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Fidelity of Translation of Satellite Tobacco Necrosis Virus Ribonucleic Acid in a Cell-Free *Escherichia coli* System†

R. Rice* and H. Fraenkel-Conrat

ABSTRACT: The polypeptides synthesized in an *E. coli* cell-free system under the direction of the small RNA of the satellite tobacco necrosis virus were compared with the authentic coat protein of this virus. Many of the tryptic peptides obtained from the two types of proteins moved identically upon high-resolution cation exchange column chromatography, but distinct qualitative and quantitative differences between the two peptide patterns were also evident. Gel electrophoresis in 9 M urea at pH 4.0 showed that the biosynthesized material was not homogeneous in regard to charge; some of it showed a mobility similar to that of the authentic coat protein. Gel electrophoresis in the presence of sodium dodecyl sulfate demonstrated the presence of a wide range of molecular weight species in the biosynthesized protein; the largest, representing about 5% of the total, coincided with authentic virus coat protein. It is concluded that much of the information on satellite tobacco necrosis virus RNA is translated into proper amino acid sequences in the *E. coli* system, but that only very little complete coat protein, and that probably carrying a terminal formyl–methionyl group, is synthesized.

Escherichia coli cell-free systems synthesize recognizable proteins when programmed with messengers from the small RNA bacteriophages (Stavis and August, 1970). These extracts generally respond better to exogenous messenger RNA, particularly in quantitative respects, than do incorporation systems from plant and animal sources. The genetic code, possibly including initiation (Stewart *et al.*, 1971; Housman *et al.*, 1970; Marcus *et al.*, 1970) and termination (Goldstein *et al.*, 1970) codons, seems to be identical in the animal, plant, and bacterial kingdoms. Hence, *E. coli* extracts have been used with animal and plant viral RNA messengers to investigate translation in these heterologous systems and to study the viral gene products *in vitro*.

The most direct test to establish whether accurate translation of a viral messenger occurs has been to ascertain whether the viral coat protein is synthesized *in vitro* since other gene products have not been as readily identifiable. Some studies have indicated considerable similarity between translation products and viral coat protein. For example, with the RNA from the satellite of tobacco necrosis virus (STNV)[1] (Clark *et al.*, 1965) and the similarly small top a component of alfalfa mosaic virus (van Ravenswaay Classen *et al.*, 1967), two-dimensional peptide maps have been reported as showing nearly complete coincidence of radioactive spots from the translation products and ninhydrin-positive spots from the coat proteins. In contrast, earlier studies with TMV-RNA had indicated no clear evidence for coat-related material being synthesized *in vitro* (Aach *et al.*, 1964; Schwartz, 1967). Studies with brome mosaic virus RNA (Stubbs and Kaesberg, 1967) and poliomyelitis virus RNA (Rekosh *et al.*, 1970) gave somewhat ambiguous results and the recent claim that avian myeloblastosis virus RNA directs the synthesis of viral antigens (Siegert *et al.*, 1972) must await substantiation.

In view of these widely varying results, it was our intention to reinvestigate one of the reports indicating positive results. We chose to characterize the translation products of STNV-RNA using more discriminating techniques which have become available since the original report appeared. The RNA of STNV is a convenient messenger for this kind of work in that it is small (0.4×10^6) and can code maximally for only one small protein in addition to the viral coat protein (Roy *et al.*, 1969). Since the completion of this work, an interesting study of the translation of STNV-RNA in *E. coli* and wheat embryo

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[1] Abbreviations used are: STNV, satellite tobacco necrosis virus; TMV, tobacco mosaic virus; TPCK, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone.

cell-free systems has appeared (Klein *et al.*, 1972; Lundquist *et al.*, 1972). The results of that study are compared to our results in the Discussion section.

Experimental Section

The strain of STNV used in this work was obtained with TNV on infected mung beans from Dr. M. E. Reichmann. After passing the viruses twice through single lesions on cowpea (*Vigna sinensis* (Torner) savi), the STNV corresponded to strain 1 of Kassanis by amino acid composition, including cysteine content (Rees *et al.*, 1970). The viruses were grown on leaves of french bean (*Phaseolus vulgaris* L., var Bountiful) and tobacco (*Nicotiana tobacum* L., var Xanthi or Turkish) and purified essentially as previously described (Horst *et al.*, 1971). Good yields of STNV, 3 mg/100 g of plant tissue, were often obtained in late autumn and early winter but seldom at other times of the year.

Tritiated virus was obtained from Turkish tobacco. Twelve hours after inoculation, 25 leaves were cut near the petiole with a razor blade and the cut stem was immersed in 50 μl (containing 10 μCi) of a neutralized solution of tritiated arginine or lysine (Schwarz/Mann, 7 Ci/mmol) in a petri dish. When the leaves had taken up the radioactive solution and a further 100 μl of water, they were allowed to float on water for 2 days and then incubated in a tightly closed plastic bag at 19° for 2 days. The leaves were frozen and mixed with 50 g of infected nonradioactive leaves before purification of the virus. Typical yields were about 100,000 cpm in the STNV fraction of a virus preparation.

STNV-RNA was obtained by phenol extraction of the purified virus in 0.1 M $(NH_4)_2CO_3$ (Wimmer *et al.*, 1968). The RNA migrated predominantly as a single band upon electrophoresis in 3% polyacrylamide gels, though after repeated handling it often appeared heterogeneous (Fowlks and Young, 1970). Fresh preparations were used to program the *E. coli* extracts.

Bacteriophage Qβ and the host *E. coli* M-27 (Silverman *et al.*, 1967) were obtained from Dr. R. J. Young, and the virus was purified by his method (Young and Fraenkel-Conrat, 1971). Qβ-RNA was prepared by phenol extraction of the phage. TMV-RNA was prepared by standard procedures (Fraenkel-Conrat *et al.*, 1961).

Cell-free S-30 extracts were prepared from *E. coli* strain A-19 (Gesteland, 1965) by the method of Capecchi (1966) with modification. The Tris–Mg buffer was made to be 10 mM Tris (pH 7.8)–10 mM $MgAc_2$–60 mM KCl–7 mM β-mercaptoethanol. This buffer was also used for dialysis of the extract. After dialysis, the extract was centrifuged a second time at 30,000*g* for 30 min, pipeted into 0.25-ml aliquots, frozen in a Dry Ice–acetone bath, and stored at −60°.

The extracts were preincubated at 36° for 20 or 45 min (the latter time gave lower background synthesis). To this end, the 0.25-ml aliquots were diluted to 0.29 ml and contained per milliliter: 12 μmol of $MgOAc_2$, 50 μmol of KCl, 20 μmol of Tris, pH 7.8, 5 μmol of NH_4Cl, 8 μmol of β-mercaptoethanol, 2 μmol of ATP, 0.2 μmol of GTP, 3.5 μmol of phosphoenolpyruvate, 35 μg of pyruvate kinase, and 20 nmol of each amino acid (L isomers). The reaction mixture for incorporation of radioactive amino acids usually contained per milliliter: 0.29 ml of preincubated S-30, 0.5 mg of STNV-RNA, 11 μmol of $MgAc_2$, 80 μmol of NH_4Cl, 15 μmol of KCl, 55 μmol of Tris, pH 7.8, 6 μmol of β-mercaptoethanol, 4 μmol of ATP, 0.3 μmol of GTP, 6.5 μmol of phosphoenolpyruvate, 20 μg of pyruvate kinase, 5 μCi of [^{14}C]arginine or [^{14}C]lysine (300 mCi/mmol), and 200 nmol of other nonradioactive amino acids. Incubation was at 36° for 20 min, at which time net incorporation of radioactive amino acids reached a maximum. Parallel incubations without added messenger were performed to measure background synthesis and are referred to as control incorporations.

For characterization of the polypeptides synthesized *in vitro*, 0.5-ml aliquots of reaction mixtures were used. Synthesis was terminated by addition of neutralized disodium EDTA to 25 mM and pancreatic RNase (Worthington, electrophoretically pure) to 200 μg/ml followed by a further 5 min of incubation. The sample was brought to 0.1 M in Tris, pH 7.4, and solid urea was added to about 8 M. This solution was then passed through a Sephadex G-25 column (Stubbs and Kaesberg, 1967), 1.1 × 25 cm run in a solution of fresh 5 M deionized (Burgess, 1969) urea brought to 10 mM in Tris, pH 7.4. The excluded peak of radioactivity was collected (from which aliquots in the urea were removed for sodium dodecyl sulfate gel electrophoresis) and dialyzed 36 hr against three changes of distilled water. The contents and two rinses of the dialysis bag with 0.4 ml of 50% formic acid were pooled and lyophilized twice.

For tryptic digests, tritiated STNV was degraded by inucbation at 37° for 24 hr in 67% formic acid (Miki and Knight, 1965) and added to the lyophilized polypeptides synthesized *in vitro*. This sample was then oxidized with performic acid (Hirs, 1967), lyophilized twice, and resuspended in 1.5 ml of 0.2 M NH_4HCO_3, pH 8.3 (adjusted with concentrated NH_4OH). Digestion was allowed to proceed for 18 hr at 37° after addition of 30 μg of TPCK trypsin (Worthington) at 0 hr and again after 6 hr. The digest was acidified with concentrated acetic acid and lyophilized twice. The tryptic peptides were separated on a 0.9 × 18 cm column of Aminex A-5 cation exchange resin (Bio-Rad) using a pyridine acetate elution gradient. The gradient consisted of 120 ml of pH 2.8 buffer 0.1 M in pyridine and 100 ml of pH 4.9 buffer 2 M pyridine (Gelfand and Hayashi, 1970). Fractions of 1.5 ml were collected at a flow rate of 40 ml/hr. Aliquots (0.75 ml) of column fractions were dried in scintillation vials at 100° and the residues were redissolved in 0.2 ml of NCS reagent per vial. After addition of 10 ml of toluene scintillation fluid [14.3 g of Omnifluor (New England Nuclear) per 3 kg of toluene] to each, the vials were held at room temperature for 12–24 hr and counted in a Beckman Model 1650 scintillation counter. Efficiencies of 80% for ^{14}C and 35% for ^{3}H were obtained with spillovers of 10% ^{14}C into the ^{3}H isoset and 2% ^{3}H into the variable isoset adjusted to register ^{14}C counts. The results were corrected for spillovers but not for efficiencies.

The methods for assay of messenger activity, gel electrophoresis in sodium dodecyl sulfate or urea, and gel filtration are given in the legends to the respective figures.

Results

Efficiency of STNV as Messenger in the E. coli System. As a preliminary step to characterization of the translation products of STNV-RNA, the relative messenger activity of this RNA was compared to the activities of Qβ-RNA and TMV-RNA. The comparison was prompted by the finding that extracts prepared as described by Capecchi (1966), quite variable in activity toward Qβ-RNA, often responded little or not at all to STNV-RNA. Modification of the extract preparation (as described in the Experimental Section) gave much more reproducibly active extracts with our strain of bacteria. These preparations still indicated that considerably higher concentrations of STNV-RNA than of the RNA from Qβ or TMV

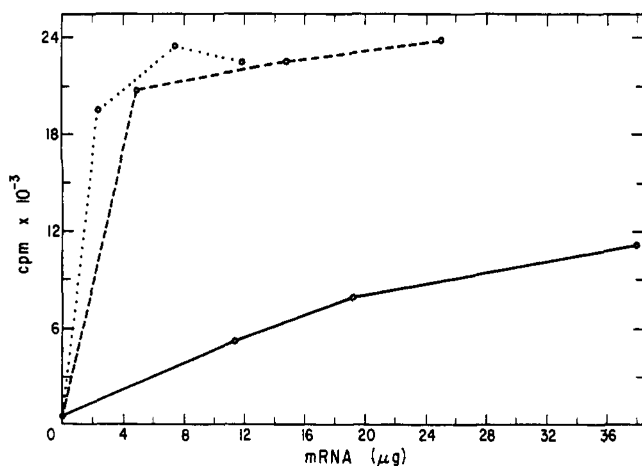

FIGURE 1: Relative messenger activities. Each tube contained 0.06 ml of preincubated reaction mixture, viral RNA as indicated, and 0.1 μCi each of [^{14}C]glycine, [^{14}C]isoleucine, and [^{14}C]valine. After incubation at 36° for 20 min, 1 ml of 0.02 N NaOH per tube was added. The tubes were held at room temperature for 15 min and then chilled in an ice bath for 15 min after adding 2 ml of 15% Cl_3CCOOH (0.1% each in ^{12}C glycine, isoleucine, and valine). The contents of the tubes were filtered with gentle suction onto GF/C disks (presoaked in 1 M NH_4Ac) and rinsed three times with cold 5% Cl_3CCOOH. The filters were glued to planchets, oven dried, and counted by gas flow counter at about 20% efficiency: solid line, STNV-RNA; dashed line, TMV-RNA; dotted line, Qβ-RNA.

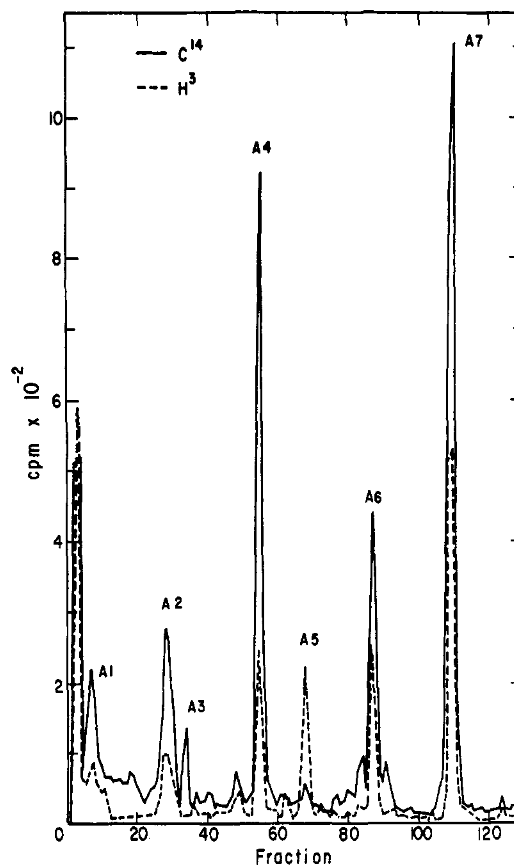

FIGURE 2: Arginine-labeled tryptic peptides from STNV protein (^{3}H) and the translation products directed by STNV-RNA (^{14}C).

were required to obtain maximal incorporation (Figure 1). This difference in activities was not significantly affected by addition of *E. coli* B unfractionated tRNA or calcium leucovorin (a formyl donor), which have been reported upon occasion to stimulate amino acid incorporation in cell-free systems (Capecchi, 1966; Klein *et al.*, 1972), nor by mild formaldehyde treatment of the RNA, as described by Lodish (1970). The magnesium ion concentration for optimal messenger activity was found to be 11 mM for all three messengers.

Comparison of Peptides Resulting from Tryptic Digestion of Translation Products of STNV-RNA with Those of STNV Coat Protein. The original report (Clark *et al.*, 1965) concerning translation of STNV-RNA in an *E. coli* cell-free system employed two-dimensional mapping of tryptic peptides, a common technique for comparing proteins in a qualitative fashion. This technique, as usually applied, is not suited for detailed quantitation, however, since relative amounts of peptides are not measured, and judgments as to similarity of patterns are open to subjective interpretation. In the present work, tryptic digests of [^{3}H]STNV protein and ^{14}C translation products were compared by high-resolution column chromatography. In order to detect as many peptides as possible while keeping the elution patterns relatively simple, arginine and lysine were used separately as the labeled amino acids.

Figure 2 gives the peptide elution pattern obtained with arginine as the labeled amino acid. Of the seven radioactive peaks designated A1 through A7, five contained both carbon and tritium label. Within the limits of detection, peak A3 contained only ^{14}C (from translation products) and peak A5 contained only ^{3}H (from coat protein of virion). Approximately 15% of the radioactivity of both isotopes applied to the column eluted in the first several tubes and was presumably contained in large peptides excluded from the 8% cross-linked resin.

The tryptic peptide pattern obtained with labeled lysine (Figure 3) gave a similar correspondence of radioactive peaks from the two sources. Of the six tritiated peaks from coat protein, four (L3, L5, L6, and L8) were accompanied by ^{14}C label. The carbon label in peak L4 is not considered significant since it was found in the same amount in digests of control incorporations (no added messenger). Peak L7 also lacked detectable carbon label from the translation products. Peak L1, which contained only carbon label, was reliably observed. Other such peaks, L2 for example, were observed occasionally but not reproducibly. The small peaks in the region of fractions 19–40 were present in digests of control incorporations and hence were not considered to arise from translation products of STNV-RNA. Of the radioactivity applied to the column, about 40% of the ^{14}C and 20% of the ^{3}H eluted in the first several tubes. Digestion of this excluded material with chymotrypsin and rechromatography gave two peaks with both ^{14}C and ^{3}H label, while two-thirds of the radioactivity was again excluded (data not shown, Rice, 1972).

The peptide elution patterns observed with arginine or lysine label, which should allow detection of all tryptic peptides except C-terminal peptides, indicate considerable similarity between the viral coat protein and the translation products of its RNA. Of the 12 peaks detected from coat protein, nine were accompanied by peaks from the translation products. This result is consistent with the original observation (Clark *et al.*, 1965) that two-dimensional peptide maps of material from the two sources were very similar and supports the idea that this RNA codes for STNV protein *in vivo*.

However, three features of the peptide patterns here presented suggest limitations to the fidelity of translation. First, in the nine matching peaks, the two isotopes were present in different ratios, indicating that the peptides may not have been

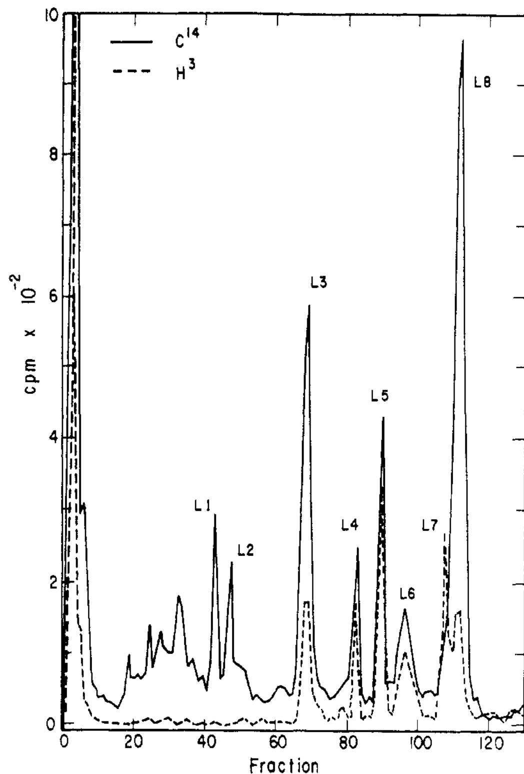

FIGURE 3: Lysine-labeled tryptic peptides from STNV protein (^{3}H) and the translation products directed by STNV-RNA (^{14}C).

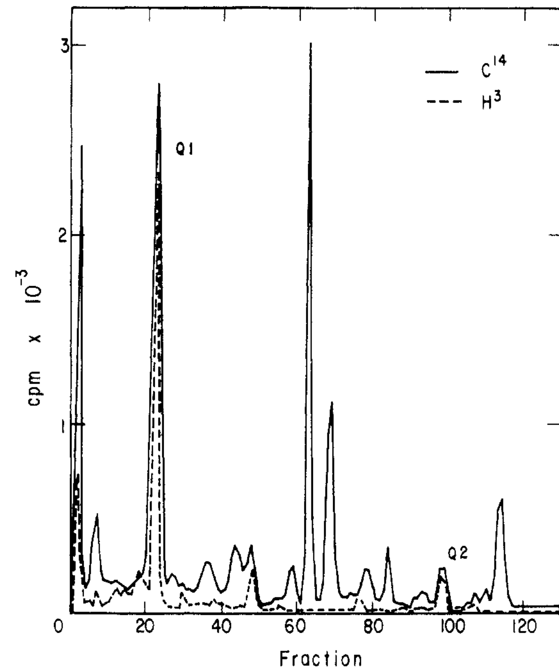

FIGURE 4: Tryptic peptides from translation products directed by Qβ-RNA. The polypeptides were synthesized in a volume of 0.26 ml containing 75 μg of Qβ-RNA, 2 μCi of [^{3}H]methionine (2.6 Ci/mmol) and 0.8 μCi of [^{14}C]lysine. Other reagents were in the usual concentrations except that nonlabeled methionine was added only during preincubation. The sample contained no Qβ coat protein added before tryptic digestion but in other respects was treated identically with samples directed by STNV-RNA.

synthesized in the same quantities as expected for complete STNV protein. Second, three peptides from the coat protein were not detectably synthesized *in vitro*. If complete coat protein were synthesized, only one such tritiated peptide, that from the N terminus of the viral coat protein, might be expected not to correspond to a carbon labeled one, because it would lack the fMet initiation group of the biosynthesized polypeptides (Lucas-Lenard and Lipmann, 1971). Third, two small ^{14}C-labeled peaks (A3 and L1) were reliably observed in which detectable ^{3}H label was absent.

Peak L1 did not seem to correspond to the formylated peptide fMet-Ala-Lys which might be expected to result from initiation of STNV coat protein in proper phase *in vitro*. The mild acid treatment employed for removal of N-terminal formyl groups (0.5 N HCl, 90°, 20 min; Osborn *et al.*, 1970) had no effect on the elution position of the material in this peak from our columns. In contrast, the methionine and lysine containing tryptic peptide obtained in good yield from the translation products of Qβ-RNA (peak Q1 in Figure 4), and presumed to be the N-terminal peptide fMet-Ala-Lys of the Qβ coat protein (Osborn *et al.*, 1970; Konigsberg *et al.*, 1970), eluted 150 ml later (coincident with peak Q2 in Figure 4) after this mild acid treatment. Arginine was noted to elute about 100 ml after *N*-acetylarginine, illustrating the effect of removal of an acyl group on the elution position of a small peptide.

Molecular Weight and Charge Properties of Translation Products. Comparison of the molecular weight distribution of the translation products of STNV-RNA with that of the viral coat protein by sodium dodecyl sulfate gel electrophoresis showed that most of the translation products migrated faster than the marker coat protein and hence were of lower molecular weight (Figure 5). The electrophoretic pattern generally showed a predominant broad peak of radioactivity with mobility corresponding to about 17,500 and two smaller peaks of slightly higher molecular weight. One of these two peaks, the highest in molecular weight observed on the gels, usually containing about 5% of the radioactivity in the sample, coelectrophoresed with marker STNV protein.

The electrophoretic patterns also showed a considerable percentage of the translation products to migrate in the range of 12,000–15,000 which consisted of several closely spaced peaks. The relative amounts of this material varied from extract to extract. It was often the major feature of the observed pattern with extracts prepared as described by Capecchi (1966).

The molecular weight distribution was not appreciably changed by variations in the magnesium ion concentration between 9 and 14 mM in the incorporation mixture. Also, the pattern was similar after 12- or 20-min incubations and was not altered by dialysis against water before addition of sodium dodecyl sulfate to the samples obtained by G-25 gel filtration (see Experimental Section). Moreover, the distribution given by the polypeptides released from ribosomes and thus remaining at the top of a sucrose gradient was quite similar to that of the unfractionated translation products. This last result suggests that the heterogeneity of the molecular weight distribution was probably not due to nascent polypeptide chains, although premature release by the ribosomes was not ruled out.

The molecular weight distribution of polypeptides synthesized *in vitro* was also examined by Sephadex G-200 gel filtration in 6 M guanidine hydrochloride (Figure 6). The results support those obtained by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. In this type of experiment, the translation products eluted in two broad peaks corresponding in molecular weights to 20–16,000 and 16–14,000 by com-

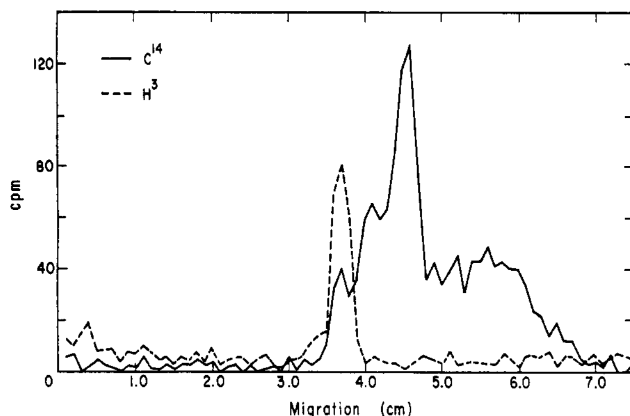

FIGURE 5: Sodium dodecyl sulfate acrylamide gel electrophoresis of translation products directed by STNV-RNA. After isolation by Sephadex G-25 gel filtration (see Experimental Section), an aliquot of [^{14}C]lysine-labeled translation products in 5 M urea and marker ^{3}H-labeled STNV were brought to 1% in sodium dodecyl sulfate, 1% in β-mercaptoethanol, and 10 mM in sodium phosphate, pH 7.2, and incubated for 2 min in a boiling water bath. Conditions of electrophoresis on 10% gels were as described by Weber and Osborn (1969). Sample volumes of 50 μl were electrophoresed for 12 hr at a current of 3 mA per gel. The gels were frozen on Dry Ice, fractionated transversely into 1-mm slices, incubated at 50° for 4 hr with 0.7 ml of 90% NCS (Zaitlin and Hariharasubramanian, 1971), and counted under double label conditions as described for tryptic peptides. Molecular weights were estimated from mobilities of marker proteins run with STNV protein on parallel gels on which the bands were detected by staining with Coomassie Blue.

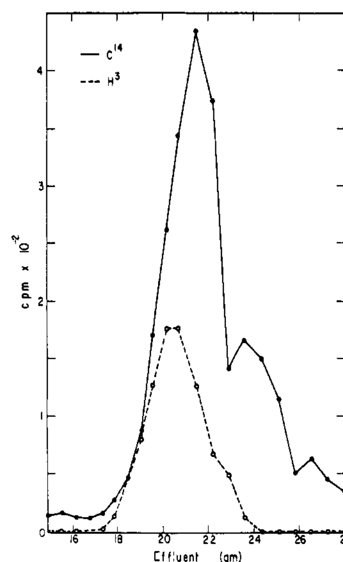

FIGURE 6: Sephadex G-200 gel filtration of STNV-RNA directed translation products in 6 M guanidine hydrochloride. [^{3}H]Lysine-labeled STNV was added to lyophilized ^{14}C-labeled lysine polypeptides synthesized *in vitro* and the mixture was dissolved and incubated for 3 hr at 37° in 0.6 ml of 6 M guanidine hydrochloride (recrystallized or Mann "Ultra Pure") which was 20 mM in Tris, pH 7.8, and 45 mM in dithiothreitol. The sample was made 10% in sucrose before application to the column, 1.1 × 30 cm, which was equilibrated and run in 6 M guanidine hydrochloride brought to 0.1% in β-mercaptoethanol. Fractions of about 0.6 g were collected in preweighed tubes at a flow rate of 1–1.5 g/hr. Aliquots of 0.05 ml were diluted with 1 ml of water and 10 ml of Aquasol (New England Nuclear) and counted under double label conditions as described for tryptic peptides.

parison with marker proteins run separately. An internal STNV protein marker (as in Figure 6) would be expected to elute with the translation products of approximately the same molecular weight. Thus, the gel filtration served as a means of isolating these polypeptides synthesized *in vitro* for further study.

Gel filtration in 8 M urea at pH 7.0 in the absence of salt did not appear as suitable as in guanidine hydrochloride in the above type of experiment. In preliminary runs, performic acid oxidized STNV protein, though soluble, eluted in the void volume of Sephadex G-200, indicating that it was aggregated under these conditions. About half the translation products, treated identically, eluted with the coat protein. This result indicates some similarity in the aggregating tendency of STNV protein and the translation products, despite the fact that only a small percentage of the latter were of the same molecular weight as the coat protein.

The translation products isolated by gel filtration in 6 M guanidine hydrochloride that eluted with marker STNV protein were examined for electrophoretic heterogeneity by polyacrylamide gel electrophoresis at pH 4.0 in 9 M urea. Under these conditions, marker STNV protein migrated as a sharp band while the translation products migrated slower than the coat protein in twice as broad a band consisting of two charge species (Figure 7). Aliquots of three consecutive column fractions were electrophoresed to determine which of these two charge species had a higher molecular weight. As seen in Figure 7, the species with faster electrophoretic mobility was dominant in the column fraction corresponding to higher molecular weight. Hence, this may be the species which coelectrophoresed with STNV protein on sodium dodecyl sulfate gels.

In these electrophoresis runs, STNV protein migrated about 4% faster than the higher molecular weight polypeptide species synthesized *in vitro*. Since STNV protein has about 20 basic amino acids and five histidines which would be positively charged at pH 4.0, this difference in mobility may correspond to a net difference of one positive charge, if one assumes that mobility of proteins under these conditions is proportional to net charge (Strauss and Kaesberg, 1970) and that carboxyl groups do not contribute much to the charge at pH 4.0.[2] If STNV protein were synthesized *in vitro*, it would presumably be formylated and thus match in electrophoretic mobility the faster migrating translation product. In view of the considerable similarity in tryptic peptide elution patterns between STNV coat protein and the polypeptides synthesized *in vitro*, it does not seem unlikely that a small amount of formylated STNV-like protein may actually have been synthesized and represents the material in the largest component shown in Figure 5 and the most charged in Figure 7.

Discussion

The results of the gel electrophoresis and filtration studies indicate that in an *E. coli* cell-free system directed by STNV-RNA only a small fraction of the translation products resembled the STNV coat protein in molecular weight, while the bulk of the material was of various smaller sizes. The tryptic peptide column elution patterns of the translation products, on the other hand, were similar to those of the viral coat protein, although certain differences were noted.

[2] This seemed to be a good approximation since STNV protein in which the sulfhydryl group was aminoethylated (Raftery and Cole, 1966) migrated 1–2 mm farther than unmodified STNV protein under these conditions.

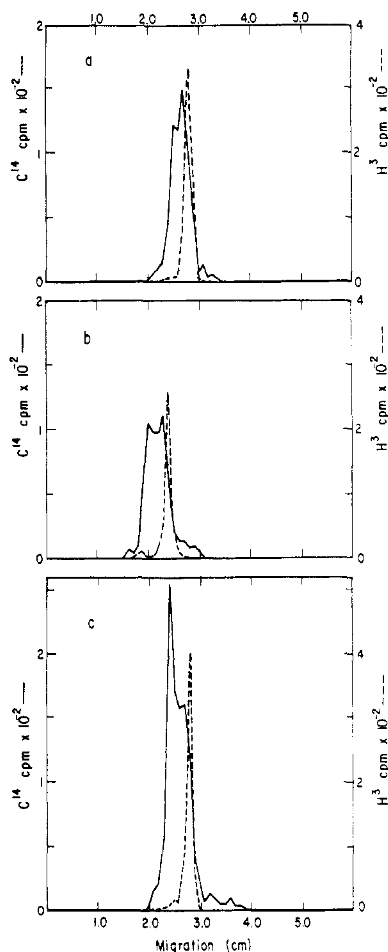

FIGURE 7: Heterogeneity of STNV-RNA directed translation products as observed by polyacrylamide gel electrophoresis in 9 M urea at pH 4.0. Electrophoresis was performed in 7.5% gels 6 cm in length cast from a solution which contained the following ingredients and which was brought to a final volume of 16 ml with fresh deionized 10 M urea: 1.2 g of acrylamide (Eastman, electrophoresis grade), 32 mg of bisacrylamide (recrystallized, a gift from Dr. R. J. Young), 0.8 ml of potassium acetate buffer (7.5 M HAc adjusted with solid KOH to pH 4.0 at a 1:20 dilution), 10 μl of *N,N,N',N'*-tetramethylethylenediamine (TEMED), and 0.16 ml of fresh ammonium persulfate (100 mg/ml). The gels were allowed to polymerize at 37° for at least 1 hr. The running buffer (pH 4.0) consisted of 8 ml of glacial HAc and 8 g of β-alanine per liter of distilled water. Samples were prepared by overnight dialysis of aliquots of the column fractions obtained by G-200 gel filtration in guanidine hydrochloride against fresh deionized 9 M urea which was 0.3% in formic acid (pH 4). Sample volumes were 50 μl and were electrophoresed for 2 hr toward the cathode with a current of 3 mA per gel. The samples electrophoresed in the figure were taken from consecutive fractions of the G-200 gel filtration (see Figure 6) eluting in order of descending molecular weights at: (a) 19.6 g, (b) 20.2 g, and (c) 20.7 g. The locations of the peaks in a and c differ slightly from those in b because the latter fraction was electrophoresed in a separate run. The gels were frozen, treated with NCS, and counted as described for sodium dodecyl sulfate gels.

These conclusions differ somewhat from those arrived at by Klein *et al.* (1972), concerning translation of STNV-RNA in *E. coli* and wheat embryo cell-free systems. On the basis of gel electrophoresis in sodium dodecyl sulfate and gel filtration in 70% formic acid, the authors suggested that the products in both systems were homogeneous and slightly smaller than STNV protein in molecular weight. The differences in the conclusions of the two laboratories might be attributable to the fact that the 10% acrylamide gels used in this laboratory offered better resolution than the presumably 5% gels used by Klein *et al.* (1972). The failure of these authors to detect the small percentage of the products showing the actual molecular weight of STNV coat protein may also be attributable to the lower resolving power of their gel procedure. The translation of STNV-RNA in the wheat embryo system might merit additional study bearing this possibility in mind.

The tryptic peptide patterns obtained by high-resolution column chromatography in the present investigation were consistent with the heterogeneity of the translation products observed by gel electrophoresis. The peptide peaks seemed to be present in widely varying amounts relative to the corresponding peaks from the coat protein. The significance of the peptides from the translation products which did not match peptides from the STNV protein is not clear. They may signify some mistranslation. More mistranslation may not be apparent because of the probably frequent occurrence of termination codons in out-of-phase translation and loss of the resulting small peptides during G-25 gel filtration.

Nonmatching peptides have been observed also in earlier reports concerning translation of RNA from brome mosaic virus (Stubbs and Kaesberg, 1967) and to a lesser extent with alfalfa mosaic virus (van Ravenswaay Claasen *et al.*, 1967) and STNV (Clark *et al.*, 1965). The assertion of Klein *et al.* (1972) that STNV-RNA serves as a monocistronic messenger for STNV protein *in vitro* is based upon two-dimensional mapping of tryptic peptides. The essentially complete coincidence of spots from viral coat protein and polypeptides synthesized *in vitro* which has been reported must be viewed with caution due to the qualitative nature of that method.

The heterogeneity of the translation products observed by us may result from the action of degradative enzymes present in *E. coli* extracts. Initiation of polypeptide synthesis at several sites on the RNA, possibly but not necessarily due to fragmentation of the messenger *in vitro*, could also account for the molecular weight heterogeneity. Yet *Qβ* coat protein was found to be formed with relatively little size heterogeneity under the same conditions as used for STNV-RNA translation (Rice, 1972; Jockusch *et al.*, 1970; Horiuchi *et al.*, 1971). This result suggests that either the translation of STNV-RNA was particularly susceptible to degradative enzyme activities or that *E. coli* ribosomes may have had difficulty recognizing the proper initiation site(s) on STNV-RNA. The relatively low messenger activity of STNV-RNA compared to Qβ-RNA may be a reflection of either possibility. (Low messenger activity has also been reported for the RNA from avian myeloblastosis virus (Siegert *et al.*, 1972) though in that instance the translation products were similar in molecular weight distribution to capsid proteins.) The relative homogeneity of the *Qβ* coat protein synthesized *in vitro* also indicates that exhaustion of components in the incorporation mixtures did not represent the explanation for the lower efficacy of STNV-RNA in our procedures of polypeptide synthesis.

Recently, Lundquist *et al.* (1972) have reported that the translation products of STNV-RNA were initiated with fMet in an *E. coli* system and that tryptic digests of these products contained one predominant formylated peptide. This peptide, they asserted, coincided with a marker fMet-Ala-Lys peptide by fingerprint analysis, corresponding *in vitro* to a hypothetically correct initiation of STNV protein *in vivo*. In the present work, however, clear and reproducible evidence for a lysine containing formylated peptide was not obtained, though it may have been present in small amount, while such a peptide was evident in translation products directed by Qβ-RNA. The reasons for this conflict remain to be elucidated. Our results

appear similar to those obtained with polio virus RNA (Rekosh *et al*,, 1970), which showed translation products and capsid proteins to differ in molecular weight distribution, while tryptic peptides from *E. coli* translation products generally matched those from viral polypeptides synthesized in animal cells. Evidence for at least eight initiation sites on the RNA was presented, although *in vivo* there seems to be only one site (Jacobson *et al.*, 1970). Initiation at a number of sites on STNV-RNA would be consistent with the results obtained in this work, though the finding of Lundquist *et al.* (1972) remains unaccounted for.

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