Translation of Satellite Tobacco Necrosis Virus Ribonucleic Acid. I. Characterization of *in Vitro* Procaryotic and Eucaryotic Translation Products†

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ABSTRACT: The *in vitro* translation of satellite tobacco necrosis virus RNA can be carried out by procaryotic (*Escherichia coli*) and eucaryotic (wheat embryo) systems. Fingerprint analyses reveal that both *in vitro* systems produce protein with the amino acid sequence of satellite tobacco necrosis virus coat protein. Gel filtration and acrylamide gel electrophoretic analyses show that both *in vitro* systems produce a single material with an average size slightly smaller than satellite tobacco necrosis virus coat protein.

The initial characterization of the satellite tobacco necrosis virus generated interest among biochemists and molecular biologists in that the viral RNA was characterized as a small, single-stranded messenger RNA that appeared to contain only enough information to code for STNV coat protein (Reichmann, 1964). This initial assumption was based upon the satellite property of STNV,[1] the immunological properties of the system, and the apparent 3:1 ratio of STNV-RNA nucleotides to STNV coat protein amino acids. The more recent observations that STNV coat protein is smaller than originally envisioned (Roy *et al.*, 1969; Lesnaw and Reichmann, 1969) force one to be aware of the possible polycistronic character of STNV-RNA. Yet the small size of the viral RNA (with its resultant limited coding potential) and the observation (Clark *et al.*, 1965) that, *in vitro*, STNV-RNA codes as a monocistronic message for STNV coat protein emphasize the uniqueness of the STNV system.

Further characterization of the STNV system can be achieved by analyses of the product(s) of translation of STNV-RNA by various *in vitro* systems. Earlier, this laboratory reported that translation of STNV-RNA by an *in vitro* procaryotic (*Escherichia coli*) system leads to production of STNV coat protein (Clark *et al.*, 1965). This paper presents details of this *in vitro* procaryotic translation of STNV-RNA and compares this translation to the *in vitro* translation of STNV-RNA by a eucaryotic system.

Experimental Procedures

Materials. Cell-free extracts of *E. coli* A-19 were prepared from cells grown and extracted as previously described (Clark *et al.*, 1965). The cell-free wheat embryo extract employed was a 23,000*g* supernatant (S-23) preparation (Marcus *et al.*, 1968). STNV-RNA was obtained from STNV by phenol extraction (Kirby, 1965) of virus grown on tobacco (Reichmann, 1964) or mung beans (Liu *et al.*, 1969). The STNV-RNAs obtained from virus isolated from these varied sources are identical in physical properties and yield identical translation produced upon fingerprint analysis. Qβ and MS2 RNAs were obtained by phenol extraction (Kirby, 1965) of virus purchased from Miles Laboratories, Inc., Elkhart, Ind. [^{14}C]Amino acids and [^{3}H]- and [^{14}C]amino acid mixtures were as sold by New England Nuclear Co., Boston, Mass. [^{14}C]STNV coat protein, a gift from Dr. M. E. Reichmann, University of Illinois, Urbana, Ill., was prepared from STNV grown (*in vivo*) on tobacco in the presence of [^{14}C]CO_2.

Enzyme Incubations. The cell-free extracts from *E. coli* A-19 cells were incubated for 20 min as previously described (Clark *et al.*, 1965) with the following exceptions. All reactions contained 0.0003 M GTP in place of 0.003 M GTP. Where indicated certain reactions utilized 30–40 μM levels of specific L-[^{14}C]amino acids supplemented with 40 μM concentrations of the other L-[^{12}C]amino acids required for protein biosynthesis. In other instances, reactions contained varied concentrations (as indicated) of a mixture of 15 L-[^{3}H]- or L-[^{14}C]amino acids supplemented with a 4×10^{-5} M level of the [^{12}C]amino acids asparagine, cysteine, glutamine, methionine, and tryptophan so as to provide all the amino acids needed for protein biosynthesis. All reactions utilized 40 μg of mRNA.

Protein synthesis studies with the wheat embryo system were run at 30° in 0.36-ml reaction volumes with optimized Mg^{2+} and K^{+} concentrations and containing 0.15 ml of S-23 extract of wheat embryos; 1 μCi of a mixture of 15 L-[^{14}C]amino acids (therefore approximately 1 μM in each) supplemented with a 3×10^{-5} M level of the [^{12}C]amino acids asparagine, cysteine, glutamine, methionine, and tryptophan so as to provide all the amino acids needed for protein biosynthesis; 0.045 M KCl; 0.003 M $Mg(OAc)_2$; 0.0046 M β-mercaptoethanol; 0.03 M Tris-Cl (pH 7.6); 40 μg of mRNA; 0.001 M ATP; 2×10^{-4} M GTP; 0.0042 M phosphoenolpyruvate; and 35 μg of pyruvic kinase. The 30-min incubations employed are sufficient to allow all reactions to go to completion.

Analytical Methods. [^{14}C]Amino acid incorporation into protein was assayed by means of scintillation counting of acid-precipitable material collected on Millipore-type HAWP filters after treatment with hot 5% trichloroacetic acid (Conway and Lipmann, 1964).

Reactions destined for gel filtration analysis were terminated by 20 min of 25° incubation in the presence of 20–100 μg of pancreatic RNase. This treatment was followed by titration

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[1] Abbreviation used is: STNV, satellite tobacco necrosis virus.

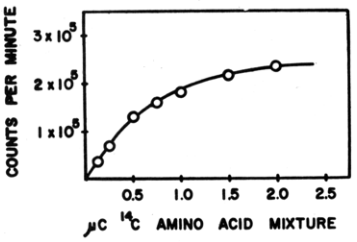

FIGURE 1: Amino acid concentration dependence of incorporation of mixture of 15 L-[^{14}C] amino acids into protein.

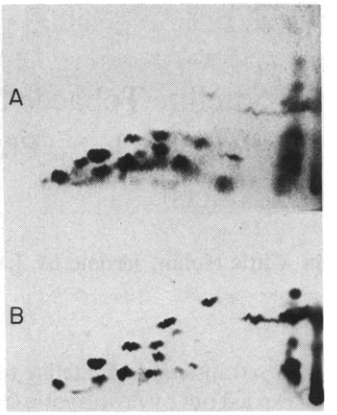

FIGURE 2: Comparative tryptic fingerprint analysis of STNV coat protein and [^{14}C]protein made during *in vitro* procaryotic (*E. coli*) translation of STNV-RNA. Part A depicts ninhydrin-positive tryptic peptides moving as cations at pH 6.5. Part B shows the radioautogram-positive tryptic peptides from [^{14}C]protein labeled with [^{14}C]Lys and [^{14}C]Arg. Similar but less obvious coincidence exists for anionic peptides. These are not shown due to difficulties in photographing the low-intensity ninhydrin reactions characteristic of this region of tryptic fingerprints of STNV coat protein.

to pH 11 with KOH, dialysis against 1% $(NH_4)_2CO_3$ and then water until no label was detected in the dialysate, and final freeze-drying of the sample. Labeled protein from one or more reaction mixtures, and carrier proteins where indicated, was taken up in 0.5 ml of 70% formic acid and placed on a 1.2 × 45 cm column of Sephadex G-200 in 70% formic acid at 4°. The column was then eluted (4°) with 70% formic acid at a flow rate of 0.05 ml/min. Indicated aliquots of 0.5-ml fractions were analyzed for radioactive label (scintillation counting) and for protein as indicated by 280-nm absorbing material (spectrophotometry). Fractions included during the above gel filtration process and destined for subsequent fingerprint or gel electrophoretic analysis were pooled and concentrated to dryness by flash evaporation. The dried product was then suspended in water and freeze-dried so as to favor subsequent solubilization.

Tryptic and chymotryptic fingerprint analysis was as previously reported (Clark *et al.*, 1965) and used either RNase and KOH-treated, dialyzed, and lyophilized material or similar material resolved by Sephadex G-200 gel filtration as above. Gel electrophoretic analysis also employed protein samples prepared as those used in fingerprint analysis studies. Aliquots (0.1 ml) of such protein(s) in 1% sodium dodecyl sulfate–0.1% β-mercaptoethanol–0.01 M sodium phosphate buffer (pH 7.2) were subjected to sodium dodecyl sulfate gel electrophoresis (Shapiro *et al.*, 1967) and then cut into 1-mm slices, each of which was analyzed by scintillation counting in toluene containing 0.5% 2,5-diphenyloxazole, 0.03% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene, 10% Beckman BBS III solubilizer.

Automatic Edman degradation of STNV coat protein was performed essentially as described by Edman and Begg (1967) with an Illinois Tool Co. sequenator. The analinothiazolinone derivatives obtained were converted to their respective amino acids by hydrolysis in 58% HI under reduced pressure at 120°. The resultant amino acids were analyzed on a Beckman Model 120C amino acid analyzer equipped with a millivolt full-scale recorder.

Results

An earlier communication from this laboratory (Clark *et al.*, 1965) presented preliminary details showing that STNV-RNA serves as a monocistronic message during *in vitro* translation by an *E. coli* system. The initial purpose of this report is to present fuller details of this *in vitro* procaryotic translation of STNV-RNA. This requires evaluation of the requirements of the *in vitro* procaryotic system and further characterization of the product protein.

The initial report of the *in vitro* translation of STNV-RNA by an *E. coli* system (Clark *et al.*, 1965) employed 0.5 μCi of a 1.8 μCi/μg of [^{14}C]amino acid mixture obtained by acid hydrolysis of [^{14}C]algal proteins. This level of amino acid is limiting when employed in the 50-μl system in the presence of saturating (40 μg) levels of STNV-RNA (Figure 1). Such amino acid limiting conditions limit the extent of STNV-RNA translation and create size nonuniformities in the product protein(s). Such nonuniformities hinder attempts to characterize the STNV-RNA translation product. Accordingly, all amino acid incorporation studies reported here involving mixtures of 15 [^{3}H]- or [^{14}C]amino acids utilize amino acid levels predetermined to be saturating for the *E. coli* system.

When operating with saturating levels of amino acids and RNA, the crude 30,000*g* supernatant system (S-30) from *E. coli* shows few requirements. Some, but not all, S-30 preparations demonstrate enhanced amino acid incorporation in the presence of (5–25 μg) *E. coli* tRNAs or in the presence of 10 ug of added calcium leucovorin. The results presented here involve incubations not supplemented with these materials.

Further proof that the protein product of this *in vitro* procaryotic system is STNV coat protein is essential to the significance of the overall STNV system. Accordingly, two approaches have been employed to establish further that the translation product in question is STNV coat protein. First, additional fingerprint studies have been performed (Figure 2) employing tryptic digestion of protein labeled with [^{14}C]-lysine and [^{14}C]arginine. Further, chymotryptic fingerprint analysis has also been performed (Figure 3) employing protein labeled with 15 L-[^{14}C]amino acids. These two studies again reveal the expected coincidence between ninhydrin-positive peptides derived from added STNV-RNA and radioactive peptides derived from the product of *in vitro* translation of STNV-RNA.

Secondly, the *in vitro* product produced by this *E. coli* system has been examined relative to size and homogeneity by use of gel filtration and use of sodium dodecyl sulfate gel electrophoresis. As seen in Figure 4, ^{3}H-labeled STNV-RNA translation product runs as a single peak of material on sodium dodecyl sulfate gel electrophoresis. This material is similar to [^{14}C]STNV coat protein produced *in vivo*. The prod-

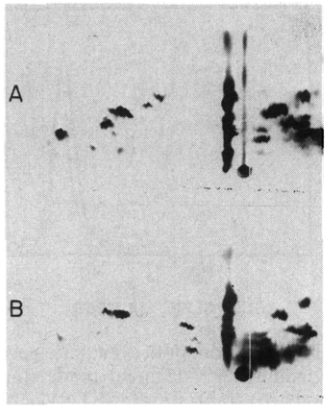

FIGURE 3: Comparative chymotryptic fingerprint analysis of STNV coat protein and [^{14}C]protein made during *in vitro* procaryotic (*E. coli*) translation of STNV-RNA. Part A depicts the ninhydrin-position chymotryptic peptides. Part B shows the radioautogram-positive chymotryptic peptides from [^{14}C]protein labeled with a mixture of 15 [^{14}C]amino acids.

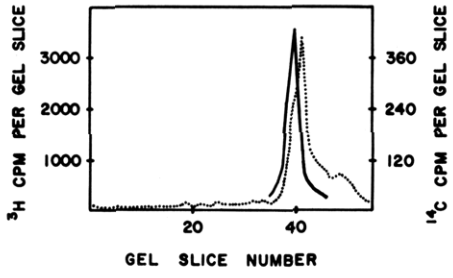

FIGURE 4: Comparative sodium dodecyl sulfate gel electrophoresis of [^{14}C]STNV coat protein (—) and [^{3}H]protein made during *in vitro* procaryotic (*E. coli*) translation of STNV-RNA (· · · · ·). *In vivo* produced [^{14}C]STNV coat protein containing 10^4 cpm was mixed with 10^5 cpm of [^{3}H]protein made during STNV-RNA translation by the *E. coli* system. The mixture was then resolved by sodium dodecyl sulfate gel electrophoresis as described in Experimental Procedures. Electrophoretic migration is from left to right.

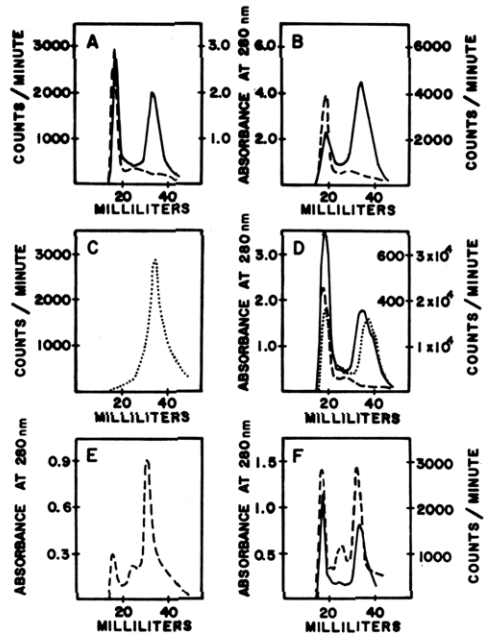

FIGURE 5: Sephadex G-200 gel filtration analyses of components of STNV system. Solid lines (——) represent ^{14}C counts from translation of STNV-RNA by the wheat embryo system. Dotted lines (· · · · ·) represent ^{3}H counts from the translation of STNV-RNA by the *E. coli* system. Dashed lines (- - -) represent protein distribution as indicated by A_{280}. (A) Elution profile of the wheat embryo system product (5 times normal incubation size). The results represent analyses of 0.1-ml aliquots of the effluent fractions. (B) Elution profile of the wheat embryo system product (10 times normal incubation size) after previous resolution on Sephadex G-200 and subsequent mixing with wheat embryo extract proteins. The results represent analyses of the 0.5-ml effluent fractions. The isolated included peak of material (*e.g.*, part A) was mixed with 1 ml of the wheat embryo S-23 extract and reanalyzed on Sephadex G-200. (C) Elution profile of the *E. coli* system product. The results represent analysis of 0.1-ml aliquots of the effluent fractions. (D) Elution profile of a mixture of the wheat embryo system product (3 times normal incubation size) and the *E. coli* system product. The results represent analysis of 0.1-ml aliquots of the effluent fractions. (E) Elution profile of 12 mg of STNV coat protein. The results represent analysis of the 0.5-ml effluent fractions. (F) Elution profile of a mixture of the wheat embryo system product and 25 mg of STNV coat protein. The results represent analyses of 0.3-ml aliquots of the effluent fractions.

uct of this procaryotic translation of STNV-RNA also migrates as a single peak of protein upon gel filtration on Sephadex G-200 (Figure 5C). Thus, the *in vitro* translation of STNV-RNA yields a single product with the fingerprint and size characteristics of STNV coat protein.

Satellite tobacco necrosis virus RNA also serves as an mRNA in a cell-free, protein synthesis system of wheat embryos. As seen in Table I, the S-23 system of wheat embryos (Marcus *et al.*, 1968) readily supports STNV-RNA-dependent amino acid incorporation into protein. Further, this *in vitro* protein biosynthesis is eucaryotic in character for, in contrast to the procaryotic *E. coli* system, it is inhibited by cycloheximide, and is insensitive to chloramphenicol. Interestingly, this eucaryotic wheat embryo system does not demonstrate enhanced amino acid incorporation in the presence of MS2 and Qβ-RNAs fully functional in the *in vitro* procaryotic (*E. coli*) system.

The ability of STNV-RNA to serve as a functional message in the wheat embryo system points out the need to character ize the protein product(s). We have employed several methods to establish that the product of STNV-RNA translation by this *in vitro* eucaryotic system is indeed STNV coat protein.

First, the product(s) of eucaryotic translation of STNV-RNA has been examined by gel filtration analysis on Sephadex G-200. As seen in Figure 5A, a RNase and KOH-treated, dialyzed wheat embryo reaction system can be resolved by gel filtration. Most of the endogenous protein, indicated by A_{280} material, elutes as a large molecular weight apparent aggregate moving at the void volume of the system. The labeled product migrates as two components, an initial peak of material moving with the apparent aggregated protein and a second included peak of material. Various studies reveal the initial peak of labeled material to represent aggregation of the protein found in the included peak. Specifically, as seen in Figure 5B, a high level of protein, for example such as employed in the wheat embryo assays, will cause the included peak of material to form some degree of apparent aggregated protein eluting at the void volume upon repassage over G-200. This potential to cause apparent aggregation is independent of the procaryotic or eucaryotic source of the original included component (Figure 5C,D). Thus, it appears that eucaryotic translation of STNV-RNA yields a single, potentially aggregatable protein.

These gel filtration studies also establish that the single

TABLE I: Characteristics of STNV-RNA-Dependent Incorporation of a Mixture of 15 [^{14}C]Amino Acids by a Cell-Free System from Wheat Embryo.

Reaction Type	Hot Acid Precipitable (cpm)	
	E. coli System	Wheat Embryo System
Complete system	146,278	33,177
Complete system minus STNV-RNA	3,223	3,279
Complete system plus chloramphenicol[a]	16,168	33,417
Complete system plus cycloheximide[a]	127,979	6,620
Complete system with MS2-RNA replacing STNV-RNA	140,153	4,186
Complete system with Qβ-RNA replacing STNV-RNA	234,832	3,316

[a] Where indicated, reactions contained either 40 μg of chloramphenicol or 2.0 μg of cycloheximide per ml.

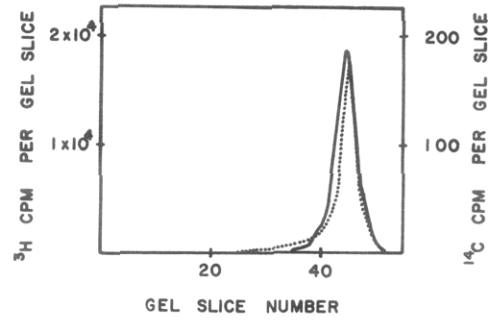

FIGURE 7: Comparative sodium dodecyl sulfate gel electrophoresis of Sephadex G-200 included [^{14}C]protein made during *in vitro* eucaryotic (wheat embryo) translation of STNV-RNA (—) and [^{3}H]-protein made during *in vitro* procaryotic (*E. coli*) translation of STNV-RNA (· · · · ·). Combined fractions containing the included peaks of [^{3}H] and [^{14}C]protein of Figure 5D were lyophilized and then resolved on sodium dodecyl sulfate gel electrophoresis and analyzed as in Experimental Procedures. Electrophoretic migration is from left to right.

protein product has size characteristics analogous to STNV coat protein. Figure 5D shows that the wheat embryo product is similar to the STNV coat protein produced during *in vitro* translation of STNV-RNA by the procaryotic (*E. coli*) system. Figures 5E and 5F also show that the non-aggregated wheat embryo product of STNV-RNA translation is analogous to added STNV coat protein. Thus gel filtration reveals that the eucaryotic product resembles STNV coat protein produced *in vitro* by the *E. coli* system or *in vivo* in plants.

Comparative tryptic fingerprint analyses of known STNV coat protein and of protein synthesized during *in vitro* eucaryotic translation of STNV-RNA provide a second means of characterizing the product of the *in vitro* wheat embryo system. The high levels of endogenous protein in the wheat embryo system must be, in part, removed in order for such analyses to be meaningful. Accordingly, all comparative fingerprint studies utilized labeled protein resolved from endogenous proteins by gel filtration of Sephadex G-200 (that is, the included peak of labeled material as shown in Figure 5A).

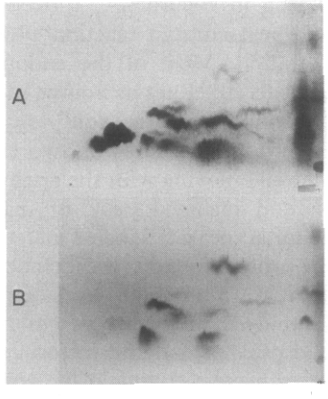

FIGURE 6: Comparative tryptic fingerprint analysis of STNV coat protein and [^{14}C]protein product of the *in vitro* eucaryotic (wheat embryo) translation of STNV-RNA. Part A depicts the ninhydrin-positive tryptic peptides moving as cations at pH 6.5. Part B shows the radioautogram-positive tryptic peptides from [^{14}C]protein labeled with a mixture of 15 [^{14}C]amino acids and then resolved as an included peak of [^{14}C]protein by gel filtration as in Figure 5A.

As seen in Figure 6, there is essentially complete coincidence between the ninhydrin-positive tryptic peptides derived from added STNV coat protein and the radioactive tryptic peptides derived from a Sephadex G-200 resolved product of eucaryotic translation of STNV-RNA. Similar radioautograms are obtained from trypsin digests of unresolved (no gel filtration) labeled protein produced by the wheat embryo system and from the initial peak of apparently aggregated protein resolved by Sephadex G-200. Thus STNV-RNA translation by the wheat embryo system yields STNV coat protein and does not yield significant quantities of any other protein product.

A third indication that the wheat embryo system yields STNV coat protein upon translation of STNV-RNA is found in sodium dodecyl sulfate gel electrophoresis studies of the eucaryotic product. As seen in Figure 7, labeled protein previously resolved on Sephadex G-200 migrates as a single component almost coincident with STNV coat protein produced *in vitro* during STNV-RNA translation by the *E. coli* system. Sodium dodecyl sulfate gel electrophoretic analysis of the product of this *E. coli* system (Figure 4) shows that this procaryotic product is similar to STNV coat protein.

The above observations establish that the product of *in vitro* translation of STNV-RNA in both the procaryotic and eucaryotic systems is a single protein analogous to STNV coat protein upon gel filtration analysis, fingerprint analysis, and sodium dodecyl sulfate gel electrophoretic analysis. This implies that STNV-RNA is a monocistronic message for its own coat protein in both the procaryotic and eucaryotic systems. The correct translation of STNV-RNA in both systems further suggests a common mechanism of protein chain initiation. Yet various *in vitro* studies (Marcker and Sanger, 1965; Adams and Cappechi, 1966; Smith and Marcker, 1970; Marcus *et al.*, 1970; Housman *et al.*, 1970) have established significant differences in protein chain initiation between procaryotes and eucaryotes. Clarification of this paradox requires knowledge of the N-terminal amino acid sequence of STNV coat protein and comparison of this sequence with initial (N-terminal) sequences obtained from procaryotic and eucaryotic translation of STNV-RNA.

Accordingly, we have utilized automated Edman degradation of STNV coat protein to determine the N-terminal amino acid sequence of STNV coat protein. Such analysis of the rather insoluble STNV coat protein results in low efficiency of reaction and as a result, significant synchronous release of amino acids for only a few cycles of stepwise degradation before the sequence becomes indeterminable (Table II). Nevertheless, an N-terminal sequence of

$$\text{Ala-Lys-}\begin{matrix}\text{Glu-}\\\text{Gln}\end{matrix}\cdots$$

is apparent from the analyses.

TABLE II: Amino Acid during Cycles of Automatic Edman Degradation of STNV Coat Protein.[a]

	nmoles of Amino Acid per Cycle					
Amino Acid	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Alanine	69	38	31	15	14	12
Lysine	4	22	41	19	6	7
Glutamic acid	2	3	37	81	60	22
Aspartic acid	7	10	9	10	33	56
Serine	12	21	11	6	6	2

[a] STNV coat protein (5.2 mg) was mixed with 2 mg of dithiothreitol, dissolved in a minimum of 0.2 M acetic acid, and vacuum dried in the spinning cup of the automatic Edman degradation machine prior to analysis. Assuming a STNV coat protein of 22000, the 69 nmoles of Ala detected on the first cycle represents only 29% detection of N-terminal Ala. Amino acids not listed all assayed at less than 15 nmoles per each listed cycle.

Discussion

The data presented here establish that STNV-RNA serves as a monocistronic message for STNV coat protein during STNV-RNA translation by *in vitro* systems from a procaryote (*E. coli*) and a eucaryote (wheat seed). Fingerprint studies with the products from both *in vitro* systems are the strongest proof of this point (Figures 2, 3, and 6). Specifically, such studies reveal essentially complete coincidence between specific peptides derived from STNV coat protein and specific peptides derived from the *in vitro* products. These studies, also fail to detect significant levels of other specific peptides, thereby supporting the monocistronic character of the STNV-RNA.

The comparative size studies involving gel filtration and sodium dodecyl sulfate gel electrophoretic analyses of STNV coat protein and the *in vitro* products also support the specific moncistronic message concept of STNV-RNA. These studies emphasize the production of a single *in vitro* product and reveal that size analogy exists between STNV coat protein and the *in vitro* products. Yet it should be pointed out that these studies also reveal that the average *in vitro* product is slightly smaller than STNV coat protein. Specifically, STNV coat protein appears slightly larger than the average wheat embryo product on gel filtration (Figure 5F). Additional gel filtration studies (Figure 5D) reveal the average wheat embryo product to be slightly larger than the average *E. coli* product. This order of slightly decreasing sizes (*i.e.*, STNV coat protein > average wheat embryo product > average *E. coli* product) is also established in the sodium dodecyl sulfate gel electrophoresis studies (Figures 4 and 7) where smaller molecules migrate further under the electrophoretic molecular sieving characteristics of sodium dodecyl sulfate systems. Such slight size deficiencies resulting from *in vitro* translation by an *E. coli* system have been detected by Lin and Fraenkel-Conrat (1967). Their careful analysis of this apparent lack of full translation revealed that C-terminal components of the *in vitro* product were deficient. This suggests that translation is incomplete due to contaminating nucleases, or shortages of components, or endogenous C-terminal proteases that reduce the average size of the product. It is likely that similar factors result in the slight average size deficiencies between STNV coat protein and the *in vitro* STNV-RNA translation products reported here.

The correct translation of STNV-RNA in both a procaryotic and a eucaryotic system suggests that there is a protein chain initiation feature of STNV-RNA that is recognized by the two different systems. It is logical to assume that this feature is a methionine codon prior to the observed *in vivo* sequence

$$\text{Ala-Lys-}\begin{matrix}\text{Glu-}\\\text{Gln}\end{matrix}\cdots$$

Yet this feature cannot be limited to a methionine codon by itself for as seen in Table I, MS2 and Qβ-RNAs, mRNAs, known to initiate cistron translation with methionine codons (Vinuela *et al.*, 1967; Skogerson *et al.*, 1971), are not translated readily by the eucaryotic system. One intriguing possibility is that it is the monocistronic character of the message that facilitates *in vitro* translation in both procaryotic and eucaryotic systems. This possibility follows from the observation that the only other reported example of a message correctly translated *in vitro* to yield a recognizable protein product in both a procaryotic and a eucaryotic system is the biosynthesis of hemoglobin (Laycock and Hunt, 1969; Schweet *et al.*, 1958). Alternately, current coding stoichiometry based upon the monocistrony of STNV-RNA, the 1200 nucleotides within STNV-RNA (Reichmann, 1964), and the approximately 200 amino acids in STNV coat protein (Roy *et al.*, 1969; Lesnaw and Reichmann, 1969; Rees *et al.*, 1970) emphasize that only half of STNV-RNA appears to be translated. Perhaps structural features of the untranslated portion(s) of STNV-RNA, acting by themselves or in combination with translated portions of STNV-RNA, provide the potential for correct *in vitro* translation in such varied systems.

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Translation of Satellite Tobacco Necrosis Virus Ribonucleic Acid. II. Initiation of *in Vitro* Translation in Procaryotic and Eucaryotic Systems†

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ABSTRACT: This paper reports the N-terminal amino acid of the product of *in vitro* translation of satellite tobacco necrosis virus ribonucleic acid (STNV-RNA) by both a procaryotic (*Escherichia coli*) and eucaryotic (wheat embryo) system. *In vitro* translation of satellite tobacco necrosis virus RNA by the procaryotic (*Escherichia coli*) system initiates with fMet-tRNA$_f^{Met}$. Specifically, deformylation of the *in vitro* product protein followed by end-group analysis with fluorodinitrobenzene reveals DNP-Met. At 6–7 mM Mg^{2+} levels, extracts from *Escherichia coli* deprived of formyl donors by the action of trimethoprim require formyltetrahydrofolic acid for translation of the RNA. The viral-RNA-dependent incorporation of [^{3}H]formate from [^{3}H]formyltetrahydrofolic acid into protein results in the preferential labeling of one tryptic fingerprint peptide. Digestion of the *in vitro* product protein with specific proteases followed by ion-exchange procedures yields *N*-formylmethionine. Similar ion-exchange analyses of the product of *in vitro* translation of STNV-RNA by the eucaryotic (wheat embryo) system fail to detect fMet in the product. In contrast, end-group analyses of the *in vitro* eucaryotic product reveal Ala as the most prevalent N-terminal amino acid. These data support the theory that the original, eucaryotic, STNV-RNA translation product has specifically lost an N-terminal methionine to yield an alanine-terminated protein.

Protein chain initiation in procaryotes (Marcker and Sanger, 1965; Adams and Cappechi, 1966; Eisenstadt and Lengyel, 1966; Horikoshi and Doi, 1968), and presumedly mitochondria and chloroplasts of higher organisms (Smith and Marcker, 1968; Galper and Darnell, 1969), utilizes the codon AUG (Thach *et al.*, 1966) as a chain-initiation signal to code for fMet-tRNA$_f^{Met}$.[1] The resultant initial product proteins of such systems, containing formylmethionine in their N termini, are then modified, if necessary, by deformylation and/or exopeptidase action, to yield the final product proteins (Takeda and Webster, 1968).

The mechanism of protein chain initiation in the cytoplasmic fraction of cells of higher organisms (eucaryotes) is not as well characterized. Current evidence indicates that a nonformylated, yet methionine-specific tRNA is uniquely involved in protein chain initiation in the cytoplasm of eucaryotic systems (Smith and Marcker, 1970; Marcus *et al.*, 1970; Housman *et al.*, 1970; Wigle and Dixon, 1970). Other evidence suggests that a deacylated, possibly methionine-specific, tRNA is involved in this initiation process (Culp *et al.*, 1970).

A role for some form of methionine-specific tRNA is central to all of these initiation schemes. Thus, the methionine codon AUG is presumedly involved. Yet protein chain initiation must require a mechanism more complex than the existence of the methionine codon sequence, AUG. Specifically, protein chain initiation must avoid "missense" initiations

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[1] Abbreviations used are: STNV, satellite tobacco necrosis virus; TYMV, turnip yellow mosaic virus; fMet, *N*-formylmethionine; fH$_4$Fol, N^{10}-formyltetrahydrofolic acid; tRNA$_f^{Met}$, methionine-specific-tRNA capable of accepting a formyl group on its methionine; Met-tRNA$_f^{Met}$, methionyl ester containing tRNA$_f^{Met}$; fMet-tRNA$_f^{Met}$, formylmethionyl containing tRNA$_f^{Met}$; tRNA$_i^{Met}$, methionine-specific initiator tRNA not capable of accepting a formyl group on its methionine; Met-tRNA$_i^{Met}$, methionyl ester containing tRNA$_i^{Met}$; FDNB, fluorodinitrobenzene.