TYMV [^{3}H]Val-RNA and cellular [^{3}H]Val-tRNAs. This is simple since the sequence at the 3′ end of the viral RNA differs from that of host plant $tRNAs^{Val}$: upon complete RNase T1 digestion and analysis of the labelled material by t.l.c. on polyethyleneimine (PEI)-cellulose plates, only [^{3}H]Val-RNA of TYMV yields a [^{3}H]Val-pentanucleotide whereas the [^{3}H]Val-tRNAs yield two longer [^{3}H]Val-oligonucleotides.

Two methods were used to define whether TYMV RNA is aminoacylated *in vivo* in the plant leaves: (1) an indirect method based on the differential *in vitro* valylation of the host plant RNAs previously submitted or not to a deacylation step; (2) a direct method of *in vivo* valylation using [^{3}H]valine.

Presence of TYMV Val-RNA in infected Chinese cabbage leaves

Total RNA extracted from uninfected or TYMV-infected Chinese cabbage leaves was aminoacylated *in vitro* with [^{3}H]valine either directly or after a prior deacylation. This material was digested with RNase T1 and analyzed by PEI-cellulose chromatography. The amount of radioactivity contained in the different [^{3}H]Val-oligonucleotides was determined and the results obtained after 0, 11, and 17 days of infection are presented in Table I. As compared with the non-deacylated material, prior deacylation of the total RNA extracted from infected Chinese cabbage leaves leads to an increase in the level of the *in vitro* valylated TYMV RNA. This indicates that the viral RNA had been aminoacylated *in vivo*. As infection proceeds the amount of *in vivo* aminoacylated TYMV RNA increases, but the total amount of TYMV RNA (reflected by the amount of [^{3}H]Val-RNA of TYMV formed *in vitro* after previous deacylation) increases more rapidly. Thus, 38% and 21% of the TYMV RNA are aminoacylated

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Table I. Valylation *in vitro* of TYMV RNA extracted from Chinese cabbage leaves

Days after infection	Deacylation prior to *in vitro* acylation	Esterification *in vitro* (fmol)		Val-(t)RNAs formed *in vivo*[a] (fmol)	
		[^{3}H]Val-RNA of TYMV	[^{3}H]Val-tRNAs	Val-RNA of TYMV	Val-tRNAs
0	+	0	3640	0	2470
	−	0	1170		
11	+	42	3862	16	2570
	−	26	1292		
17	+	239	3535	51	2343
	−	188	1192		

Results are expressed as fmol of valine esterified per 10 μg of total RNA. The values contained in the Val-pentanucleotide correspond to [^{3}H]Val-RNA of TYMV, and those contained in the two longer Val-oligonucleotides to [^{3}H]Val-tRNAs.
[a]fmol of Val-(t)RNAs formed *in vivo* = [fmol of [^{3}H]Val-(t)RNAs obtained by *in vitro* aminoacylation of totally pre-deacylated (t)RNAs] − [fmol of [^{3}H]Val-(t)RNAs obtained by *in vitro* aminoacylation of non-deacylated (t)RNAs].

in vivo after 11 and 17 days of infection, respectively (Table II). Val-RNA of TYMV, formed after 11 and 17 days, represents respectively 0.6% and 2.1% of the total aminoacylated Val-(t)RNAs. The percentage of cellular Val-tRNAs aminoacylated *in vivo* as compared with total tRNAs served as control; it remains constant (~67%, see Table II).

In vivo aminoacylation of TYMV RNA

Before searching for aminoacylation of the viral RNA in infected leaves, uninfected leaf slivers were incubated with [^{3}H]valine, the [^{3}H]Val-tRNAs isolated, totally digested with RNase T1, and the behaviour of the resulting labelled material examined by PEI-cellulose chromatography. As shown in Figure 1 (left lane), a considerable amount of RNase T1-resistant material was present in the *in vivo* labelled material that masked the Val-oligonucleotides and hindered their proper evaluation. We could efficiently separate the [^{3}H]Val-oligonucleotides from this contaminating material by filtration through a Sephadex G-50 column: the contaminant of high mol. wt. is excluded from such a column (fractions 13−15) whereas the Val-oligonucleotides are delayed (fractions 23−45). This purification step was therefore adopted for subsequent experiments of direct *in vivo* aminoacylation.

Aminoacylation *in vivo* was checked in the 14th leaf from the outside of three Chinese cabbage plants. On different days (0−32) after infection the leaf tissue was excised, incubated in the presence of [^{3}H]valine and the RNAs extracted. After RNase T1 digestion the material was analyzed by PEI-cellulose chromatography and the fluorogram obtained is presented in Figure 2. A [^{3}H]Val-pentanucleotide corresponding to Val-RNA of TYMV appears already after 6 days of infection. To better quantitate the amount of Val-RNA of TYMV formed, the material corresponding to the [^{3}H]Val-pentanucleotide and to the two longer [^{3}H]Val-oligonucleotides (resulting from the digestion of TYMV [^{3}H]Val-RNA and of endogenous [^{3}H]Val-tRNAs, respectively) was cut out and its radioactivity determined. In Figure 3 the percentage of c.p.m. corresponding to the [^{3}H]Val-RNA of TYMV as compared to the [^{3}H]Val-tRNAs + [^{3}H]Val-RNA of TYMV is presented as a function of days after infection. The results obtained in two independent experiments are shown. The percentage of Val-RNA of TYMV as compared with the total Val-(t)RNAs formed *in vivo* increases with time and reaches ~9% after 25 days of infection.

Table II. Percentage of TYMV RNA and of tRNAs aminoacylated *in vivo*

Days after infection	Val-RNA of TYMV / total TYMV RNA (%)	Val-tRNAs / total tRNAs (%)	Val-RNA of TYMV / Val-tRNA + Val-RNA of TYMV (%)
0	0	68	0
11	38	67	0.6
17	21	66	2.1

The amount of total TYMV RNA or of total tRNA^{Val} (fmol of TYMV [^{3}H]Val-RNA or of [^{3}H]Val-tRNAs obtained by *in vitro* aminoacylation of pre-deacylated RNAs) and that of Val-(t)RNAs formed *in vivo* are from Table I. The percentage of Val-RNA of TYMV as compared with total Val-(t)RNAs formed *in vivo* is also presented.

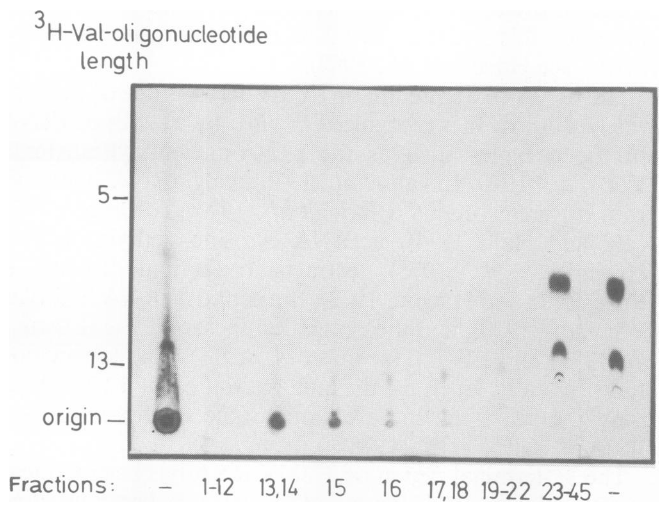

Fig. 1. Purification of [^{3}H]Val-oligonucleotides formed *in vivo*. The RNase T1 digests of the *in vivo* aminoacylated material formed in uninfected Chinese cabbage leaves were analyzed by PEI-cellulose chromatography. The fluorogram obtained after 2 weeks exposure shows the RNase T1 digests of the [^{3}H]Val-tRNAs (10 000 c.p.m.) charged *in vivo* (**left lane**), *in vitro* (**right lane**), and of a mixture of these two samples (combined so as to decrease duration of fluorography) purified by filtration through a Sephadex G-50 column (see Materials and methods). The resulting fractions were pooled as indicated, lyophilized, and analyzed. The positions of the [^{3}H]Val-penta- and tredecanucleotides are indicated to the left; they were established by analyzing the RNase T1 digests of TYMV [^{3}H]Val-RNA and of *Escherichia coli* [^{3}H]Val-tRNAs (not shown).

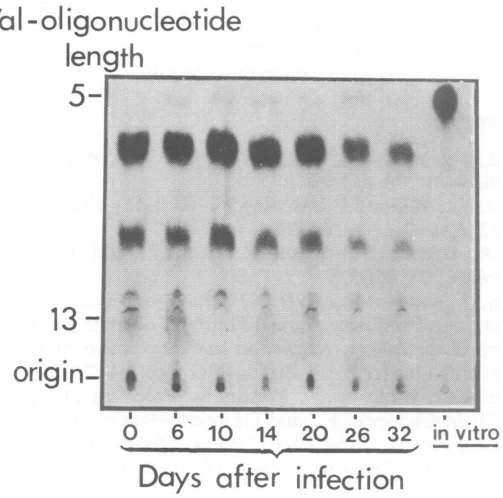

Fig. 2. Analysis of the [^{3}H]Val-oligonucleotides obtained by RNase T1 digestion of the [^{3}H]Val-(t)RNAs (2500 – 10 000 c.p.m.) formed *in vivo* in infected Chinese cabbage leaves. The conditions for aminoacylation *in vivo* and analysis were as described under Materials and methods. The fluorogram (developed after 75 days of exposure) indicates the material obtained after *in vivo* aminoacylation using Chinese cabbage leaves infected for 0, 6, 10, 14, 20, 26, and 32 days and that obtained after *in vitro* aminoacylation of TYMV RNA. The position of the [^{3}H]Val-penta- and tredecanucleotides are indicated in the left-hand margin.

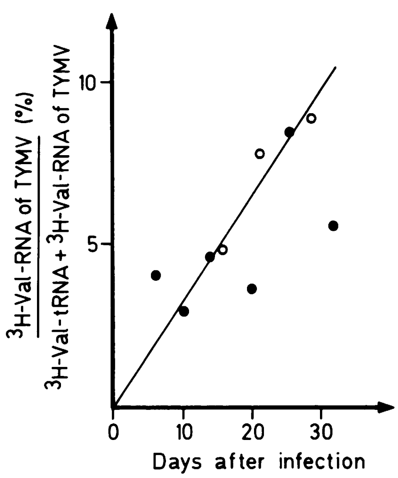

Fig. 3. Kinetics of *in vivo* aminoacylation of TYMV RNA in infected Chinese cabbage leaves. Results are expressed in percentage of [^{3}H]Val-RNA of TYMV/[^{3}H]Val-tRNA + [^{3}H]Val-RNA of TYMV, as a function of days after infection. ● and ○ correspond to values obtained in two independent experiments; the values corresponding to the closed circles result from the experiment presented in Figure 2.

Discussion

Using two different approaches we have demonstrated that TYMV RNA is aminoacylated *in vivo* in its natural host, Chinese cabbage leaves. The first approach based on differential *in vitro* valylation permits an evaluation of the TYMV RNA that is aminoacylated from the beginning of infection to the day when the leaves are collected for analysis. The second approach based on direct *in vivo* valylation allows the determination of the TYMV RNA that is aminoacylated only during the *in vivo* labelling period (5 h), but on different days after infection.

Although the system used (infected leaves) is asynchronous, as infection proceeds increasing amounts of viral RNA are aminoacylated (Table I). More than 20% of the total amount of TYMV RNA contained in the infected leaves is valylated *in vivo* (Table II). Late after infection Val-RNA of TYMV represents ~9% of the total valylated material (Figure 3).

The following previous indications pointed to a possible aminoacylation of TYMV RNA in its natural host: (1) TYMV RNA is aminoacylated *in vitro* by leaf extracts (Kohl and Hall, 1974); (2) its kinetic constants (K_m and V_{max}) for aminoacylation *in vitro* are comparable to those of yeast $tRNA^{Val}$ (Giégé *et al.*, 1978); and (3) it is aminoacylated *in vivo* in *X. laevis* oocytes (Joshi *et al.*, 1978). Since the viral RNA as extracted from the virions contains neither valine nor the 3′-terminal A residue, the Val-RNA must have undergone adenylation prior to aminoacylation in the infected leaves.

For technical reasons it has as yet not been possible to determine the length of the TYMV RNA that is valylated *in vivo*. However, it was previously demonstrated (Joshi *et al.*, 1982) that cabbage leaf extracts contain RNase(s) capable of liberating fragments 112 and 117 nucleotides long deriving from the 3′ end of the viral RNA genome, and indications existed (Yot *et al.*, 1971) that a viral RNA fragment of 4 – 5S capable of accepting valine could be recovered from infected leaves. It is thus likely that such a TYMV Val-RNA fragment 112 (or 117) nucleotides long is formed upon infection, as is the case when TYMV RNA is microinjected into *X. laevis* oocytes (Joshi *et al.*, 1978). The fragment 112 – 117 nucleotides long represents <5% of the total length of the genomic RNA. Such a fragment could also derive from the subgenomic TYMV RNA in which the tRNA-like structure is likewise present (Guilley and Briand, 1978).

The fact that both the genomic and the subgenomic RNAs present in the virions have escaped adenylation and valylation *in vivo* could mean that in order to react with the corresponding enzymes, the aminoacylatable fragment must leave the highly protected compartment where replication of the RNA and encapsidation take place. This suggests that after acylation (outside the compartment) the 3′-terminal RNA fragment acts for the benefit of the virus in one of the metabolic pathways of the cell by an as yet unknown mechanism; an important fraction of the genomic and/or of the subgenomic RNA would be sacrificed for this function.

At the present state of investigation another hypothesis, although less likely, cannot be excluded: adenylation and aminoacylation *in vivo* would not be restricted to the 3′-terminal fragment(s) but would occur on intact genomic and/or subgenomic RNAs. Some of these Val-RNA molecules would remain unencapsidated because of hinderance by the terminal Val-AMP, whereas others would lose the Val-AMP by a specific mechanism before encapsidation. Valylation of the unencapsidated RNA could play a role either directly, such as in the regulation of replication, or after nucleolytic processing as proposed above.

During the course of these studies a communication was presented indicating that aminoacylation of brome mosaic virus and barley stripe mosaic virus RNAs occurs *in vivo* in infected barley protoplasts (Loesch-Fries *et al.*, 1981).

Materials and methods

TYMV RNA and enzymes

Healthy and TYMV-infected Chinese cabbage leaves were generously supplied by S.Astier-Manifacier and P.Cornuet (INRA, Versailles). TYMV was purified by the method of Leberman (1966), and the viral RNA extracted under RNase-free conditions (Porter *et al.*, 1974) and kept at $-70°C$. *E. coli* extracts devoid of tRNAs were prepared as described previously (Yot *et al.*, 1970). RNase T1 was purchased from Sankyo.

Extraction of RNAs from Chinese cabbage leaves

Three Chinese cabbage plants (2 months old, bearing ~20 leaves each) were used for each experiment. Three external leaves of each plant were inoculated using 100 μl/leaf of a virus suspension (500 μg/ml) and carborundum as abrasive. At 0 (just prior to inoculation), 11, and 17 days after infection, the 10th, 11th, and 12th leaves respectively from the outside of each plant were removed and combined. In all cases the central nerves of the leaves were excised, the leaves weighed (2–5 g/pool of three leaves) and kept at $-20°C$. All further steps were performed at 4°C unless otherwise stated. For total RNA extraction, uninfected and TYMV-infected leaves were homogenized using a Sorvall Omni-mixer and a Kontes potter in a solution (6 ml/g of leaf) containing 200 mM sodium acetate pH 5, 1.25 mM EDTA, 1% SDS, and 50% phenol. The aqueous phase was ethanol precipitated three times and the resulting RNA pellet dissolved in sterile water and kept at $-70°C$.

Deacylation of RNAs

When indicated, the RNA was deacylated in 100 mM Tris-HCl pH 8.7 for 3 h at 37°C, ethanol precipitated, resuspended in sterile water, and kept at $-70°C$. It was verified by electrophoretic analyses on 12% polyacrylamide-7 M urea gels (Joshi *et al.*, 1982) that this treatment had no deleterious effect on the RNA since no RNA fragments were produced (not shown).

Aminoacylation in vitro

Aminoacylation *in vitro* using an *E. coli* extract devoid of tRNAs was as described previously (Joshi *et al.*, 1982), except that 200 μg/ml of total RNA and 2.6 μM [^3H]valine (28 Ci/mmol, C.E.A. Sacley, France) were used. After 15 min at 37°C, aliquots were removed to determine the cold TCA precipitable counts. The remaining material was ethanol precipitated and the dried pellets dissolved in water and kept at $-70°C$. Under these conditions the extent of aminoacylation of the viral RNA (whether previously deacylated or not) was ~40% (not shown).

Aminoacylation in vivo

The age of the plants and the inoculation conditions were as stated above. The 14th leaf from the outside of three plants was used to check for aminoacylation *in vivo* at different times after infection. On day 0 (before infection) or on different days after infection, a fragment (10 mg) was removed from the same leaf of each plant, the fragments combined, rinsed with distilled water, cut into 1 mm slivers and vacuum-infiltrated using 200 μl of incubation medium (Zaitlin and Hariharsubramanian, 1972) containing 10 mM KH_2PO_4, 60 μg/ml of actinomycin D (Sigma) and 35.7 μM (1 mCi/ml) of [^3H]valine. After incubation in the presence of light (10 000 lux) for 6 h at 28°C, the leaf strips were washed three times with distilled water and placed at $-70°C$. Extraction of total RNA was performed as indicated above except that 300 μl phenol-buffer solution was used per 30 mg of leaf. After three successive ethanol precipitations, the dried pellets were resuspended in distilled water and kept at $-70°C$.

RNase T1 digestion and analysis of [^3H]Val-oligonucleotides

The [^3H]Val-(t)RNAs aminoacylated *in vitro* (10 μg; 5000 c.p.m.; when previously deacylated: 15 000 c.p.m.) and *in vivo* (~70 μg; 20 000–80 000 c.p.m.) were digested with RNase T1 as described previously (Joshi *et al.*, 1978). When aminoacylated *in vivo*, the [^3H]Val-oligonucleotides were then purified as follows. The RNase T1 digests (15–20 μl) were brought to 0.4% with blue dextran 2000 and filterd through a Sephadex G-50 ('medium', Pharmacia) column (10 x 0.5 cm^2) equilibrated with 10 mM acetic acid; fractions containing 2 drops (~100 μl) were collected (5 drops/min). Fractions 23–45 containing the [^3H]Val-oligonucleotides were assembled, lyophilized, and dissolved in 15 μl water. In all cases, the samples were analyzed by t.l.c. on PEI-cellulose plates (20 x 20 cm^2, plastic backed with fluorescent indicator, Schleicher and Schüll) as described previously (Joshi *et al.*, 1978). The chromatogram was soaked in 7% PPO in ether (Randerath, 1970) and exposed at $-70°C$ using a flash-activated (Laskey and Mills, 1975) Kodak X-Omat R film. Where indicated, after 25–75 days exposure, the regions of the PEI-cellulose plates containing the [^3H]valine-labelled material were cut out and counted in the presence of Biofluor (New England Nuclear; 15 ml). The counting efficiency was 27% of that obtained in the absence of PEI-cellulose. A blank value (~150 c.p.m.) corresponding to the radioactivity present at the level of the Val-pentanucleotide in the uninfected samples was subtracted.

Acknowledgements

The help of R.Schwartzmann in the preparation of the manuscript is gratefully acknowledged. This work benefitted from grants from the Action Thématique Programmée Phytopathologie (No. 3608), the Ecole Pratique des Hautes Etudes, and from a Stipendium to S.J. from the Délégation Générale à la Recherche Scientifique et Technique. The manuscript was written whilst S.J. was holder of an EMBO long-term fellowship.

References

Briand,J.P., Jonard,G., Guilley,H., Richards,K., and Hirth,L. (1977) *Eur. J. Biochem.*, **72**, 453-463.
Florentz,C., Briand,J.P., Romby,P., Hirth,L., Ebel,J.P., and Giégé,R. (1982) *EMBO J.*, **1**, 269-276.
Giégé,R., Briand,J.P., Mengual,R., Ebel,J.P., and Hirth,L. (1978) *Eur. J. Biochem.*, **84**, 251-276.
Guilley,H., and Briand,J.P. (1978) *Cell*, **15**, 113-122.
Haenni,A.L., Prochiantz,A., and Yot,P. (1974) in Richter,D. (ed.), *Lipmann Symposium: Energy, Regulation and Biosynthesis in Molecular Biology*, Walter de Gruyter Press, Berlin, FRG, pp. 264-276.
Haenni,A.L., Bénicourt,C., Teixeira,S., Prochiantz,A., and Chapeville,F. (1975) in Chapeville,F., and Grunberg-Manago,M. (eds.), *Organization and Expression of the Viral Genome, Molecular Interactions in Genetic Translation, FEBS Proc. Meet.*, **39**, North Holland/American Elsevier, Amsterdam, The Netherlands, pp. 121-131.
Haenni,A.L., and Chapeville,F. (1980) in Söll,D., Abelson,J.N., and Schimmel,P.R. (eds.), *Transfer RNA: Biological Aspects*, Cold Spring Harbor Laboratory Press, NY, USA, pp. 539-556.
Haenni,A.L., Joshi,S., and Chapeville,F. (1982) in Davidson,I.N., and Cohn,W.E. (eds.), *Progress in Nucleic Acid Research and Molecular Biology*, **27**, Academic Press, NY, USA, pp. 85-102.
Joshi,S., Haenni,A.L., Hubert,E., Huez,G., and Marbaix,G. (1978) *Nature*, **275**, 339-341.
Joshi,S., Chapeville,F., and Haenni,A.L. (1982) *Nucleic Acids Res.*, **10**, 1947-1962.
Kim,S.H., Suddath,F.L., Quigley,G.J., McPherson,A., Sussman,J.L., Wang,A.H.J., Seeman,N.C., and Rich,A. (1974) *Science (Wash.)*, **185**, 435-440.
Kohl,R.J., and Hall,T.C. (1974) *J. Gen. Virol.*, **25**, 257-261.
Ladner,J.E., Jack,A., Robertus,I.D., Brown,R., Rhodes,D., Clark,B.F.C., and Klug,A. (1975) *Proc. Natl. Acad. Sci. USA*, **72**, 4414-4418.
Laskey,R.A., and Mills,A.D. (1975) *Eur. J. Biochem.*, **56**, 335-341.
Leberman,R. (1966) *Virology*, **30**, 341-347.
Litvak,S., Carré,D.S., and Chapeville,F. (1970) *FEBS Lett.*, **11**, 316-329.
Litvak,S., Tarrago,A., Tarrago-Litvak,L., and Allende,J.E. (1973) *Nature New Biol.*, **241**, 88-90.
Loesch-Fries,L.S., Kiberstis,P.A., and Hall,T.C. (1981) *Fifth International Congress of Virology*, Strasbourg, France, W20/04.
Pinck,M., Yot,P., Chapeville,F., and Duranton,H.M. (1970) *Nature*, **226**, 954-956.
Porter,A., Carey,N., and Fellner,P. (1974) *Nature*, **248**, 675-678.
Prochiantz,A., and Haenni,A.L. (1973) *Nature New Biol.*, **241**, 168-170.
Prochiantz,A. (1976) Ph.D. Thesis, University Paris, France.
Randerath,K. (1970) *Anal. Biochem.*, **34**, 188-215.
Rietveld,K., Van Poelgeest,R., Pleij,C.W.A., Van Boom,J.H., and Bosch,L. (1982) *Nucleic Acids Res.*, **10**, 1929-1946.
Silberklang,M., Prochiantz,A., Haenni,A.L., and RajBhandary,U.L. (1977) *Eur. J. Biochem.*, **74**, 465-478.
Yot,P. Pinck,M., Haenni,A.L., Duranton,H.M., and Chapeville,F. (1970) *Proc. Natl. Acad. Sci. USA*, **67**, 1345-1352.
Yot,P., Czosnek,H., and Haenni,A.L. (1971) *Seventh FEBS Meeting*, Varna, Bulgaria, Abstract 292.
Zaitlin,M., and Hariharsubramanian,V. (1972) *Virology*, **47**, 296-305.