[18] Goldman, P., A. W. Alberts, and P. R. Vagelos, *Biochem. Biophys. Research Commun.*, **5**, 280 (1961).
[19] Hofmann, K., R. A. Lucas, and S. M. Sax, *J. Biol. Chem.*, **195**, 473 (1952).
[20] Kaneshiro, T., and A. G. Marr, *J. Biol. Chem.*, **236**, 2615 (1961).
[21] O'Leary, W. M., *J. Bacteriol.*, **77**, 367 (1959).
[22] Liu, T. Y., and K. Hofmann, *Biochemistry*, **1**, 189 (1962).
[23] Lennarz, W. J., G. Scheuerbrandt, and K. Bloch, *J. Biol. Chem.*, **237**, 664 (1962).
[24] A. T. Norris, unpublished.
[25] Baronowsky, P. E., W. J. Lennarz, and K. Bloch, *Federation Proc.*, **21**, 288 (1962).

---

*DEMONSTRATION OF THE MESSENGER ROLE OF VIRAL RNA**

BY A. TSUGITA,† H. FRAENKEL-CONRAT, M. W. NIRENBERG, AND J. H. MATTHAEI‡

VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY, AND NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, BETHESDA

Communicated by W. M. Stanley, March 30, 1962

Recently, it has been demonstrated that the amino acid incorporation in cell-free *E. coli* extracts which is dependent upon the presence of sRNA, transfer enzyme, GTP, and ribosomes is greatly stimulated by the addition of macromolecular RNA from various sources, including TMV.[1, 2] That such added RNA served as messenger or template was strongly suggested by the finding that only phenylalanine was polymerized if polyuridylic acid (polyU) was added,[1] whereas different nucleotide copolymers directed many amino acids into protein with distinct specificity.[3, 4] It thus seemed possible that any protein could be synthesized by this system if the correct informational RNA were added. Since TMV-RNA stimulated amino acid incorporation in this system, we compared the nature of the protein so synthesized with authentic TMV-protein. The purpose of this communication is to report the results of such experiments.

Methods and Materials.—Preparation of the *E. coli* enzyme extracts corresponding to "preincubated S-30" fractions have been described, as well as the other components of reaction mixtures, methods of washing precipitated protein and plating and counting radioactive samples.[1]

Uniformly labeled C^{14}-L-phenylalanine having a specific radioactivity of either 5–10 or 200–250 mC/mM was obtained from Nuclear-Chicago Corporation and New England Nuclear Company, respectively. C^{14}-L-tyrosine having a specific radioactivity of 200 mC/mM was obtained from New England Nuclear Company. The C^{14}-algal protein hydrolysate had a specific radioactivity of 12.5 mC/m atom of carbon and was prepared from *Chlorella* grown upon C^{14}-$Ba(CO_3)$.[2] Yeast RNA was obtained by the method of Crestfield *et al.*[5]

Results.—A. *Incorporation studies:* The dependence of C^{14}-valine incorporation into protein upon TMV-RNA is shown in Figure 1. Incorporation increased roughly in proportion to the amount of TMV-RNA added from very low values to 75-fold when 0.2 mg RNA was present in the 0.25 ml of reaction mixture.

C^{14}-valine incorporation in the presence and absence of TMV-RNA is plotted against time in Figure 2. As before, little C^{14}-valine incorporation into protein occurred in the absence of TMV-RNA. In the presence of 50 μg of TMV-RNA per 0.25 ml reaction mixture, C^{14}-valine incorporation was linear between 5 and 30 min. These results show that viral RNA stimulates amino acid incorporation as does *E. coli* RNA and synthetic polynucleotides.

The proportion of C^{14}-valine incorporated into ribosomal-bound protein as compared to incorporation into soluble protein in the presence and absence of TMV-RNA was determined after

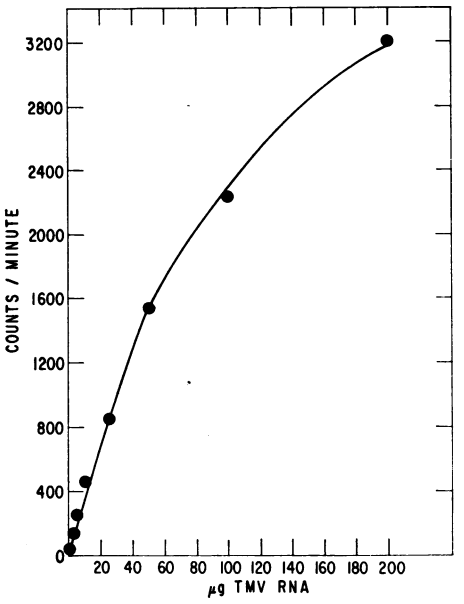

FIG. 1.—Stimulation of C^{14}-L-valine incorporation into protein by TMV-RNA. 0.25 ml reaction mixtures contained described components (see legend to Table 1, also ref. 1) and were incubated at 37° for 90 min before deproteinization.

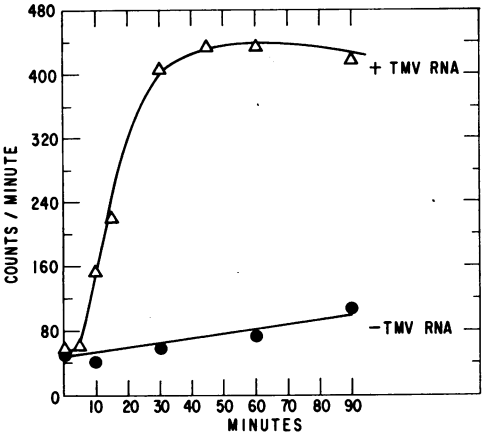

FIG. 2.—C^{14}-L-valine incorporation into protein, plotted as a function of time. Δ + 50 μg TMV-RNA. 0.25 ml reaction mixtures containing described components (legend to Table 1) were incubated at 37° for 90 min before deproteinization.

centrifugal separation of the particles (Table 1). Of the C^{14}-valine incorporated into protein under the influence of TMV-RNA, 55 per cent was found in the soluble protein.

Table 2 illustrates the marked inhibitory effect of puromycin, a known inhibitor of cellular and cell-free protein synthesis upon C^{14}-amino acid incorporation into soluble protein as directed by viral RNA.

Both methionine and histidine are absent from wild-type TMV protein but are present in the HR strain. It therefore was of interest to determine whether the RNA of wild-type TMV-RNA differed from HR-RNA in relative ability to stimulate the incorporation of either of these amino acids. The results of Table 3 demonstrate that both TMV-RNA and HR-RNA stimulate the incorporation of both C^{14}-methionine and C^{14}-histidine to a similar extent.

B. *Identification of products:* To test the working hypothesis that the C^{14}-labeled protein synthesized under the influence of TMV-RNA would resemble TMV-protein, native unlabeled TMV-protein was added to the incubated and centrifuged reaction mixture. The protein was then reisolated, and an attempt was made to ascertain by various methods whether it carried the radioactive label in a manner sufficiently constant, specific, and stable to justify the conclusion

TABLE 1

C^{14}-VALINE INCORPORATION INTO SOLUBLE AND RIBOSOMAL BOUND PROTEIN

Protein fraction	Addition	Counts/min
Soluble	None	85
	+ 0.5 mg TMV-RNA	881
Ribosomal bound	None	60
	+ 0.5 mg TMV-RNA	740

1.0 ml reaction mixtures containing 0.025 μmole each of 19 L-amino acids minus valine, 1.8 mg of preincubated S-30 protein, 0.02 μmole of C^{14}-L-valine (98,000 cpm), in addition to previously described components,[1] were incubated at 37° for 90 min. Each reaction mxture was then diluted with 9.0 ml of 0.01 *M* Tris, pH 7.8, 0.01 *M* magnesium acetate, 0.05 *M* KCl, and 6×10^{-3} *M* mercaptoethanol and was centrifuged at 100,000 × *g* for 2 hr at 2°. Supernatant solutions were decanted and the protein precipitated by the addition of trichloracetic acid. Ribosomal pellets were resuspended in the above buffer and were also precipitated with trichloracetic acid. The washed precipitates were plated and counted.[1]

TABLE 2

EFFECT OF TMV-RNA UPON C^{14}-AMINO ACID INCORPORATION INTO PROTEIN

Experiment no.	C^{14}-isotope	Addition	Counts/min/mg soluble protein	Counts/min/mg soluble protein minus puromycin control
1	C^{14}-protein hydrolysate	None	1,872	601
		+0.5 mg TMV-RNA	10,905	9,634
		+0.5 mg TMV-RNA + 0.015 μmole puromycin	1,271	—
2	C^{14}-L-phenylalanine	None	150	57
		+0.5 mg TMV-RNA	1,604	1,511
		+0.5 mg TMV-RNA + 0.015 μmole puromycin	93	—

In Experiment 1, each 0.5 ml reaction mixture contained approximately 12.5 μg. C^{14}-algal protein hydrolysate (~500,000 cpm); 0.006 μmole each of 20 C^{12}-amino acids and 0.84 mg of preincubated S-30 enzyme protein as well as described components.[1] In experiment 2, each 0.25 ml reaction mixture contained 0.02 μmole C^{14}-L-phenylalanine (~110,000 cpm), 0.025 μmole each of 19 C^{12}-amino acids minus phenylalanine, and 0.84 mg of preincubated S-30 enzyme protein as well as described components.[1] After incubating reaction mixtures at 37° for 90 min, samples were chilled and ribosomes were removed as described under Table 1. The figures in the table represent counts/min of C^{14}-amino acids incorporated into *soluble* protein, as counted after trichloracetic acid precipitation and washing.

TABLE 3

EFFECT OF WILD-TYPE TMV AND HR-MUTANT RNA UPON AMINO ACID INCORPORATION

C^{14}-amino acid	Addition	Counts/min
C^{14}-Valine	None	40
	+50 μg TMV-RNA	1,210
	+50 μg HR-RNA	1,430
C^{14}-Methionine	None	220
	+50 μg TMV-RNA	902
	+50 μg HR-RNA	674
C^{14}-Histidine	None	28
	+50 μg TMV-RNA	120
	+50 μg HR-RNA	111

0.25 ml reaction mixtures contained 0.02 μmole of the appropriate C^{14}-amino acid, 0.025 μmole of each of 19 L-amino acids minus the C^{14}-amino acid, 0.84 mg of preincubated S-30 protein and previously described components.[1] Reaction mixtures were incubated at 37° for 60 min and then were precipitated and counted as usual.

that the added viral protein acted as carrier for similar or identical material synthesized under the influence of viral RNA.

The methods used in reisolation and characterization of the protein were (1) precipitation of the protein by anti-TMV serum, (2) aggregation and sedimentation of the protein at pH 4.6, (3) fractionation of the protein on DEAE cellulose columns, (4) reconstitution of the protein with added TMV-RNA to form typically infectious and sedimentable virus rods, (5) degradation of the protein by trypsin and identification of specific peptides. Methods 2 and 3 which serve for the purification of the viral protein were usually used prior to applying the more selective tests #4 and 5.

(1) *Precipitation of the protein with anti-TMV serum:* The diluted and centrifuged amino acid incorporation mixtures were treated with TMV-protein and anti-TMV serum as described in Table 4. The counts obtained after the extensive washing procedure indicated that an appreciable fraction of the label incorporated into protein under the direction of TMV-RNA was precipitated by anti-TMV serum, but much less in the control without added RNA, and still less in the presence of puromycin. When amino acid incorporation was stimulated to a similar extent with yeast RNA and then TMV-protein and antiserum were added, the TMV-antibody precipitate contained only about one third of the radioactivity, compared to reaction mixtures containing TMV-RNA.

(2) *Isoelectric protein aggregation:* The reaction mixtures containing about 2–10 mg of added TMV-protein per ml became opalescent upon addition of acetate (to pH 4.6, 0.1 *M*), and a sediment obtained upon ultracentrifugation could largely be redissolved in water at about pH 8, clarified by low-speed centrifugation, and again sedimented at pH 4.6. The redissolved material showed in different experiments UV absorption spectra ranging from being qualitatively and quantitatively typical of the added TMV-protein to indicating the presence of excessive amounts of a nucleoprotein. The total and the specific radioactivity of this material was usually somewhat higher in the experimental sample (containing TMV-RNA in the incubation mixture) than in the

TABLE 4

REACTION OF SYNTHESIZED C^{14}-PROTEIN WITH TMV-ANTISERUM*

Additions	Counts/min	Counts/min minus puromycin†	Counts/min in antibody-antigen precipitate‡
None	1,870	530	99
+0.5 mg TMV-RNA	10,900	9,560	2,250
+0.5 mg TMV-RNA + puromycin	1,340	0	71
+0.5 mg yeast RNA	13,250	11,910	782

Reaction mixtures are described under Table 2, experiment 1. After a 90 min incubation at 37°, ribosomes were removed as described under Table 1.

* Kindly given us by C. A. Knight.

† 3.0 ml of reaction mixture precipitated with trichloracetic acid, washed, and plated as usual.

‡ To 3.0 ml of 100,000 × *g* supernatant solution were added 2.0 ml of buffer solution, 0.5 mg purified TMV-protein, and 0.5 ml rabbit TMV-antiserum. Samples were incubated at 37° for 60 min, and precipitates were centrifuged at 10,000 × *g* for 15 min at 2°. Supernatant solutions were discarded and pellets were resuspended in 5 ml of 0.9 per cent NaCl. Precipitates were washed in this manner 3 additional times. After the last wash, protein was denatured by the addition of trichloracetic acid, and the precipitated protein was washed and plated as has been described.[1]

controls, but there was enough radioactivity also in the latter to illustrate that this method was not very selective in isolating only TMV-protein. However, this method served well to remove any free C^{14} amino acids, for it was observed that the specific radioactivity (C^{14}-phenylalanine) in such material was decreased by no more than 50 per cent by extensive dialysis against water, 0.05 *M* phenylalanine (pH 9) or 67 per cent acetic acid. In different experiments, a total of 50,000–300,000 cpm were recovered in this macromolecular fraction.

(3) *Column fractionation of TMV-protein:* A chromatographic system was developed for the separation of native TMV-protein from other reaction products. A DEAE cellulose column (0.9 × 20 cm) was used at 5°, with a gradient of 2×10^{-3} to 1 *M* sodium acetate (pH 7.3). Besides the main protein peak emerging after about 220 ml, a small peak appeared frequently at 310 ml which is tentatively attributed to partly denatured material. In some experiments, a good part of the added protein (up to 60 per cent) did not chromatograph but could only be recovered by means of 0.1 *N* alkali, and this is regarded as completely denatured material. The recovery of native protein seemed to depend on the amount of RNA contaminating the applied sample. Recovery of control protein was close to 100 per cent. Even if the UV spectrum of the applied sample was that of a nucleoprotein, the material appearing in the early peaks showed a typical protein spectrum indistinguishable from that of TMV-protein. Recovery of radioactivity in the main protein peak ranged from 0.5–10 per cent of that applied to the column in the various experiments, and its specific radioactivity has ranged from 20 to 200 cpm/mg.

(4) *Reconstitution:* When a solution of 0.1 per cent TMV-protein and 0.005 per cent TMV-RNA in 0.1 *M* pH 7.3 pyrophosphate is held for 4–6 hr at 30°, about half of the two viral components usually combine to form stable, sedimentable, and infectious TMV rods.[6] TMV-protein reisolated from control reaction mixtures, which had been incubated either with *E. coli* RNA or without added RNA, behaved normally in this respect (30–40 per cent yield of reconstituted virus after two cycles of differential centrifugation). In contrast, the protein reisolated from TMV-RNA directed reaction mixtures reconstituted to only about one tenth of that extent. The radioactivity in such pellets was 10–20 cpm/mg, about 15 per cent of that in the protein, but, owing to the low yields in countable material (usually less than 1 mg), the actual counts were as low as 10 per cent above background, and the repeated centrifugal cycling performed to establish the stability and constancy of these counts caused further loss of material and thus of net, though not of specific counts. In contrast, the reconstitutions with protein from control reaction mixtures presented no technical problem and showed only 0.6 to 2 cpm/mg after the same number of cycles of differential centrifugation.

(5) *Location of C^{14}-phenylalanine and C^{14}-tyrosine on tryptic peptides from TMV-protein:* The amino acid sequence of the TMV-protein being known,[7] it should be possible to establish beyond doubt the relationship of the C^{14}-labeled material to the carrier protein by the methods of protein degradation and peptide analysis. TMV-protein is degraded by trypsin into 12 peptides of well established chromatographic properties and of known amino acid composition. Thus, when phenylalanine was the only radioactive amino acid used for incorporation, only the phenylalanine containing peptides in the tryptic digest should be labeled, in proportion to their phenylalanine content, if the radioactivity of the product was entirely due to newly synthesized TMV-protein.

On the other hand, with tyrosine as label, only certain other peptides should carry the radioactivity.

To ascertain the chemical nature of the incorporated label in this manner, the TMV-protein reisolated, as described above, by isoelectric precipitations and chromatography was digested with trypsin. The insoluble peptide (#1)[8] was removed by centrifugation of the digests adjusted to pH 4.6. The soluble peptides were separated by chromatography on Dowex 1 × 2,[9] and each peptide was subjected to amino acid analysis and radioactivity counting. Data obtained by this method are summarized in Table 5. The small amounts of labeled material available for peptide fractionation and the seeming presence of non-TMV proteins have interfered with the isolation of all peptides in pure form, but the basic peptides emerging early from the column have presented no technical problems. It is evident that of these peptides only peptide #3 is appreciably labeled by the phenylalanine marker, while with tyrosine as marker this peptide is almost free from count, and its neighboring peaks (peptide #5 and #11) carry the label, in accord with the composition of the TMV peptides chromatographing in this position. Altogether 78 and 50 per cent, respectively, of the radioactivity in the applied samples were found associated with the 4 and 3 soluble TMV peptides containing phenylalanine and tyrosine. The distribution of the rest of the radioactivity over other fractions and particularly its association with several minor peaks which could not be identified with any TMV peptides indicate that some labeled protein is present in the isolated material which bears no clear relationship to TMV-protein.[10] *Note added in proof:* Upon separation of peptides from control digests (carrier protein from other than TMV-RNA directed experiments), only 0.1 to 0.5 per cent of the applied count appeared in peptides #2, 3, 5, 7, and 11.

One of the 4 tyrosines of the TMV-protein occurs in the insoluble peptide #1, from which it can be split off by chymotrypsin in form of the singular nonamphoteric peptide N-acetyl-seryl tyrosine which marks the N-terminus of the virus protein.[11] With C^{14}-tyrosine as the marker, this peptide, surprisingly, contained almost no label. Oddly enough, from the presumably denatured protein eluted from the same DEAE column with alkali, radioactive N-acetyl-seryl tyrosine was obtained by the same procedure (60 cpm).[10]

Discussion and Conclusions.—The thesis under study is that TMV-protein is synthesized *in vitro* by an *E. coli* enzyme system subjected to the influence of purified TMV-RNA. The first indication that this occurred was obtained in tests with an anti-TMV serum performed by one of us (M.W.N.) in May 1961. However, some nonspecific precipitation of radioactivity from the control reaction mixtures showed that the system was complex and more refined tests had to be applied before the above thesis could be regarded as established.

The results of the reconstitution experiments suggest that at most about 10 per cent of the protein synthesized by the *E. coli* system *in vitro* is identical with TMV-protein. Yet, the observation of a strong interference with the reconstitution reaction exerted by the reaction product, seemingly a negative result, appears to carry strong positive implications. Since this interfering action was shown only by protein isolated from TMV-RNA directed reaction mixtures, it certainly appears to represent a specific effect. If one assumed that the synthesis of the TMV-protein proceeded to a point, short of completion, which did not enable it to form its proper 3-dimensional structure, one could well envisage stages which would actively interfere with the reconstitution of the added authentic protein. Thus, one may compare the protein aggregation leading to rod formation with an antigen-antibody system, and may then regard the interfering material as a monovalent antigen. Obviously, a tiny amount of such material would, through premature termination, effectively interfere with the ordered aggregation of the 2,200 subunits required for complete rod formation.

We must now consider the results of the peptide analyses after tryptic digestion

TABLE 5

AMINO ACID COMPOSITION AND RADIOACTIVITY OF PEPTIDES OBTAINED BY TRYPSIN FROM C^{14}-PHENYLALANINE-LABELED TMV-PROTEIN*

Peak no.	Amino acid composition†	Sequential peptide no.	Recovery‡ of: Peptide (μM)	Recovery‡ of: Amino acid μM	Total, cpm
1	$Thr_{0.9}$,$Glu_{1.0}$,$Val_{1.0}$(Val)(Arg)	2	3.1	9	8
2	$Asp_{0.3}$,$Thr_{0.1}$,$Ser_{0.1}$,$Glu_{0.2}$,$Val_{0.3}$,*Phe*$_{0.1}$(Arg)	—	—	0.6	20
3	$Asp_{1.0}$(Arg)	7	2.2	2.2	8
4	$Thr_{0.7}$,$Ser_{1.4}$,$Glu_{3.0}$,$Pro_{2.1}$,$Val_{2.7}$,*Phe*$_{1.0}$(Try,Lys,Arg)	3	2.9	32	73
5	$Asp_{1.0}$,$Thr_{0.9}$,$Ser_{0.8}$,$Gly_{2.0}$,$Tyr_{1.0}$(Arg)	11	3.0	18	10
6	$Val_{1.0}$,$Tyr_{1.0}$(Arg)	5	3.3	6.6	3
7	$Ser_{0.1}$,$Glu_{0.05}$,$Gly_{0.3}$,$Ileu_{0.2}$,$Leu_{0.05}$,*Phe*$_{0.3}$	—	—	1.0	20
8	$Asp_{2.0}$,$Thr_{1.0}$,$Ala_{2.0}$,$Val_{1.8}$,$Ileu_{0.9}$(Arg_2) $Asp_{1.0}$,$Thr_{2.6}$,$Ser_{2.6}$,$Gly_{1.9}$,$Ala_{1.6}$,$Leu_{0.1}$,*Phe*$_{0.9}$	9 —	2.5 —	19 11	40
9	$Asp_{0.01}$,$Ser_{0.12}$,$Gly_{0.01}$,$Val_{0.01}$,$Leu_{0.01}$	—	—	0.2	4
10	Small amount uncharacteristic (less than peak No. 9)	—	—		9
11	$Asp_{3.1}$,$Thr_{3.6}$,$Glu_{4.0}$,$Pro_{1.0}$,$Ala_{3.1}$,$Val_{0.9}$,$Ileu_{0.6}$,$Leu_{0.9}$(Ileu)(Arg)	8	0.2	3.5	3
12	$Asp_{2.7}$,$Thr_{3.8}$,$Glu_{3.7}$,$Pro_{1.2}$,$Ala_{2.9}$,$Val_{0.6}$,$Ileu_{0.7}$,$Leu_{1.0}$(Ileu)(Arg) $Asp_{2.0}$,$Ser_{1.0}$,$Pro_{1.0}$,*Phe*$_{1.8}$(Lys)	8 4	1.4 0.8	23 4.6	50
13	$Asp_{2.0}$,$Ser_{0.9}$,$Pro_{1.0}$,*Phe*$_{1.8}$(Lys)	4	1.9	11	96
14	$Asp_{2.0}$,$Ser_{1.0}$,$Pro_{1.0}$,*Phe*$_{2.0}$(Lys) $Thr_{1.3}$,$Ser_{5.2}$,$Glu_{0.9}$,$Pro_{0.8}$,$Gly_{1.6}$,$Ala_{1.0}$,$Val_{0.6}$,$Leu_{0.9}$,*Phe*$_{0.6}$(Try)	4 12	0.3 0.3	1.8 1.6	64
15	$Thr_{1.7}$,$Ser_{5.7}$,$Gly_{1.0}$,$Pro_{1.1}$,$Gly_{2.0}$,$Ala_{1.0}$,$Val_{0.9}$,$Leu_{1.0}$,*Phe*$_{0.9}$(Try)	12	1.4	21	40
16	$Asp_{3.0}$,$Thr_{1.6}$,$Pro_{1.0}$,$Gly_{1.1}$,$Ala_{3.0}$,$Val_{1.7}$,$Leu_{3.2}$,$Tyr_{0.5}$,*Phe*$_{0.9}$(Arg)	6	1.2	20	30
17	$Ser_{5.2}$,$Glu_{1.0}$,$Gly_{0.9}$,$Leu_{1.3}$,*Phe*$_{0.7}$	—	—	2.3	14
18	($Asp_{2.0}$,$Ser_{1.1}$,$Glu_{1.1}$,$Ala_{2.1}$,$Val_{1.0}$,$Ileu_{3.3}$,$Leu_{1.8}$(Arg))	—	—	0.5	17

Summation of counts of peptides separated (Phe = 5)		509	
Counts in typical Phe-containing TMV peptides	3, 4, 6, 12	393 =	78 per cent
Counts in mixture applied to chromatography		642	
Insoluble fraction (I-peptide) (Phe = 3)	1	280	
Total sample before digestion (Phe = 8)		1,050	

* Experiments of this type, in which the digests were fractionated by column chromatography, were performed twice each with phenylalanine and tyrosine as label. The more thoroughly analyzed phenylalanine experiment only is given *in extenso* for the sake of preservation of space, since all four experiments agreed in all principal features. With C^{14} tyrosine, sequential peptides Nos. 2, 7, 3, and 9 contained 0, 0, 7, and 10 cpm; Nos. 8 and 4, 11 cpm; Nos. 11 and 5, 42 and 39 cpm; No. 6 was not obtained pure and 73 cpm were found in fractions containing this peptide as well as 129 cpm in various fractions the amino acid composition of which did not correspond to any typical TMV peptide. Peptide No. 1 (N-acetyl-seryl tyrosine) contained 2 cpm.

† Amino acids listed in parentheses are those which according to expectation were not detected or not analyzed for, but which are known to be present in the particular peptide, as identified by sequential number in the adjacent column.

‡ The peptide recovery figure of identified peptides is derived from their known amino acid analyses, using the most reliable analytical values of column 2. The amino acid recovery represents a summation of the actually observed analytical data of identified or unknown peptides. It thus is not corrected for destruction of tryptophane and serine or slow release of valine or isoleucine, and it lacks the basic amino acids which were not at all determined in these experiments.

of the labeled product isolated with carrier TMV-protein. It is evident that the bulk of the C^{14}-phenylalanine of the product is, in roughly proportional amounts, associated with those typical TMV peptides which contain this amino acid. Some of the peptides which lack phenylalanine and radioactivity in these experiments show in the experiments with C^{14}-tyrosine the label expected due to their tyrosine content. These data supply strong evidence that part of the protein synthesized under the influence of TMV-RNA closely resembles TMV-protein in its structure. However, the absence of label from the N-acetyl-seryl tyrosine definitely shows that the N-terminal part of the biosynthetic product is not identical with this protein. This may signify only the absence of the acetyl groups. Regardless of the exact nature and extent of difference it supplies a chemical basis for the conclusion derived from the reconstitution experiments that the bulk of the biosynthetic virus protein is not quite identical with the functional protein.

The evidence for marked structural similarity between proteins synthesized by the *E. coli* system and by the tobacco plant under the direction of the same RNA clearly indicates that the genetic code is quite similar for widely divergent organisms and thus probably is universal.

TMV-RNA directs the incorporation of histidine and methionine to the same extent as does HR-RNA, even though common TMV in contrast to HR lacks those amino acids in its protein coat. The simplest explanation for this, namely, that TMV-RNA carries the code for several proteins, is in line with current concepts based on the ratio of nucleotides to amino acids in the viral coat protein (6,400:158), as well as on the finding of many mutants showing the same amino acid composition.[12, 13] This conclusion obviously complicates the task of identifying the products of virus protein synthesis.

Summary.—The addition of TMV-RNA to a cell-free amino acid incorporating system derived from *E. coli* caused up to 75-fold stimulation in protein synthesis (C^{14}-incorporation). Part of the protein synthesized formed a specific precipitate with anti-TMV serum. Labeled proteinaceous material isolated from the reaction mixture by means of carrier TMV-protein showed marked similarities in amino acid sequences to TMV-protein, but differed in the nature of the N-terminus of the peptide chain. Reconstitution experiments also suggested the presence of C^{14}-labeled protein which was virus specific and yet not identical with the native virus protein, although a small fraction of the synthesized protein may actually be functionally complete. It appears justified to conclude that TMV-RNA directs the synthesis of a protein similar to TMV-protein in a cell-free *E. coli* system. These data suggest that at least part of the genetic code may be universal.

* These investigations were supported in part by Grant CRTY-5028 of the National Institutes of Health and Grant G-9745 of the National Science Foundation.

† Present address: Research Institute for Protein Chemistry, Osaka University, Osaka, Japan.

‡ NATO postdoctoral fellow.

[1] Nirenberg, M. W., and J. H. Matthaei, these PROCEEDINGS, **47**, 1588 (1961).

[2] Nirenberg, M. W., J. H. Matthaei, and O. W. Jones, these PROCEEDINGS, **48**, 104 (1962).

[3] Martin, R. G., J. H. Matthaei, O. W. Jones, and M. W. Nirenberg, *Biochem. Biophys. Res. Comm.*, **6**, 410 (1962).

[4] Lengyel, P., J. F. Speyer, C. Basilio, and S. Ochoa, these PROCEEDINGS, **48**, 282 (1962).

[5] Crestfield, A. M., K. C. Smith, and F. W. Allen, *J. Biol. Chem.*, **216**, 185 (1955).

[6] Fraenkel-Conrat, H., and B. Singer, *Biochim. Biophys. Acta*, **33**, 359 (1959).

[7] Tsugita, A., D. T. Gish, J. Young, H. Fraenkel-Conrat, C. A. Knight, and W. M. Stanley, these PROCEEDINGS, **46**, 1463 (1960).

[8] Sequential numbers refer to the location of peptides, starting with the N-acetyl terminal peptide, #1.

[9] By a modification of the method of H. G. Wittmann and G. Braunitzer (*Virology*, **9**, 762 (1959)) as described by A. Tsugita and H. Fraenkel-Conrat, *Molecular Genetics*, ed. J. H. Taylor (New York: Academic Press, 1962), in press.

[10] Many of the peptides isolated by column chromatography were subsequently subjected to 2-dimensional electrophoresis-chromatography (mapping, according to B. Woody and C. A. Knight, *Arch. Biochem. Biophys.*, **78**, 460 (1958)) to further verify their identity and their intrinsic radioactivity. The quantitation of small amounts of C^{14}-labeled peptides by counting either segments of the paper maps (3MM) or plated eluates was poor for technical reasons which remain to be resolved. Attempts to use the mapping procedure directly on the digests were quite unsuccessful, probably because of above-mentioned quantitative aspects.

[11] Narita, K., *Biochim. Biophys. Acta*, **28**, 184 (1958).

[12] Wittmann, H. G., *Virology*, **11**, 505 (1960).

[13] Tsugita, A , and H. Fraenkel-Conrat, *J. Mol. Biol.* (in press).

---

*SOME OBSERVATIONS ON DIFFERENCES IN COMPOSITION BETWEEN THE NUCLEUS AND CYTOPLASM OF THE FROG OOCYTE**

BY HIROTO NAORA,† HATSUKO NAORA,† M. IZAWA, V. G. ALLFREY, AND A. E. MIRSKY

THE ROCKEFELLER INSTITUTE

Communicated March 29, 1962

Much of what is known about the chemistry of the cell nucleus has come from investigations of isolated nuclei. In this work a difficulty that constantly must be borne in mind is that nuclei may be changed in the course of isolation; materials may be lost from the nuclei, and materials from the cytoplasm may contaminate the nuclei. We have found a way of isolating nuclei from frog oocytes in which we may be reasonably confident that nuclear composition remains unchanged. In this procedure the cells are frozen quickly and the nuclei are cleanly dissected out while they are still frozen. Nuclei can be removed only from those cells in which on freezing there appears a crack extending from the cell surface to the nucleus. Such a crack occurs in about one tenth of the frozen cells. With the finest-gauge cold hypodermic needles the frozen oocyte is opened up along the crack as far as the nucleus, which is then removed in one piece.

Using nuclei isolated by this procedure we have determined the concentration of sodium and potassium in the nucleus and cytoplasm and the penetration of radioactive sodium (Na^{22}) and potassium (K^{42}) ions into the nucleus and cytoplasm. We have also studied the penetration of C^{14}-labeled amino acids into both the nucleus and cytoplasm.

The sodium content of the nucleus was of special interest to us because several years ago we had found that the penetration of amino acids into isolated thymus nuclei is dependent upon the presence of sodium ions in the medium in which the nuclei are suspended (Allfrey *et al.*).[1] The maximum rate of penetration for