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Translation of Virus mRNA: Protein Synthesis Directed by Several Virus RNAs in a Cell-free Extract from Wheat Germ

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SUMMARY

The ability of wheat germ cell-free extracts to translate several virus RNAs has been surveyed, using RNA from viruses with different host ranges, and which do not normally infect wheat. Synthesis of specific coat protein-like products was achieved with RNAs from cowpea chlorotic mottle virus, broad bean mottle virus, cucumber mosaic virus, and alfalfa mosaic virus. It appears to be mainly the small RNAs (which contain the coat protein cistron) that are translated with most fidelity. The larger RNA components of these multicomponent viruses direct amino acid incorporation quite efficiently, but the products are heterogenous. Other larger RNAs, such as tobacco mosaic virus RNA, also produce a heterogenous (polydisperse) mixture of polypeptides, rather than virus products. The RNAs of Maus–Elberfeld virus and avian myoblastosis virus stimulate a little amino acid incorporation without directing the synthesis of any recognizable virus protein-sized products. The relationship of RNA size with translatability is discussed.

INTRODUCTION

Previously, we have shown that RNA from brome mosaic virus (BMV) can be translated accurately and efficiently in wheat embryo cell-free extracts prepared from wheat seeds or commercial wheat germ (Davies & Kaesberg, 1973; Shih & Kaesberg, 1973). Because BMV can infect wheat, we consider BMV RNAs to be model homologous messengers. In contrast, Qβ bacteriophage RNA, which we regard as a heterologous RNA because Qβ does not infect wheat, is a poor messenger in this *in vitro* system in terms of efficiency, although it is probably translated accurately (Davies & Kaesberg, 1973).

An initial step in the establishment of virus infection is the formation of polysomes and translation of at least part of the RNA genome. Is host specificity to some extent determined at the translation level? If so, we might expect that in the wheat system efficient translation would be restricted to homologous messengers. We thus wished to know if wheat ribosomes can efficiently translate RNA from viruses which are not known to infect wheat. If some virus RNAs can be translated and others cannot, is there any general criterion common to the translatable messages?

We have investigated the ability of the wheat germ cell-free system to translate RNAs from various viruses whose host ranges differ from BMV. (*a*) Viruses belonging to the bromovirus group were chosen as examples of viruses closely related to BMV. These were cowpea chlorotic mottle virus (CCMV), which is serologically related to BMV although its

host range is very different, and broad bean mottle virus (BBMV). (*b*) Cucumber mosaic virus (CMV), which is a member of the cucumovirus group, and not currently considered to be a bromovirus. It has a spectrum of RNAs similar to that of bromoviruses (Lot *et al.* 1974). (*c*) Alfalfa mosaic virus (AMV), which is less BMV-like, but does have a divided genome including a small, presumably monocistronic, RNA. (*d*) Cowpea mosaic virus (CPMV), an example of the comovirus group, which has two large RNAs. (*e*) Tobacco mosaic virus (TMV) (tobamovirus group). This was chosen as an example of a different structural type, having a single large RNA molecule.

Although this study emphasizes work with plant virus RNAs, we have tested RNA from two animal viruses with quite large RNAs. These were avian myoblastosis virus, a leukovirus, and the picornavirus Maus–Elberfeld (MEV). We have also included some further comparative data with RNA from phage Qβ. More detailed studies of individual viruses will be published elsewhere.

We find that the RNAs of the divided genome viruses are translated quite efficiently. However, it is the coat protein cistrons that are most readily translated and, like BMV, the coat protein cistrons are on the smallest RNAs. The largest RNA components of CCMV, for example, direct *in vitro* amino acid incorporation efficiently, but into somewhat polydisperse products rather than distinct proteins. Similarly large plant virus messages such as that of TMV RNA are translated into 'heterogenous' products. RNAs from avian myoblastosis virus and MEV are inefficient at amino acid incorporation and moreover are not translated into virus proteins under the conditions used.

There is a general trend of correlation of RNA size with efficiency or fidelity of translation. The wheat germ *in vitro* system has a preference for small monocistronic messengers. If this is a property of the eukaryotic ribosome system, then the existence of multicomponent viruses with a genome divided into smaller RNAs may represent a type of evolutionary adaptation of such viruses to the functional properties of the ribosome system.

METHODS

Viruses and RNA preparations. BMV (wild type) was grown in barley (*Hordeum vulgare*), CCMV (wild type) in cowpea (*Vigna sinensis*) and TMV in tobacco (*Nicotiana tabacum* var. Havana 38). BBMV (Hull, 1972) was a gift from R. Fulton. Maus–Elberfeld (MEV) was a gift from R. Rueckert (Rueckert & Schafer, 1965). Avian myoblastosis virus (here abbreviated AvMV to avoid confusion with AMV) RNA was a gift from R. Wells.

BMV, CCMV, BBMV and TMV RNAs were prepared by dissociating the virus particles with SDS, 2 mg/mg virus, in the presence of purified bentonite, followed by removal of protein by phenol extraction. Phenol was removed by ether extraction and the RNA precipitated with ethanol. RNA was redissolved in 89 mM-tris, made pH 8·3 with boric acid, and containing 2·75 mM-EDTA (disodium). The RNA was then again precipitated with ethanol, and redissolved.

Alfalfa mosaic virus (AMV) RNA and CMV RNA were gifts from L. van Vloten-Doting. CPMV RNA was a gift from J. S. Semancik. MEV RNA was prepared by phenol extraction from virus suspended in 10 mM-tris, pH 7·5, 0·14 M-NaCl, 5 mM-EDTA (disodium), and SDS, 5 mg/l to 2 mg virus.

The individual components from multicomponent RNAs were purified by two cycles of sucrose gradient sedimentation, 5 to 20% sucrose (RNase-free) in 100 mM-glycine, pH 8·5, with KOH, containing 100 mM-KCl and 1 mM-EDTA. BBMV RNA components were similarly prepared by T. C. Hall. Sucrose was dialysed from the selected fractions, the

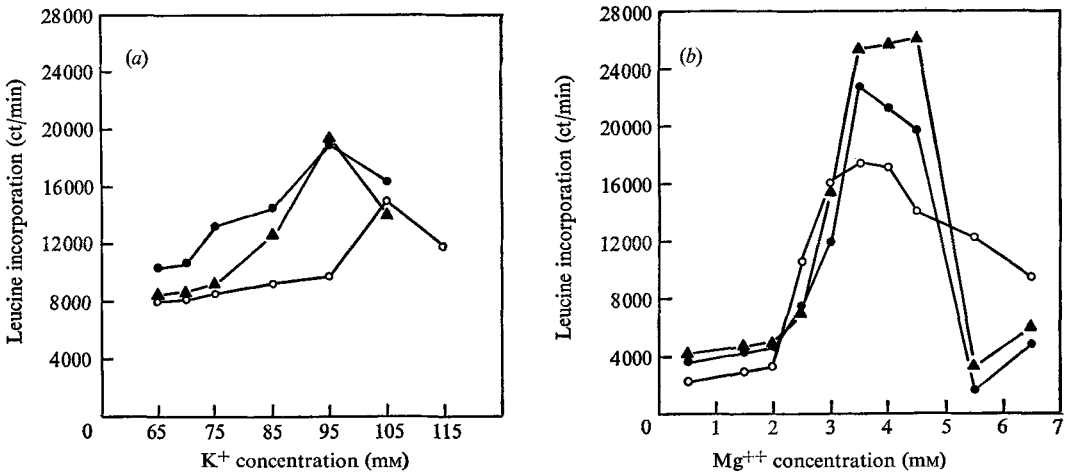

Fig. 1. Monovalent and divalent cation requirement for various virus RNAs. [^{14}C]-leucine was incorporated into trichloroacetic acid-precipitable material, at 31 °C for 60 min. (*a*) Potassium ion concentration curves (magnesium concentration constant at 4 mM). ●—●, BMV RNA 1 to 4 (ct/min × ½); ▲—▲, BBMV RNA *1* to *4*; ○—○, TMV RNA (ct/min × 2). (*b*) Magnesium ion concentration curves (potassium concentration constant at 90 mM). ●—●, BMV RNA 1 to 4; ▲—▲, CCMV RNA 1 to 4; ○—○, TMV RNA.

RNA precipitated with ethanol, and redissolved in 89 mM-tris-borate, pH 8·3, containing 2·75 mM-EDTA. This buffer was diluted at least 10-fold when the RNA was added to the *in vitro* reaction mixture.

Preparation of cell-free extracts and in vitro *incubation procedures.* The wheat embryo S-23 extract used in these studies was prepared from commercial wheat germ, after flotation purification as previously described (Davies & Kaesberg, 1973). The wheat germ was obtained from General Mills, Inc. (Vallejo, Cal., U.S.A.) and consists mainly of a blend of hard red spring and winter wheat from Montana, Washington and Idaho. The *in vitro* reaction mixture was as described previously (Davies & Kaesberg, 1973). Electrophoresis was for 16 to 24 h, as indicated in the text. The gel patterns are not necessarily quantitatively comparable, because the adjustment to 6 to 8 M-urea is approximate, resulting in differential volume changes during dialysis.

RESULTS

Amino acid incorporation directed by various virus RNAs: monovalent and divalent cation requirements

The amino acid incorporation ability of the wheat germ cell-free extracts was tested with various virus RNAs, under several ionic conditions. The optimum potassium and magnesium ion concentrations for BMV RNA were found to be similar for the other RNAs tested (Fig. 1). Since there was little variation in optimum ionic requirements for these RNAs, further studies were made under the same conditions, namely 100 mM [K^+] and 4 mM [Mg^{++}].

Polyacrylamide gel analysis of the in vitro *products directed by several virus RNAs*

CCMV RNA was found to be an excellent messenger in the wheat germ system. Fig. 2 shows the products of translation of CCMV RNA. The mixture of all four RNAs produced

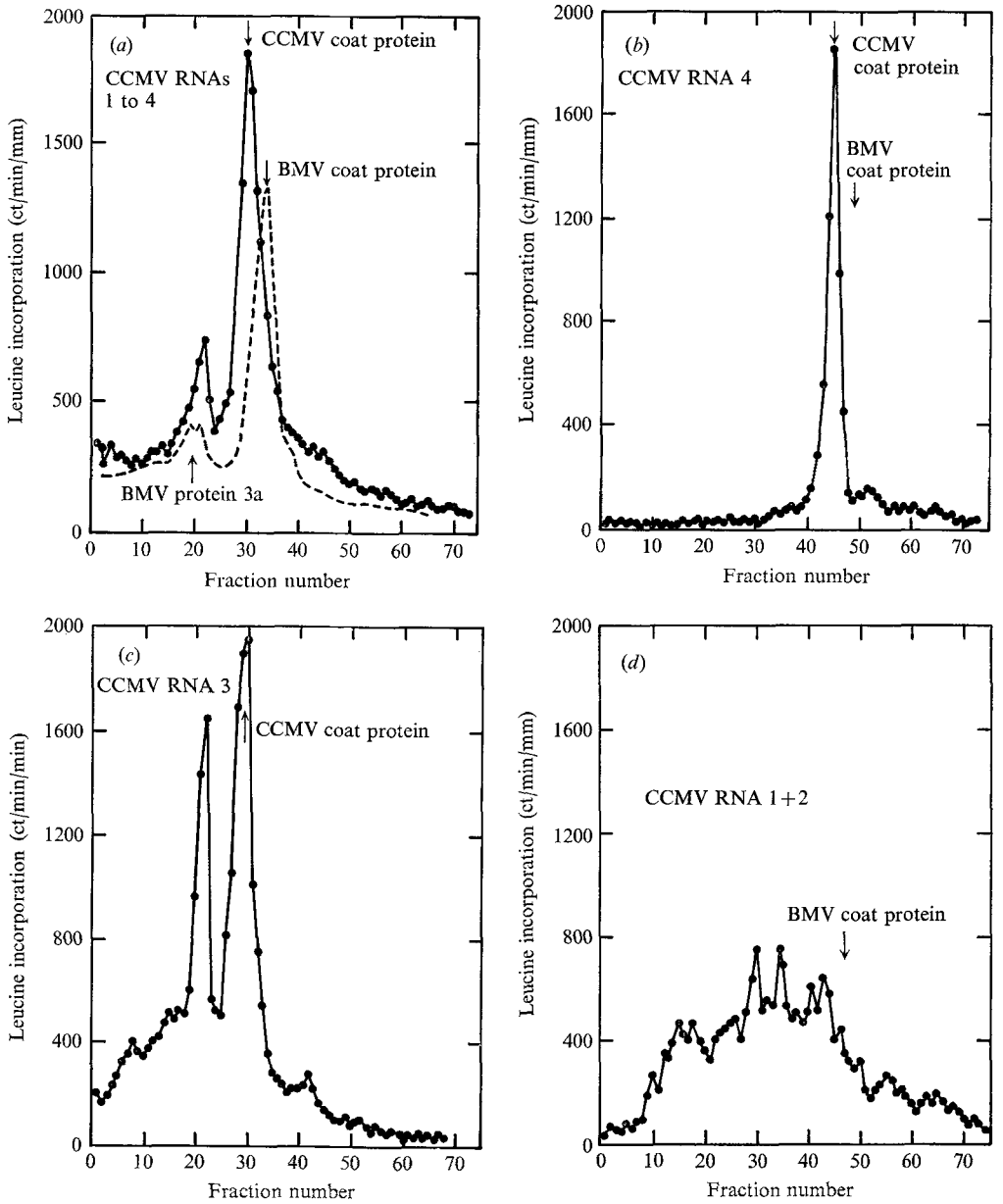

Fig. 2. SDS-polyacrylamide gel analysis of the products of translation of CCMV RNAs. (*a*) CCMV RNA 1 to 4 natural mixture, 20 μg RNA/200 μl reaction mixture. Incorporation of [^{3}H]-leucine (15 Ci/mmol): the other 19 amino acids were present, each at 0·025 mM. ●—●, CCMV RNA 1 to 4 products; - - -, BMV RNA products co-electrophoresed (16 h) as mol. wt. markers. (*b*) CCMV RNA 4 product. 8 μg RNA/200 μl; [^{14}C]-leucine (311 μCi/μmol). 50 μl of the 200 μl reaction mixture was applied to the gel. BMV authentic coat protein (labelled with [^{3}H]-leucine *in vivo*) which was co-electrophoresed (for 24 h) as a marker, is indicated by an arrow. (*c*) CCMV RNA 3 products. 10·2 μg RNA/200 μl; [^{14}C]-leucine (311 μCi/μmol). 100 μl of a 200 μl reaction mixture was applied to the gel, which was electrophoresed for 16 h. (*d*) CCMV RNA 1+2 products. 10 μg RNA/200 μl (approx. equimolar amounts of RNA 1 and RNA 2). [^{14}C]-leucine, 311 μCi/μmol. The products of BMV RNA 1 to 4, labelled with [^{3}H]-leucine, were co-electrophoresed for 24 h. The migration position of BMV coat protein is indicated by an arrow. Migration is from left to right.

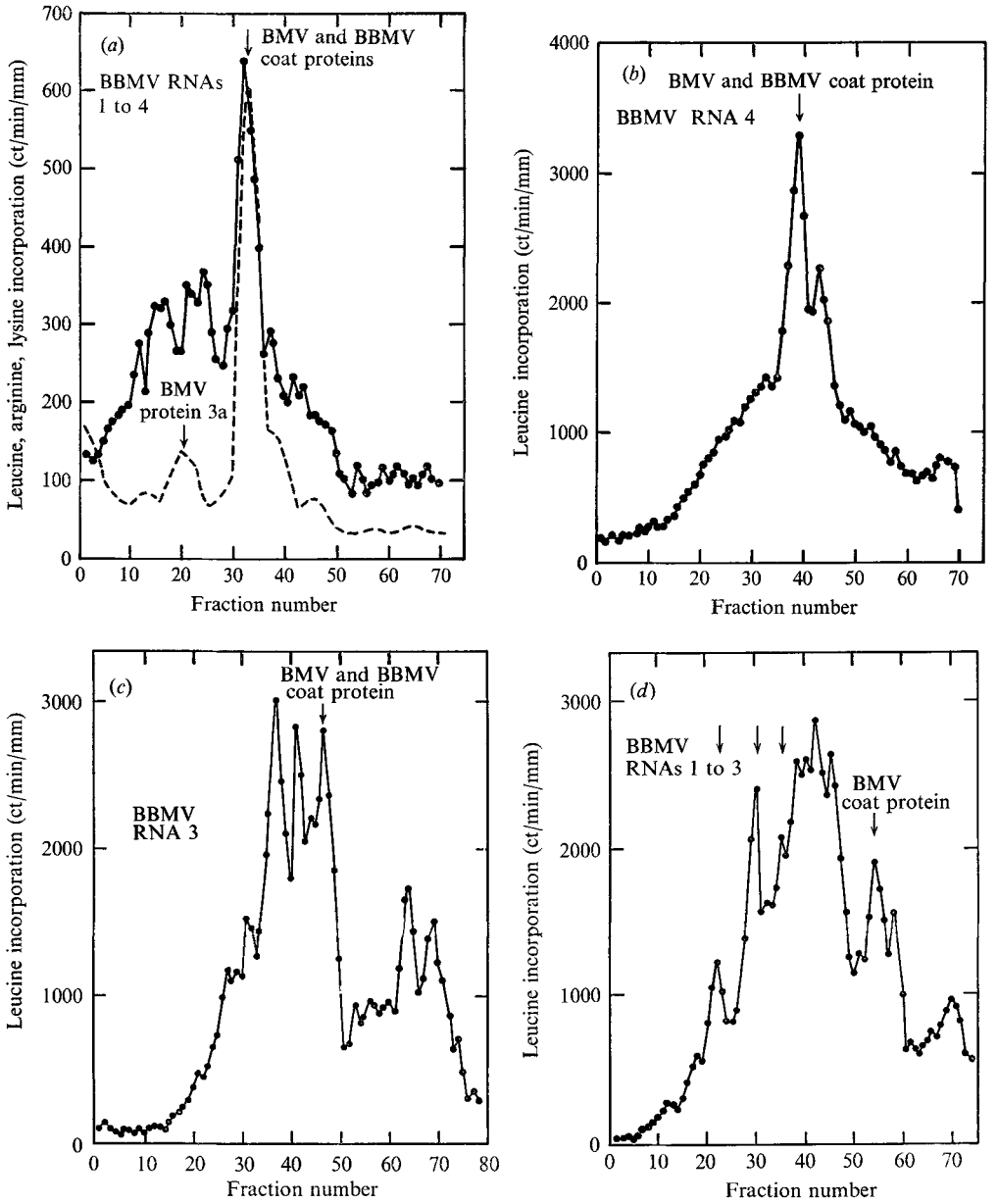

Fig. 3. SDS-polyacrylamide gel analysis of the products of translation of BBMV RNAs. (*a*) BBMV RNA 1 to 4 natural mixture, 10 μg RNA/200 μl. A 100 μl sample of reaction mixture (see Fig. 2) was applied to the gel. BBMV RNA products, labelled with [^{3}H]-arginine, -lysine and -leucine were co-electrophoresed for 16 h with BMV RNA 1 to 4 products labelled with [^{14}C]-leucine (50 μl sample). ●—●, BBMV RNA products; - - -, BMV RNA products. (*b*) BBMV RNA 4 products, 8 μg RNA/200 μl reaction mixture; [^{3}H]-leucine (50 Ci/mmol). A 50 μl sample was electrophoresed for 16 h. (*c*) BBMV RNA 3 products, 8·9 μg RNA/200 μl reaction mixture; [^{3}H]-leucine (50 Ci/mmol). A 100 μl sample was co-electrophoresed with [^{14}C]-labelled authentic BMV coat protein (migration position indicated by arrow). (*d*) BBMV RNA 1+2+3 products. Approx. equal amounts of RNAs 1, 2 and 3, 10 μg RNA/200 μl reaction mixture; [^{3}H]-leucine (50 Ci/mmol). A 100 μl sample was co-electrophoresed (for 24 h) with [^{14}C]-labelled authentic BMV coat protein, to indicate the position of BBMV coat protein, which is the same size (see arrow). Other arrows indicate the positions of the products of *in vitro* translation of BMV RNA 1+2, synthesized in a separate experiment. Migration is from left to right.

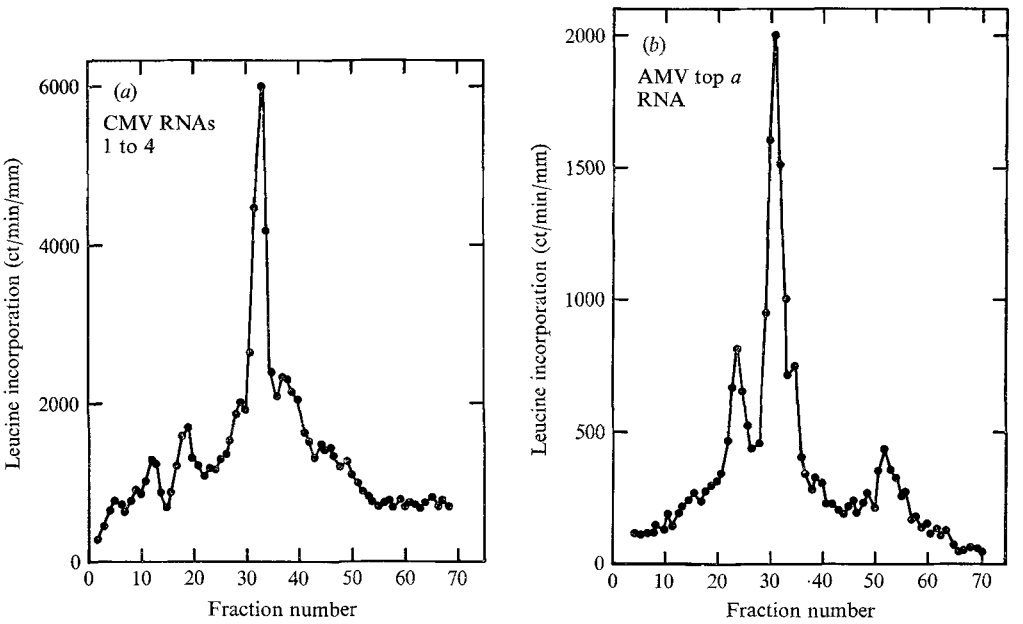

Fig. 4. Translation of CMV RNA 1 to 4 and AMV tc*a* RNA. (*a*) CMV RNA natural mixture, 10 μg RNA/200 μl reaction mixture (see Fig. 2); [^{14}C]-leucine (311 μCi/μmol). A 100 μl sample was electrophoresed for 16 h. (*b*) AMV RNA, top component *a*, 8 μg RNA/200 μl reaction mixture. Incorporation of [^{14}C]-leucine (311 μCi/μmol). A 100 μl sample was electrophoresed for 16 h. Migration is from left to right.

a minor band and one major product (Fig. 2*a*), whose electrophoretic mobility was identical to that of authentic CCMV coat protein. The small CCMV RNA component (RNA 4) also induced this product (Fig. 2*b*). CCMV RNA 3 is translated into two products, the putative coat protein and a larger polypeptide the same size as BMV RNA 3 product 3a (Fig. 2*c*). CCMV RNAs 1 and 2 together are not translated into products that are clearly defined bands on acrylamide gels (Fig. 2*d*). In general, the products are large, but heterogenous. Possibly these larger RNAs ($1{\cdot}15$ and $1{\cdot}07 \times 10^6$) are not translated as well as the smaller RNA 3 ($0{\cdot}85 \times 10^6$) and RNA 4 ($0{\cdot}23 \times 10^6$) in terms of fidelity of the products.

BBMV RNA mixed and separated components are translated in a similar manner to CCMV RNA (Fig. 3). The major product of the RNA mixture migrates in electrophoresis as does authentic BBMV coat protein and co-electrophoreses with *in vitro* synthesized BMV coat protein (Fig. 3*a*). BMV and BBMV coat proteins are the same size (Hull, 1972). A coat protein-sized polypeptide is the predominant product of BBMV RNA 4 (Fig. 3*b*) although a heterogenous collection of products is also obtained. BBMV RNA 3 produces several products (Fig. 3*c*). One of these products electrophoreses in the position of coat protein. Faster migrating polypeptides are believed to be incomplete products. The products of BBMV RNA 1, 2 and 3 together (Fig. 3*d*) are similar to those of RNA 3, with the addition of at least two larger products, which coincide in mobility with two of the products of BMV RNA 1+2 translation. However, other products of BMV RNA 1+2 (not shown) have no counterparts among the BBMV RNA 1 and 2 translation products. It is possible that some cistrons on BBMV RNA 1 and 2 cannot be translated by wheat germ ribosomes. In several experiments, the radioactivity incorporated into BBMV RNA products was less than with BMV and CCMV RNAs, and all of the BBMV gel patterns indicate some hetero-

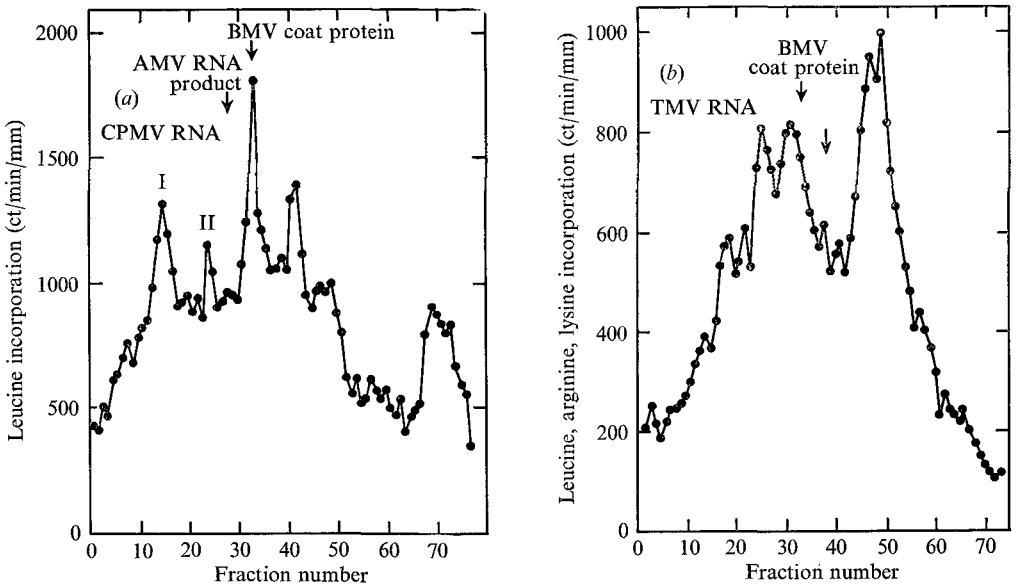

Fig. 5. Translation of large plant virus RNAs. (*a*) Multicomponent: CPMV RNAs 1+2. 5·6 μg CPMV RNA (natural mixture) was translated in a 100 μl reaction mixture, containing [^{3}H]-arginine, lysine and leucine. The product was co-electrophoresed (for 18 h) with the *in vitro* product of AMV tc*a* RNA (see Fig. 4*b*). In a similar experiment, BMV coat protein was used as a marker, and its position is also shown. (*b*) Single component: TMV RNA. 34 μg TMV RNA was translated in a 200 μl reaction mixture, containing [^{3}H]-arginine, lysine and leucine. A 100 μl sample was co-electrophoresed ($\sim$16 h) with [^{14}C]-labelled BMV RNA 1 to 4 products as mole. wt. markers. An arrow indicates the position of BMV coat protein, and another indicates the expected position of TMV coat protein. Migration is from left to right.

geneity of the products. Under the conditions used, BBMV RNA does not seem to be such a good *in vitro* messenger as BMV and CCMV RNAs, either in efficiency or fidelity.

CMV RNA, a mixture of four components (mol. wt. 1·25, 1·19, 0·96 and $0{\cdot}5\times10^6$) was translated into a major product (Fig. 4*a*) of mol. wt. about 25000, which is consistent with one of the reported mol. wt. for CMV coat protein (24000) although there is some uncertainty as to the CMV coat protein size (Brown & Hull, 1973). Unfortunately we did not have authentic CMV coat protein available as a marker. Fig. 4*a* also shows unidentified minor products, of larger mol. wt. The pattern resembles that of BMV and CCMV RNAs 1 to 4. Presumably, the coat protein-like major polypeptide is a product of the smallest RNA component but this was not tested directly using purified CMV RNA 4.

AMV RNA efficiently directs amino acid incorporation in a wheat germ cell-free system (L. van Vloten-Doting, personal communication). We have done several incorporations, especially with AMV top component *a* (tc*a*) and concur with this conclusion. For example, our results (Fig. 4*b*) show a polypeptide of mol. wt. about 25000, consistent with AMV coat protein (Kraal *et al.* 1972). A smaller quantity of a larger polypeptide was also evident which may be a product of top component *b*, of which there is approx. 10% contamination in our AMV top component *a* preparation.

The above experiments relate to multicomponent plant viruses, one genome component of which is a very small RNA ($0{\cdot}25$ to $0{\cdot}5\times10^6$). We wished also to test RNAs from viruses which are multicomponent, but which do not have a very small RNA so that the coat protein cistron(s) must be on larger ($>1{\cdot}0\times10^6$) RNAs. Cowpea mosaic virus (CPMV) has

Table 1. *Comparison of amino acid incorporation directed by various virus RNAs*

Virus RNA	Mol. wt. RNA $\times 10^{-6}$*	pmol RNA	Lysine incorporation ct/min	Lysine incorporation pmol per pmol RNA
		Expt. A		
BMV 4	0·28	14·2	32990	78·1
CCMV 4	0·23	12·5	32070	65·9
BBMV 4	0·36	11·1	9213	28·6
AMV tc*a*	0·33	12·1	8919	25·4
Qβ	1·5	13·3	1970	5·1
TMV	2·0	12·5	8445	23·3
AvMV 35S	3·0	6·6	1010	5·3
MEV	2·6	7·6	935	4·2
		Expt. B		
BMV 4	0·28	14·2	32454	74·2
BMV 3	0·75	10·6	12303	37·6
BMV 1+2	1·00	10·0	45856	148·8
CCMV 4	0·23	17·3	39237	73·6
CCMV 3	0·85	9·4	12437	42·9
CCMV 1+2	1·00	10·0	38359	124·5
BBMV 4	0·36	11·1	7429	21·7
BBMV 3	0·90	9·9	3707	12·1
BBMV 1+2(+3)	1·00	10·0	9995	32·5

In vitro reaction mixtures were incubated for 90 min. Sp. act. of [^{14}C]-lysine 26·3 μCi/μmol for Expt. A; 28 μCi/μmol for Expt. B. Counting efficiency on Whatman 3 MM discs approx. 50 %.

* The mol. wt. for the RNAs are not necessarily exact: see Brown & Hull (1973). For bromovirus RNAs 1+2, an average mol. wt. of $1{\cdot}0 \times 10^6$ was assumed.

RNA components of about 2·6 and $1{\cdot}5 \times 10^6$ mol. wt. and it has two coat proteins of mol. wt. 49000 and 27500. The gel pattern for CPMV is quite complex (Fig. 5*a*), showing at least six resolved bands together with a heterogenous collection of products. Two of the bands (labelled I and II) correspond with the expected positions of the two CPMV coat proteins.

TMV RNA was chosen as an example of a large, single component RNA. Some experiments with TMV RNA have already been reported. Efron & Marcus (1973*b*) used wheat embryo extracts (not commercial wheat germ extracts) and Roberts, Mathews & Bruton (1973) used a wheat germ extract under rather different conditions from ours. They showed that TMV coat protein-like peptides, but not homogenous coat protein, is synthesized. In agreement with these other reports, we find that TMV RNA directs the incorporation of amino acids efficiently, but the acrylamide gel patterns suggest a disperse, heterogeneous mixture of products (Fig. 5*b*) without a distinct coat protein-sized peak.

For purposes of comparison we also tested two examples of larger RNAs from animal viruses, Maus–Elberfeld (MEV) and avian myoblastosis virus (AvMV). AvMV 70S RNA was used, and also AvMV 35S plus 4 to 5S RNA produced by heating 70S RNA at 70 °C for 3 min. These RNAs induced an incorporation of amino acids into trichloroacetic acid precipitable material, increasing with incubation time. However, the incorporated radioactivity was low, and when analysed on SDS polyacrylamide gels, no specific products were found.

Comparison of translation efficiencies

From the experiments described above, it was evident that some heterologous RNAs are more efficient than others at radioactive amino acid incorporation, and production of distinct virus protein-like products. This variation in efficiency was evident quantitatively in experiments in which various RNAs were translated under the same conditions with the same radioactive amino acid. An example is given in Table 1 (Expt. A), in which the incorporation of [^{14}C]-L-lysine is compared. The small RNA components of BMV, CCMV, BBMV and AMV were compared with the polycistronic RNAs of TMV, AvMV, MEV and Qβ bacteriophage, using similar quantities of virus RNA. The efficiency of CCMV RNA 4 is similar to BMV RNA 4. BBMV RNA 4, AMV top component *a* and TMV RNA are less efficient, but comparable to each other. AvMV and MEV RNA show a similar incorporation efficiency to Qβ RNA. Table 1 (Expt. A) also shows the approximate mol. wt. of the RNAs used. There is a suggestion of a correlation of amino acid incorporation efficiency with size of the RNA. Similar results were also obtained with amino acids other than lysine, including mixtures of several amino acids.

In Table 1, Expt. B, amino acid incorporation efficiency directed by the various RNA components of the bromovirus group is compared. Here again it is evident that the small (RNA 4) components are more efficient than the intermediate sized RNA 3 components. The large RNA 1 and 2 components are apparently more efficient than the small RNAs in terms of amino acid incorporation, although as shown above (Fig. 2*d*) the products are heterogeneous and disperse.

DISCUSSION

We have previously shown that Qβ RNA is translated with poor efficiency in wheat embryo or wheat germ cell-free extracts (Davies & Kaesberg, 1973). However, rabbit globin RNA can also be translated in a wheat embryo extract (Efron & Marcus, 1973*a*), as can the RNA of the satellite tobacco necrosis virus (Klein *et al.* 1972), both quite efficiently. This suggested that a heterologous virus RNA need not necessarily be a poor messenger in this cell-free system. Our results clearly indicate that several RNAs from viruses not known to infect wheat can indeed be efficient messages. CCMV RNA is particularly good, and AMV top component *a*, CMV and BBMV RNAs are also quite well translated. The coat protein cistrons of these viruses appear to be the most successful messengers, and probably the least specific for ribosomes from their natural host range plants. If host specificity is determined to some extent at the translation level, it may involve cistrons other than the coat protein cistrons.

CCMV RNA is translated with a similar efficiency to BMV RNA (Table 1). BBMV RNA, although a member of the bromovirus group, is less efficiently translated by wheat germ ribosomes. TMV RNA shows a similar incorporation activity to BBMV RNA 4 and AMV top component *a* RNA (Table 1) but the products are polydisperse, and are mostly polypeptides which do not correspond to known virus proteins. AvMV and MEV RNAs show a similar incorporation efficiency to Qβ RNA (Table 1), but whereas the bacteriophage RNA is translated with fidelity (see Davies & Kaesberg, 1973), the animal virus RNAs do not produce any AvMV or MEV proteins at all. This may not necessarily be due to the more distant phylogenetic relationship between animal viruses and wheat plants. Rabbit globin RNA is correctly translated by wheat embryo extract (Efron & Marcus, 1973*a*) and recently, we have found that reovirus ssRNAs are translated with good efficiency by this wheat germ system (unpublished results). Globin RNA is a small monocistronic

message, and reovirus has a divided genome which includes small, monocistronic RNAs. Our results suggest that there is a general trend for small messengers to be translated more efficiently than larger ones (see Table 1). A correlation of messenger size with translatability is perhaps somewhat surprising. It possibly reflects a relationship not with size directly, but with properties associated with RNA size, such as complexity of RNA conformation and number of cistrons. All of the RNAs efficiently translated into definite polypeptides are thought to be monocistronic, or dicistronic and contain the coat protein cistron. Coat protein translation seems to be intrinsically easy to initiate, except perhaps in cases where the cistron is inaccessible due to the complex conformation of large RNAs.

We conclude that the coat protein cistrons of the multicomponent virus RNAs display less species (or host) specificity than cistrons for other virus proteins, and that this eukaryote (wheat) protein synthesizing system has a preference for monocistronic messengers, particularly small RNAs. It is interesting in this respect that the multicomponent bromoviruses which have a coat protein cistron on the RNA 3 component have evolved a monocistronic form of the coat protein cistron, the cistron for the virus protein required in the largest quantity.

We are indebted to Dr Lous van Vloten-Doting for providing us with CMV and AMV tc*a* RNAs and for discussing with us some preliminary results. We thank Dr T. C. Hall, R. Fulton, J. S. Semancik, R. Rueckert and R. Wells for gifts of virus or RNA. We also thank Claudia Benike for her expert technical assistance.

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