JOURNAL OF VIROLOGY, Dec. 1973, p. 1434-1441
Vol. 12, No. 6

Translation of Virus mRNA: Synthesis of Bacteriophage Qβ Proteins in a Cell-Free Extract from Wheat Embryo

JEFFREY W. DAVIES AND PAUL KAESBERG

Biophysics Laboratory and Biochemistry Department, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 24 August 1973

The RNA from bacteriophage Qβ can be translated by cell-free extracts from wheat embryos. This translation, by 80S ribosomes, occurs at a low magnesium ion concentration. Three products are synthesized which coelectrophorese with Qβ proteins synthesized in *Escherichia coli* extracts. The smallest of these has been identified as coat protein. Although the polycistronic bacteriophage message is translated with fidelity, the efficiency is much less than when the monocistronic brome mosaic virus coat protein message is translated.

Recently there has been considerable interest in the translation of viral messengers in cell-free extracts from eukaryotic organisms, particularly messengers from animal viruses (1, 6, 7, 11). However, the mammalian extracts used in such studies are not convenient to prepare and do not always produce authentic products. We have investigated the use of plant extracts as an alternative to mammalian cell extracts. Extracts from wheat embryo (10, 13) are easily prepared and very active. We are currently using such extracts to study the translation of the RNA components of brome mosaic virus (BMV) (17), a virus which can infect wheat plants and may therefore be regarded as a model homologous messenger. Translation of BMV RNA 4, a monocistronic messenger, gives an incorporation of L-leucine (into BMV coat protein) of about 200 pmol/μg of RNA added, indicating that each messenger RNA is read about four times.

We wished to determine whether heterologous virus messengers, particularly polycistronic ones, can be translated in the wheat embryo system. For our initial studies, we chose bacteriophage Qβ RNA since its structure and products are well characterized and it has been tested in several extracts from mammalian cells (2, 14, 16). We find that Qβ RNA is translated with fidelity in wheat embryo extracts, yielding the same products as in *Escherichia coli* extracts. This suggests that the plant ribosomes can recognize bacterial initiation and termination codons) and that a heterologous polycistronic message can be translated accurately, although with less efficiency than a homologous monocistronic viral message.

MATERIALS AND METHODS

Throughout the preparations described here, precautions were taken to maintain bacteria-free and ribonuclease-free conditions.

Preparation of wheat embryo cell-free extracts. The method for preparation of wheat embryo cell-free extracts was as previously described (17), with a few modifications. Dry embryos from wheat seeds (*Triticum vulgare* L., cultivar Kenosha) or from wheat germ (General Mills Inc., Vallejo, Calif.) were purified by flotation on a cyclohexane-carbontetrachloride mixture (9, 17). Dry embryo (1 g) was ground to a paste in a mortar with 2 ml of "grinding solution" (1 mM magnesium acetate, 2 mM calcium acetate, 90 mM potassium acetate; no buffer or sulfhydryl reagent was added at this stage). Broken pieces of a sterile, glass capillary tube were used as abrasive. Grinding solution was added in 2-ml portions during continued grinding to make a 10-ml extract. DNase (RNase-free; Worthington Biochemical Corp.) was added (~5 μg/ml). The extract was centrifuged at 23,000 × *g* (average) for 10 min. Tris-acetate buffer (pH 7.6, 1:100 by volume, 1 M) and magnesium acetate (1:100 by volume, 100 mM) were added to the supernatant fluid, which was centrifuged again at 23,000 × *g*. The supernatant fluid was clear yellow. Pellicle material and the cloudy portion of supernatant fluid (about 1 ml) above the pellet were discarded. The extract was then dialyzed overnight against 10 mM Tris (acetate; pH 7.6) containing 1 mM magnesium acetate, 90 mM potassium acetate, and 1 mM dithiothreitol (DTT), kept in ice. The dialyzed extract (S-23) was less clear after dialysis at 0 C), but this did not affect the activity (at 31 C). S-23 prepared in this way contains about 15 to 20 mg

of protein per ml, 2 to 3 mg of ribosomes, and about 4 mg of soluble nucleic acid per ml.

Wheat embryo in vitro reaction mixture. The in vitro reaction mixture consisted of 10 μliters of Mix IV (ATP [25 mM], GTP [3.75 mM], phosphoenolpyruvate [PEP] [50 mM], and amino acids [0.25 mM each]), 10 μliters of radioactive amino acid, 10 μliters of HKM (consisting of 200 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid] buffered to pH 7.5 with KOH, magnesium acetate [35 mM], and potassium acetate [500 mM]), 50 μliters of S-23, 20 μliters of water, and an appropriate concentration of mRNA. For product analysis experiments, the ingredient volumes were doubled to give a 200-μliter reaction mixture. The S-23 extract was diluted by half in the reaction mixture, such that the final concentrations were 20 mM HEPES, 5 mM Tris, 4 mM Mg^{2+}, approximately 100 mM K^+ (95 mM from potassium acetate and 5 to 8 mM from the KOH), 0.25 mM for each amino acid, 2.5 mM ATP, 0.375 mM GTP, 5 mM PEP (but no PEP kinase), 0.5 mM DTT, 0.75 to 1.0 mg of S-23 protein, and 2 to 10 μg of BMV RNA or 10 to 25 μg Qβ RNA. These concentrations were determined as optima for BMV RNA translation and were kept as such for Qβ RNA experiments, since the efficiency could not be markedly increased by changing ionic conditions.

Preparation of E. coli cell-free extract. *E. coli* (Q13) cells were grown in Penassay broth to an optical density at 600 nm of 0.5 to 0.6, and then washed in buffer I (20 mM Tris [pH 7.5], 5 mM magnesium acetate, 100 mM ammonium chloride and 6 mM 2-mercaptoethanol). Extracts (S-30) were prepared essentially by the method of Capecchi (3), except that the washed alumina was equilibrated with buffer I before autoclaving, and the alumina cell pellet after the first grinding and centrifugation was ground a second time (adding more alumina) and reextracted with buffer I. The S-30 extracts were combined and dialyzed against buffer I. This contained 5 mM Mg^{2+} (rather than the conventional 10 mM) to allow more dissociation of 70*S* ribosomes into subunits) required for initiation. *E. coli* S-30 (25 μliters) was added to a 100-μliter reaction mixture which contained finally: 45 mM HEPES, and 5 mM Tris (pH 7.5), 80 mM NH_4Cl, ~20 mM k^+, 10 mM magnesium acetate, 2 mM ATP, 0.2 mM GTP (stock solutions of these adjusted to pH 7.0), 3 mM 2-mercaptoethanol, 0.75 to 1.0 mg of S-30 protein, and 0.1 mM for each amino acid. Phosphoenolpyruvate and pyruvate kinase were unnecessary; indeed some batches of the latter inhibit. Usually no other additions are necessary, but each new S-30 preparation was tested for formyl donor (as calcium leucovorin) and extra sulfhydryl reagent (DTT) requirements. These were added if necessary.

Radioactive amino acids. Labeled amino acids were obtained from Amersham-Searle and Co. Leucine and sometimes lysine or arginine were used. For routine incorporation experiments, they were diluted with unlabeled amino acid to give a ^{14}C specific activity of about 20 μCi/μmol (using 0.2 μCi/100 μliter reaction). For analysis of in vitro products, specific activities (^{14}C-amino acids) of up to 300 μCi/μmol were used. Tritiated amino acids were used at a specific activity of about 250 μCi/μmol for most experiments.

Radioactivity was counted on Whatman 3 MM disks (washed in hot trichloroacetic acid [90 C] and then in ethanol and ether) in a toluene-based scintillator; ^{14}C counting efficiency was about 50%, ^{3}H counting efficiency was about 15%.

Viral RNA preparations. Qβ bacteriophage was grown and isolated as described previously (8). RNA was purified by the method of Strauss and Sinsheimer (20), but the phenol was removed by an ether extraction and there was only one ethanol precipitation. The precipitate was dissolved in 10 mM Tris, pH 7.0.

BMV RNA and its separated components were prepared from BMV (Russian strain) (18; L. Lane, Ph.D. thesis, Univ. of Wisconsin, Madison, 1971). The RNA was finally dissolved in 10 mM Tris-borate, pH 8.3, containing 1.5 mM EDTA.

SDS-urea polyacrylamide gel electrophoresis. Amino acid incorporation was measured during incubation by removing 10- or 20-μliter samples from a 200-μliter reaction mixture. To the 120 to 160 μliters remaining, 50 μliters of a mixture containing 250 mM EDTA, 5% (wt/vol) casein amino acids, and 50 μg of RNase/ml was added; the mixture was then incubated at 37 C for 10 min. Solid urea (to give 6 to 8 M) and 2 μliters of 2-mercaptoethanol were added. After 5 min, 20 μliters of 10% sodium dodecyl sulfate (SDS) was added, and the mixture was heated at 60 C for 15 min and dialyzed for 24 h (against 6 M urea, 0.1% SDS, 1% 2-mercaptoethanol, and 100 mM sodium phosphate buffer, pH 7.6). A sample prepared in this way (200 μliters) was applied to a 10% polyacrylamide gel (6 by 80 mm) (19) made in 100 mM phosphate buffer (pH 7.6) containing 6 M urea, 0.1% SDS, and 10 mM 3-mercaptopropionic acid. Electrophoresis was for about 18 h (3 mA/gel) at room temperature. Bromophenol blue was used as a tracker dye.

Acid gel electrophoresis. In vitro incubated reaction mixtures were treated with EDTA, RNase, amino acids, and urea as described above, but SDS was omitted. The EDTA and amino acids were removed by dialysis against 200 mM glycine adjusted to pH 4.0 with lactic acid and containing 6 M urea. The gels consisted of 7% polyacrylamide (38 parts acrylamide-1 part bisacrylamide) buffered with 0.125 M NaOH adjusted to pH 4.2 with lactic acid, and containing 6 M urea. The running buffer was 200 mM glycine-lactate, pH 4.0. Electrophoresis was for about 4 h at 3 mA/gel. Methyl green was used as a tracker dye.

Fractionation and analysis of polyacrylamide gels. Gels were fractionated with a Gilson gel fractionator, which crushes the gel to fine particles through a stainless-steel gauze. Each fraction was equivalent to 1 mm of gel and was eluted with 0.33 ml of water. These fractions were incubated at 60 C for 3 h, or at room temperature overnight, before addition of dioxan-napthalene-Triton X-100 scintillation fluid. Radioactivity was counted on a Beckman LS-330 liquid scintillation spectrometer. For dual isotope counting, the ^{3}H channel had a ^{14}C "cross-talk" of 10%, under these quench conditions.

Trypsin digestion and peptide analysis. SDS-urea polyacrylamide gels were subjected to electro-

phoresis as described above. A 50-μliter sample from each 0.33-ml fraction was counted. Those fractions corresponding to the viral coat protein peak (five to six gels) were pooled. Gel particles were removed by centrifugation, and the supernatant fluid was then dialyzed against water for 48 h to remove the SDS. The sample was then adjusted to 0.2 M NH_4CO_3, and trypsin (Calbiochem, TPCK-treated, 10 mg/ml of 0.1 N HCl) was added to give a concentration of about 1.5 mg/ml. After 6 h of digestion at 37 C, a further 0.7 mg/ml was added, and the sample was incubated for an additional 12 h. The digest was then lyophilized, dissolved in 1 ml of 0.2 M pyridine (pH 3.1 with acetate), and eluted thorugh a Dowex column (50 by 8 mm) (4) with pyridine buffer. The fractions were lyophilized, dissolved in 0.5 ml of water, and mixed with dioxan-napthalene-triton X-100 scintillation fluid.

RESULTS

Qβ RNA stimulation of amino acid incorporation in the wheat embryo cell-free system. When Qβ RNA is used as messenger in the wheat embryo cell-free extract, there is a three- to fourfold stimulation of the amino acid incorporation activity above the control. This is not a large stimulation, although it is readily and consistently detectable. Similar stimulation is obtained with the heterologous system (*E. coli* extracts programmed with BMV RNA). The homologous systems were more active. Qβ RNA stimulated the *E. coli* extract activity over 82-fold, and even with very small quantities of BMV RNA (less than the optimum) amino acid incorporation of the wheat embryo extract was stimulated by about 30-fold. Typical data are presented in Table 1.

The kinetics of Qβ RNA-directed amino acid incorporation by wheat embryo S-23 is shown in Fig. 1. Usually there is a lag for 5 to 10 min, then the incorporation increases for about 60 min and reaches a plateau at 90 to 120 min. (Fig. 1). The kinetics are similar for BMV RNA and, indeed, are characteristic of the wheat system. The kinetics of incorporation are different in the *E. coli* system; there is no time lag for either messenger and the plateau is reached between 40 and 60 min (results not shown).

Table 1 shows that high activity of the wheat embryo S-23 can be attained with only small amounts of mRNA. Various concentrations of the homologous monocistronic message (BMV RNA 4) and the heterologous polycistronic message (Qβ RNA) were added in the wheat embryo cell-free system (Fig. 2). The wheat embryo S-23 became saturated (Fig. 2b) with only 4 μg of BMV RNA 4 per 100 μliters. This is unlike the *E. coli* system, which requires over 100 μg RNA per 100 μliters (results not shown). With Qβ RNA in the wheat system, the maximal activity is reached at about 20 μg of Qβ RNA per 100 μliters. Addition of more of this messenger inhibits the incorporation (Fig. 2a).

The wheat embryo system is quite efficient at protein synthesis. We calculate that with BMV RNA at the saturating concentration each message is read on average three to four times (Table 2). Under the same conditions and at the saturating concentration of Qβ RNA, 4.8 pmol of leucine is incorporated into protein per μg of Qβ RNA in the reaction mixture. This is equivalent to an average "messenger reading number" of 0.6 (Table 2). Either not all messages are read, due to a limiting factor (such as a specific initiation factor), or a considerable percentage of the messenger molecules is not available to be translated, perhaps owing to nuclease digestion.

TABLE 1. *Viral RNA-directed incorporation into protein, in extracts from wheat embryo and E. coli: comparison of heterologous and homologous messengers*[a]

Determination	Extract	Viral RNA	Radioactivity (counts/min/ 100 μliters)	Stimulation (fold)
Heterologous systems	Wheat embryo	Qβ (13.5 μg)	8,820	3.4
	E. coli	BMV 4 (7 μg)	8,660	2.8
	E. coli	BMV 3 (44 μg)	15,495	5.0
Controls	Wheat embryo		2,560	
	E. coli		3,070	
Homologous systems	Wheat embryo	BMV 4 (1.4 μg)	73,885	28.8
	Wheat embryo	BMV 3 (4.4 μg)	90,940	35.5
	E. coli	Qβ (27 μg)	253,860	82.6

[a] *E. coli* S-30 and wheat S-23 extracts and incubations were as described in Materials and Methods. Radioactive labeling was with ^{3}H-arginine, -lysine, and -leucine (0.033 mM each, 248 μCi/μmol). The counts per minute indicated are at ~15% counting efficiency. The *E. coli* extracts were incubated at 37 C for 40 min; the wheat embryo extracts were incubated at 31 C for 60 min.

Table 2 also shows that commercially available wheat germ (see Materials and Methods) gives preparations as active as embryos obtained from wheat seeds. However, food-store wheat germ obtained locally, containing a considerable amount of endosperm and "chaff" yet claimed to be "untreated," does not give active preparations.

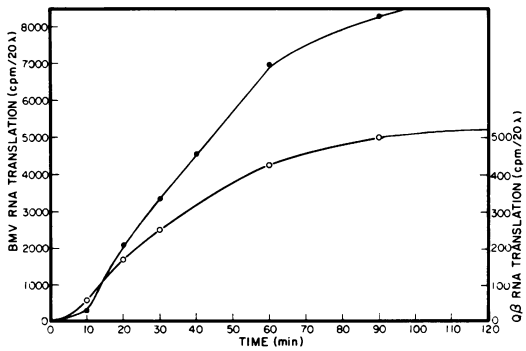

FIG. 1. *Kinetics of wheat embryo in vitro protein synthesis directed by Qβ and BMV messengers. The wheat embryo in vitro reaction mixture was as described in Materials and Methods. A 200-μliter mixture was used, and 20-μliter samples were removed at time intervals, onto 3 MM filters previously treated with a few drops of 10% trichloroacetic acid. The incorporation of ^{14}C-leucine directed by 20 μg (10 μg/100 μliters) of BMV RNA is compared to ^{3}H-leucine incorporation directed by 30 μg (15 μg/100 μliters) of Qβ RNA. Symbols: ●, BMV RNA translation; ○, Qβ RNA translation.*

The effects of cycloheximide and chloramphenicol on Qβ RNA-directed protein synthesis in wheat embryo extract were tested. If Qβ RNA is being translated by 80*S* ribosomes in the wheat extract and not by plastid or contaminating bacterial 70*S* ribosomes, then the activity should be inhibited by the antibiotic cycloheximide, but not by chloramphenicol. This was, indeed, found to be true (Table 3). As a control, Qβ RNA was also translated in *E. coli* S-30, in which it was inhibited by chloramphenicol instead of cycloheximide.

Products of Qβ RNA-directed translation in wheat embryo extracts. On SDS-urea polyacrylamide gels, the product was found to consist of one major polypeptide band (accounting for over 90% of the total activity) and other very small, poorly resolved bands. A sample, labeled with ^{14}C-arginine, -lysine, and -leucine, was mixed with the in vitro products from the *E. coli* S-30-Qβ RNA system, labeled with the ^{3}H-arginine. After SDS-urea treatment, the sample was subjected to electrophoresis and the gel fractions were assayed by dual isotope scintillation counting (Fig. 3a). The major polypeptide product made in the wheat system coincides with the major band made in the *E. coli* system and known to be the Qβ coat protein. Coincidence of the other bands is also evident, including band I of the Qβ replicase polypeptide. Two of the minor bands from the wheat embryo synthesis correspond in position to those of proteins I and II, which have been identified in the *E. coli* system to be replicase

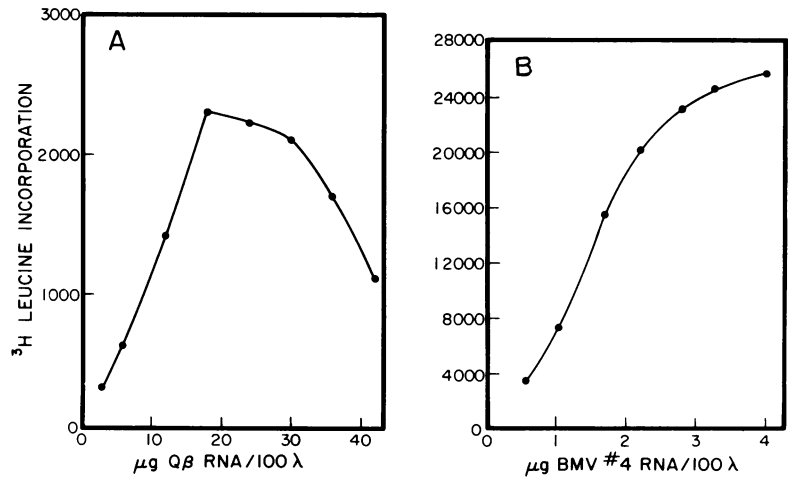

FIG. 2. *Effect of changes in the mRNA concentration for wheat embryo in vitro protein synthesis. A comparison of Qβ RNA with the monocistronic homologous messenger, BMV RNA 4. ^{3}H-leucine (125 μCi/μ mol) was incorporated into the trichloroacetic acid-insoluble products of the wheat embryo system as described in Materials and Methods. Various amounts of Qβ RNA and BMV RNA 4 were added to each of two series of incubations, respectively. (A) Qβ RNA concentration curve; incorporation as ^{3}H-leucine counts per minute per 50 μliters. (B) BMV RNA 4 concentration curve.*

subunit and read-through protein, respectively (21). A third, corresponding minor band that is frequently found between bands II and III in Qβ RNA-programmed *E. coli* synthesis is of unknown origin. Products from the in vitro syntheses were also subjected to electrophoresis in the pH 4.0/4.2 polyacrylamide gel electrophoresis system described above. The products of *E. coli* S-30 were ^{3}H-arginine labeled, and the wheat embryo S-23 products were labeled with ^{14}C-arginine, -lysine, and -leucine. There was one major broad band derived from the wheat extracts, which we judge must be polypeptide III. It migrates slightly slower (~2%) than its counterpart from *E. coli* extracts.

The major product of the *E. coli* in vitro translation of Qβ RNA, polypeptide III, is known to be coat protein, some of which still retains (formyl) methionine at its N terminus.

TABLE 2. *Efficiency of translation by wheat embryo from seed and wheat germ cell-free extracts programmed with Qβ and BMV messages*[a]

Messenger	Wheat seed embryo extract		Wheat germ embryo extract	
	pmol of leucine per μg of RNA	R(av)[b]	pmol of leucine per μg of RNA	R(av)[b]
Qβ RNA (tricistronic) ...	4.8	0.6	2.4	0.3
BMV RNA 3 (dicistronic)	87.9	4.4	57.4	2.8
BMV RNA 4 (monocistronic)	155.2	2.9	197.6	3.6

[a] Wheat germ from a commercial source was used to make a wheat germ S-23 by a method similar to that described for wheat embryo. Incubation mixtures (100 μliters) were as described in Materials and Methods, containing 0.26 pmol of L-[^{14}C]leucine (26 μCi/μmol). Messenger RNAs were added in the following amounts: BMV RNA 4, 3.75 μg; BMV RNA 3, 8.85 μg; and Qβ RNA, 25 μg. Incubation was for 60 min at 31 C.

[b] R(av) = the number of times a messenger is read (on average), calculated as pico moles of protein per pico moles of message. BMV RNA 4 makes only BMV coat protein. Qβ RNA was assumed to make mostly (90%) Qβ coat protein. The amino acid composition of the major product of RNA 3 is not yet known; it was assumed to have 15 leucine residues as an approximation.

TABLE 3. *Effect of chloramphenicol and cycloheximide on Qβ RNA-directed protein synthesis in wheat embryo and E. coli cell-free extracts*[a]

Extract	Treatment	pmol of leucine per 100 μliters	pmol of leucine per μg of RNA	Inhibition (%)
Wheat embryo	Control	122	4.8	0
	+CAP	108	4.3	10.5
	+CH	0	0	100.0
E. coli	Control	1672	37.1	0
	+CAP	101	2.2	94.1
	+CH	1572	34.9	5.9

[a] Wheat embryo S-23 was incubated with 25 μg of Qβ RNA, as described in Materials and Methods. *E. coli* ribosomes (700 μg) and S-100 supernatant fluid (350 μg of protein) were incubated with 45 μg Qβ RNA as in Materials and Methods. Specific activity of ^{14}C-leucine was 26 μCi/μmol. CAP, Chloramphenicol (D-isomer) at 43.7 μg/ml; CH, cycloheximide at 3.0 μg/ml.

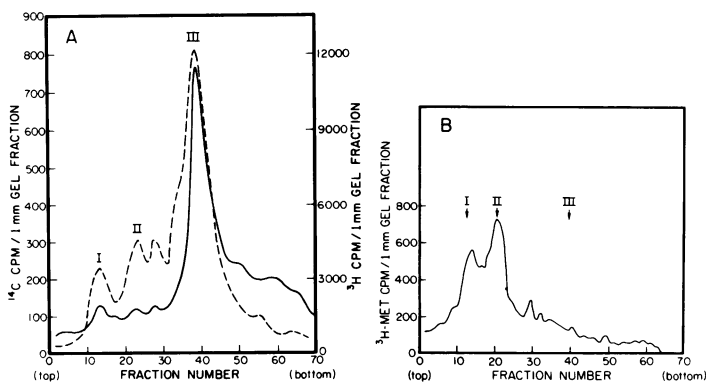

FIG. 3. *Analysis of the products of wheat embryo S-23 in vitro protein synthesis by directed Qβ RNA. (A) SDS-urea polyacrylamide gel patterns. (——) Qβ RNA translation by wheat embryo S-23: incorporation of ^{14}C-arginine, -lysine, and -leucine. (- - -) Qβ RNA translation by E. coli S-30: incorporation of ^{3}H-arginine. (The products were co-electrophoresed and analyzed by dual isotope liquid scintillation counting as described in Materials and Methods.) (B) SDS-urea polyacrylamide gel pattern. (——) Incorporation of ^{3}H-methionine (0.7 Ci/mmol) in wheat S-23.*

Mature coat protein lacks methionine. When the products of ^{3}H-methionine incorporation in the wheat embryo system were subjected to electrophoresis on SDS-urea acrylamide gels, the major readioactive band (III) corresponding to coat protein was missing (Fig. 3b). Bands I and II were still present. This result also indicates that there is no N-terminal methionine on this product. To provide further evidence that the polypeptide product of the same size, synthesized in wheat embryo extracts, is coat protein-like, the tryptic peptides of this product were analyzed. The Qβ RNA products in the wheat embryo system were labeled with ^{14}C-leucine. These were treated as described above, along with ^{3}H-leucine-labeled *E. coli* in vitro product. After dual-label scintillation counting of the fractions from the Dowex column, the tryptic peptide pattern shown in Fig. 4 was obtained. There were 7 major leucine-labeled peaks, and all of these from the wheat system coincided with those from the *E. coli* system, indicating substantial similarity of the two products. However the *E. coli* in vitro product had one additional peak (peak 7 of Fig. 4). We believe, but have not shown, that this is the N-terminal tryptic peptide from the Qβ coat proteins which have retained their initiating formyl (f) methionine residue, namely, f Met-Ala-Lys-Leu-Glu-Thr-Val-Thr-Leu-Gly-Asn-Ile-Gly-Lys, and which would be expected to have no counterpart from the wheat embryo synthesis. (It should be noted that this tryptic peptide exists because tryptic cleavage at the lysine in position 3 fails [12].)

We also investigated the translation of two Qβ amber mutant RNAs in the wheat embryo extracts. The mutants chosen were amB86 and amB37, whose amber mutations (UAG termination codon) are at the codon of amino acids 86 and 37, respectively, of the coat protein (15). RNA from these mutants was from phage grown in Su$^+$ cells. The products from the translation of these RNAs in the wheat embryo S-23 were labeled with ^{14}C-arginine, -lysine, and -leucine (Fig. 5). Products smaller than the coat protein were detected. In the case of *amB86*, there was a single, major band corresponding to a protein of molecular weight approximately 11,000, as would be expected for an amber fragment 85 amino acid residues long. The electrophoretic

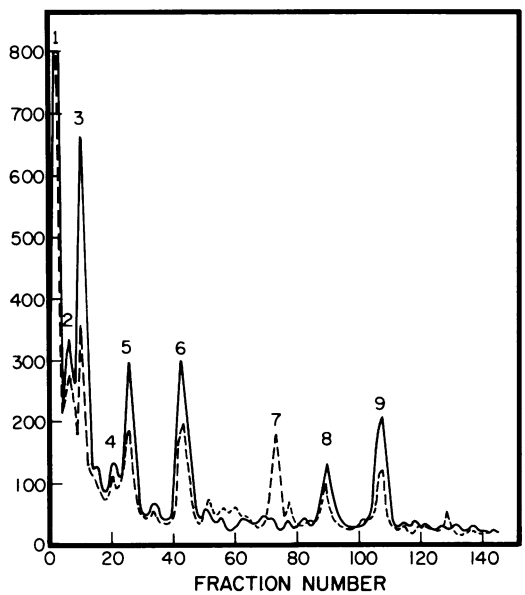

FIG. 4. *Analysis of the tryptic peptides of Qβ RNA-directed protein synthesis in wheat embryo S-23; comparison with the tryptic peptides of Qβ coat protein synthesized in vitro by E. coli S-30. The in vitro products were labeled with ^{14}C-leucine (wheat embryo) and ^{3}H-leucine (E. coli), and treated as described in Materials and Methods. Peptides were eluted from the Dowex column with pyridine-acetate buffer (gradient 0.2 to 2.0 M, pH 3.1 to 5.0), lyophilized, dissolved in 0.5 ml water, and counted by dual isotope liquid scintillation counting. (——) Tryptic peptide of the major Qβ RNA product synthesized by wheat embryo S-23. (- - -) Tryptic peptides of Qβ coat protein synthesized by E. coli S-30.*

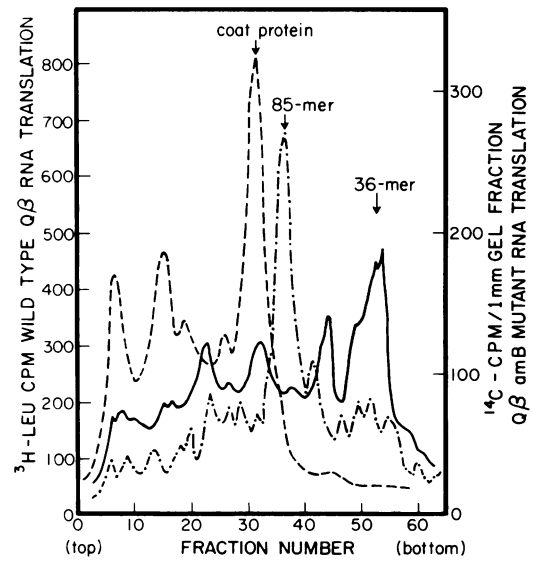

FIG. 5. *Wheat embryo S-23 translation of Qβ amber mutant RNA; estimation of the size of polypeptide products by SDS-urea polyacrylamide gel electrophoresis. (——) Products of amB37 RNA. (-·-·-) Products of amB86 RNA. (- - -) Products of wild-type RNA translated in an E. coli cell-free extract, for comparison of "authentic" coat protein position.*

pattern in the case of *amB37* was complex and not well resolved. Nevertheless, the most radioactive band corresponded to a protein of molecular weight 4500, consistent with an amber fragment of 36 amino acid residues.

DISCUSSION

Extracts from wheat embryo prepared as described in this paper, show enhanced amino acid incorporation due to Qβ RNA. The stimulation, however, is usually quite small (two- to fourfold) compared with the homologous system, Qβ RNA translation by *E. coli* extracts (50- to 100-fold). Synthesis continues for about 60 min after an initial lag of 5 to 10 min, and then levels off. Such kinetics are characteristic of the wheat embryo system, since both homologous and heterologous, monocistronic and polycistronic messages have similar kinetics of translation. However, the effects of messenger concentration depend on the message: the monocistronic homologous BMV RNA 4 saturates at 4 μg/100 μliters and levels off at higher concentration, whereas the heterologous Qβ RNA produces maximal polypeptide synthesis at 20 μg/100 μliters; higher concentrations inhibit. It is interesting to note that inhibition and, indeed, the same optimal concentration is obtained when Qβ RNA is translated by extracts from Krebs II mouse ascites cells (14).

It is not yet known how many times a messenger is read. We estimate that, unlike the homologous messenger system (BMV RNA), in which each messenger is read on average three to four times, the Qβ messages are read on average only 0.6 times, indicating that some are not translated at all. Possibly only a few messages are translated several times. Perhaps the Qβ RNA is degraded by nucleases in the wheat embryo extract. However, preliminary investigations of messenger degradation in wheat embryo S-23 indicate that there is little nuclease activity: 80 to 90% of most viral messages remains intact after in vitro translation, as judged by gel electrophoresis of ^{32}P-labeled messengers. Also, if there was messenger degradation, there would probably be some translation of messenger fragments, particularly those intact over the initiation region. However, there was little synthesis of polypeptides smaller than the coat protein. The products translated from Qβ RNA in wheat S-23 are the same size as Qβ coat protein synthesized in *E. coli* S-30. It seems more likely that all Qβ messages are not translated because of limiting components, such as specific initiation factors. If there are different cistron specific factors in the wheat S-23, varying in pool size, a very small pool of factors capable of recognizing Qβ RNA cistrons would explain the low efficiency of the system. Functional heterogeneity of the ribosome population could also account for such results. We rule out the possibility that a small proportion of contaminating 70*S* ribosomes in the eukaryotic cell extract translate the Qβ RNA on the basis of antibiotic inhibition experiments of the type shown in Table 3. Using ^{14}C-leucine incorporation into Qβ RNA products, we calculated that the maximal incorporation under our conditions is 4 to 5 pmol of leucine per μg of RNA added (Table 2). Reactions with the optimal message concentration (20 μg/100 μliters) incorporate a total of 80 to 100 pmol of leucine per 100-μliter reaction. In the Krebs II mouse ascites cell extracts (14), about 25×10^3 to 50×10^3 counts of ^{3}H-leucine per min are incorporated per 25 to 50 μliters of reaction mixture, which is equivalent to about 1.5 to 3.0 pmol (taking a specific activity of 40 Ci/mol and an assumed counting efficiency of 20%). Aviv et al. (2) report a ^{3}H-leucine incorporation by Krebs ascites extracts of 0.85 to 1.27 pmol/60-μliter reaction mixture. The wheat embryo cell-free extract, therefore, synthesizes more Qβ protein than these mammalian systems.

The products of Qβ RNA translation have the same molecular weights as the products synthesized by the *E. coli* system. The major product was shown to be similar to, and probably identical, coat protein. Methionine was not found in the synthesized coat protein, indicating that the N-terminal methionine is removed. In *E. coli* extracts, a proportion of the coat protein molecules retain *N*-formylmethionine at the N-terminal end. We believe this can account for the slight difference in charge of the wheat embryo *E. coli* products and the difference in just one tryptic peptide.

The fact that Qβ RNA is translated with fidelity in the wheat embryo cell-free system tells us several interesting facts. First, the 80*S* ribosomes of wheat plants can translate a polycistronic message, although not as efficiently as a monocistronic message like BMV RNA 4. The Qβ coat protein cistron is correctly initiated and terminated even though it is near the middle of the messenger molecule. The ribosomes also recognize the termination sequences UGA and UAG, since the system can not only synthesize the complete Qβ authentic coat protein, but also the polypeptides of amber mutants with UAG termination codons at positions 37 and 86 of the coat protein molecule. This may provide an assay system for plant termination factors since it is likely that UAG is a termination sequence in plants as well as animals and

bacteria. This result also shows that wheat embryo extracts probably do not have a suppressor tRNA; thus, like the mammalian cell-free extracts (2, 14, 16) this provides a model system for testing and identifying eukaryotic cell suppressors.

Our results present further evidence for the universality of the genetic code, including initiation and termination sequences. Although the system translates with good fidelity, the efficiency of heterologous messenger translation is low. This provides a model with which we can investigate some aspects of quantity control in protein synthesis, by finding what components (such as specific initiation factors, ribosomal proteins, or transfer RNAs) account for these differences.

Recently, the wheat embryo system has been shown to translate rabbit globin RNA (5). Since we have shown that polycystronic messengers can also be translated in wheat extracts, it is likely that under suitable conditions it may also be able to translate other heterologous RNAs, including those of some animal viruses.

ACKNOWLEDGMENTS

We thank Claudia Benike for technical assistance, and D. Shih and M. Bastin for useful discussions.

This work was supported by Public Health Service grants 5R01-AI-01466 and 5K06-AI-21,942 from the Institute of Allergy and Infectious Diseases, and by grant AT-11-1-1033 from the Biology Division of the U.S. Atomic Energy Commission.

LITERATURE CITED

1. Aviv, H., I. Boime, and P. Leder. 1971. Protein synthesis directed by encephalomyocarditis virus RNA: properties of a transfer RNA-dependent system. Proc. Nat. Acad. Sci. U.S.A. **68**:2303-2307.
2. Aviv, H., I. Boime, B. Loyd, and P. Leder. 1972. Translation of bacteriophage Qβ messenger RNA in a murine Krebs 2 ascites tumor cell-free system. Science **178**:1293-1295.
3. Capecchi, M. R. 1966. Cell-free protein synthesis programmed with R17 RNA; identification of two phage proteins. J. Mol. Biol. **31**:178-193.
4. Craven, G. R., P. Voynow, J. S. Hardy, and C. G. Kurland. 1969. The ribosomal proteins of *Escherichia coli*. II. Chemical and physical characterization of the 30s ribosomal proteins. Biochemistry **8**:2906-2195.
5. Efron, D., and A. Marcus. 1973. Efficient synthesis of rabbit globin in a cell-free system from wheat embryo. FEBS Lett. **33**:23-27.
6. Eggen, K. L., and A. J. Shatkin. 1972. In vitro translation of cardiovirus ribonucleic acid by mammalian cell-free extracts. J. Virol. **9**:636-645.
7. Graziadei, W. D., and P. Lengyel. 1972. Translation of *in vitro* synthesized reovirus messenger RNAs into protein of the size of reovirus capsid proteins in a mouse L cell extract. Biochem. Biophys. Res. Commun. **46**: 1816-1823.
8. Jockusch, H., L. A. Ball, and P. Kaesberg. 1970. Synthesis of polypeptides directed by the RNA of phage Qβ. Virology **42**:401-414.
9. Johnston, F. B., and H. Stern. 1957. Mass isolation of viable wheat embryo. Nature (London) **179**:160-161.
10. Klein, W. H., C. Nolan, J. M. Lazar, and J. M. Clark. 1972. Translation of satellite tobacco necrosis ribonucleic acid. I. Characterization of *in vitro* procaryotic and eukaryotic translation products. Proc. Nat. Acad. Sci. U.S.A. **11**:2009-2014.
11. McDowell, M. J., W. K. Joklik, L. Villa-Komaroff, and H. F. Lodish. 1972. Translation of reovirus messenger RNAs synthesized *in vitro* into reovirus polypeptides by several mammalian cell-free extracts. Proc. Nat. Acad. Sci. U.S.A. **69**:2649-2653.
12. Maita, T. and W. Konigsberg. 1971. The amino acid sequence of the Qβ coat protein. J. Biol. Chem. **246**:5003-5024.
13. Marcus, A., B. Luginbill, and J. Feeley. 1968. Polysome formation with tobacco mosaic virus RNA. Proc. Nat. Acad. Sci. U.S.A. **59**:1243-1250.
14. Morrison, T. G., and H. Lodish. 1973. Translation of bacteriophage Qβ RNA by cytoplasmic extract of mammalian cells. Proc. Nat. Acad. Sci. U.S.A. **70**:315-319.
15. Palmenberg, A., and P. Kaesberg. 1973. Amber mutant of bacteriophage Qβ capable of causing overproduction of Qβ replicase. J. Virol. **11**:603-605.
16. Schreier, M. H., T. Staehelin, R. F. Gesteland, and P. F. Spahr. 1973. Translation of bacteriophage R 17 and Qβ RNA in a mammalian cell-free system. J. Mol. Biol. **75**:575-578.
17. Shih, D. S., and P. Kaesberg. 1973. Translation of brome mosaic viral ribonucleic acid in a cell-free system derived from wheat embryo. Proc. Nat. Acad. Sci. U.S.A. **70**:1799-1803.
18. Shih, D. S., L. C. Lane, and P. Kaesberg. 1972. Origin of the small component of brome mosaic virus RNA. J. Mol. Biol. **64**:353-362.
19. Strauss, E. A., and P. Kaesberg. 1970. Acrylamide gel electrophoresis of the intact virions and of the viral proteins. Virology **42**:437-452.
20. Strauss, J. H., and R. L. Sinsheimer. 1963. Purification and properties of bacteriophage MS2 and of its ribonucleic acid. J. Mol. Biol. **7**:43-44.
21. Weiner, A. M., and K. Weber. 1971. Natural read-through at the UGA termination signal of Qβ coat protein cistron. Nature N. Biol. **234**:206-209.