TRANSLATION OF VIRUS mRNA: COMPARISON OF REOVIRUS AND BROME MOSAIC VIRUS SINGLE-STRANDED RNAs IN A WHEAT GERM CELL-FREE SYSTEM

Jeffrey W. Davies[1] and Charles E. Samuel[2]

[1]Biophysics Laboratory of the Graduate School
University of Wisconsin
1525 Linden Drive
Madison, Wisconsin 53706

[2]Section of Biochemistry and Molecular Biology
Department of Biological Sciences
University of California Santa Barbara
Santa Barbara, California 93106

Received June 9,1975

SUMMARY: Wheat germ cell-free extracts translate reovirus ssRNA as efficiently as brome mosaic virus ssRNA. The magnesium optima, kinetics, dependence on messenger RNA concentration, and high level amino acid incorporation are similar for the animal virus and plant virus messenger RNAs. With unfractionated mRNAs from either virus, the largest messengers are not translated, or are very poorly translated. The smaller mRNAs, including the reovirus messengers for polypeptides σ_1, σ_3, σ_2 and μ_1, are translated with fidelity. Evidence suggests that after several translation cycles, the ribosomes are not efficiently released, perhaps creating a "ribosome-jam" resulting in the synthesis of incomplete polypeptides.

Brome mosaic virus and reovirus are representatives of different plant and animal virus groups whose genetic information is distributed among several RNAs. The reovirus genome consists of ten segments of double-stranded RNA; a virion core-associated RNA-dependent RNA polymerase catalyzes the synthesis *in vitro* of 10 single-stranded RNA species of three size classes: small (*s*), molecular weight 0.3 to 0.4 x 10^6; medium (*m*), 0.6 to 0.7 x 10^6; and large (*l*), 1.3 to 1.4 x 10^6 (1,2). The reovirion consists of seven polypeptides (3), whereas reovirus-infected cells contain nine detectable virus-specific polypeptides of three size classes: λ (molecular weight 140 to 155,000); μ (72 to 88,000); and σ (34 to 42,000) (4). The brome mosaic virus genome consists of four species of single-stranded RNA (5) which are of the same polarity as mRNA. The two smallest BMV ssRNAs, RNA3 and RNA4, code for the coat protein; RNAs 1, 2 and 3 also code for other polypeptides (6). The regulation of the translation

of the individual messenger RNAs of plant or animal viruses such as BMV and reovirus is not yet understood. It is possible that some RNAs are intrinsically better messengers than others (7) and that some RNAs prevent the translation of others by being themselves preferentially selected. An efficient cell-free translation system would be useful in investigating these problems.

Reticulocyte extracts efficiently synthesize reovirus polypeptides, but also contain globin mRNA and make large quantities of globin. Mouse L cell or ascites tumor cell extracts catalyze the synthesis of five to seven virus-specific polypeptides, with an overall stimulation of up to 10-fold (8,9). We wished to know how well reovirus RNAs are translated in the heterologous wheat germ system (6,10-15), and how this compares with translation of mRNA from a virus which can infect that plant. We have previously reported that wheat germ translates reovirus mRNAs (12). In this communication, we identify the products, make a comparison with the ascites system, and in particular, compare the *in vitro* translation of unfractionated reovirus (heterologous) and brome mosaic virus mRNAs (homologous) in the plant cell-free extracts.

RESULTS AND DISCUSSION: As shown in Table I, unfractionated reovirus ssRNA stimulates amino acid incorporation in wheat germ extracts to the same extent as does BMV ssRNA. The activity is about 10-fold greater than with mouse ascites cell extract. More than 1000 *p*moles leucine are incorporated (100 µl reaction), the efficiency being >100 *p*moles leucine per µg of added animal or plant virus ssRNA. The magnesium ion optimum concentration (3.5 mM) is comparable for the translation of both reovirus and BMV RNAs (Fig. 1a). Wheat germ S-23 extracts show an optimum concentration of unfractionated reovirus ssRNA mixture of about 20 µg per 100 µl reaction mixture, similar to that for BMV RNA (Fig. 1b). In the absence of exogenously added animal or plant virus mRNA, the background activity of the non-preincubated wheat germ system is very low (Fig. 1c). The kinetics of amino acid incorporation directed by similar quantities of unfractionated reovirus and BMV RNAs are nearly identical; the reaction continues for about 60 min, after which there is only minimal

TABLE 1

COMPARISON OF AMINO ACID INCORPORATION EFFICIENCY DIRECTED BY REOVIRUS ssRNA AND BMV RNAs 1-4

	Radioactivity cpm/100 µl	Stimulation due to viral RNA	*p*moles leucine/ 100 µl	*p*moles leucine/ µg RNA
no RNA	215	--	7	--
BMV RNAs (10 µg)	39,340	182	1356	135
REO ssRNA (6 µg)	29,624	137	1021	170
REO ssRNA (12 µg)	46,267	215	1595	133

The wheat germ *in vitro* system (10) contained the quantity of viral RNA indicated in the table. Low specific activity L-[^{14}C]-leucine was used (26 µCi per µmole). Reaction mixtures (100 µl) were incubated at 31° for 60 min, then pipetted onto TCA pretreated (dried) disks. After washing in hot trichloroacetic acid, then ethanol and ether, the disks were scintillation counted at 50% efficiency.

The radioactivity (cpm) has been corrected for a zero time control of 546 cpm (complete reaction mixture). Stimulation is calculated as the increase in radioactivity due to the viral RNA, compared with the control with no added RNA.

activity (Fig. 1c). In this time, however, over 1000 *p*moles leucine are incorporated.

The products of reovirus and BMV ssRNA translation in extracts of commercial wheat germ were analyzed by polyacrylamide gel electrophoresis (Fig. 2). With unfractionated BMV RNA, the major product, as established for dry seed derived wheat embryo extracts (6), is the viral coat protein (Fig. 2a). Despite the high efficiency of overall amino acid incorporation in response to unfractionated reovirus mRNA, there is apparently not a close agreement between the electrophoretic mobilities of the *in vitro* products and reovirion polypeptides (Fig. 2a). There is a conspicuous absence of large polypeptides, but

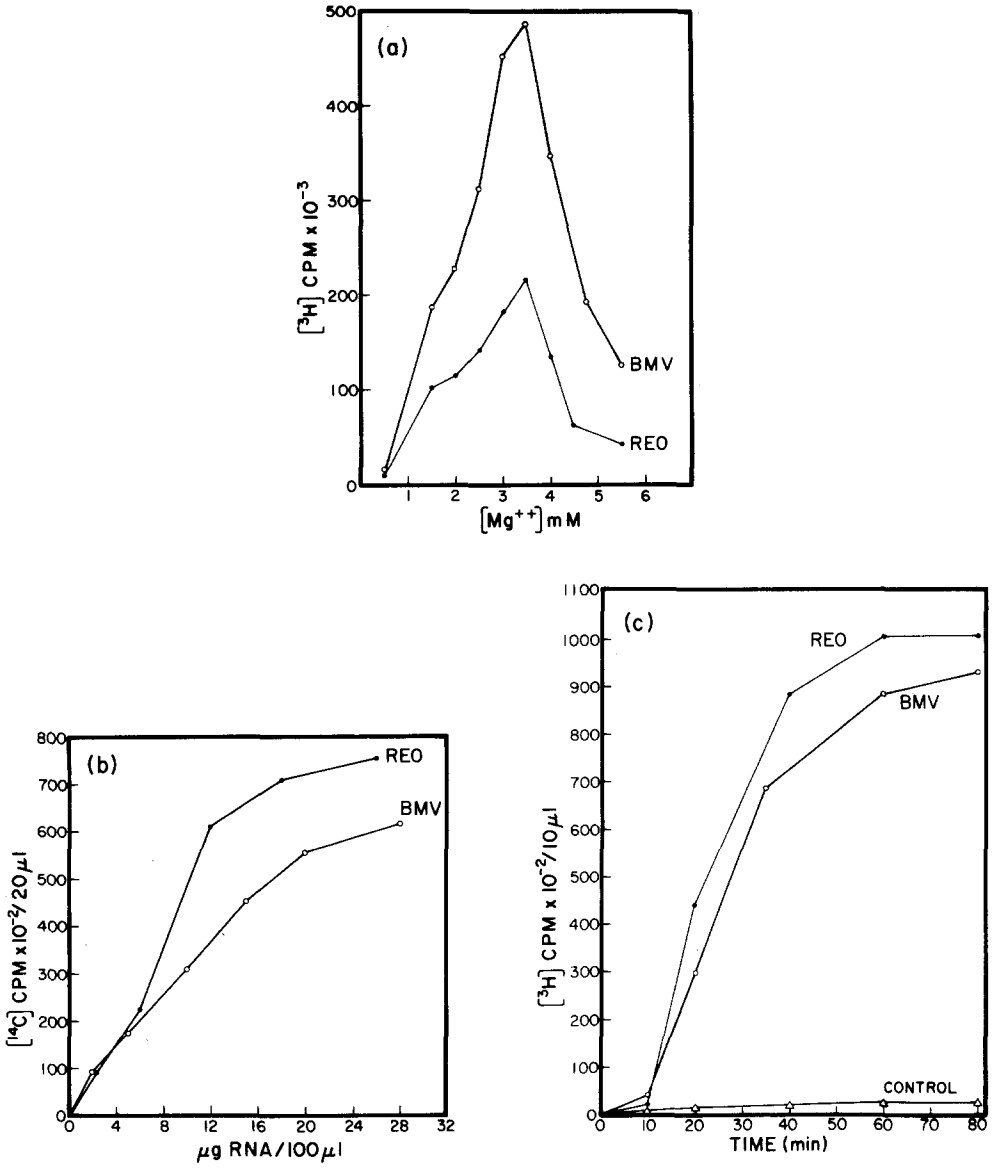

Fig. 1. Some characteristics of amino acid incorporation in wheat germ extracts directed by reovirus and brome mosaic virus ssRNAs.
(a) Effect of magnesium ion concentration. The wheat germ system was as previously described (10), except various levels of magnesium acetate were added to the reaction mixture. Reovirus ssRNA, 6 µg per 100 µl. BMV RNA, 10 µg per 100 µl. The radioisotope was L-[^{3}H]lysine, 55 Ci per mmole. Aliquots of 10 µl were counted on filter paper disks.
(b) Effect of messenger RNA concentration. The reaction was as in (a), but with 3.5 mM [Mg^{++}] and using L-[^{14}C]leucine, 348 mCi per mmole.
(c) Kinetics of incorporation. The reaction was as in (b), but with 12 µg per 100 µl reovirus ssRNA.

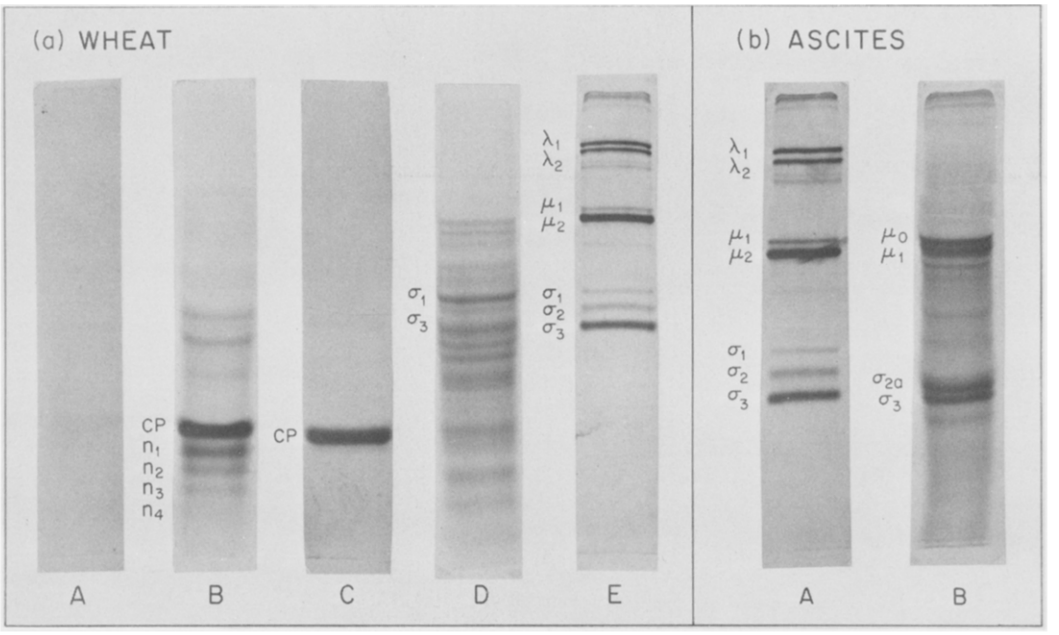

Fig. 2. Autoradiograms of brome mosaic virus and reovirus polypeptides synthesized *in vitro*. Polypeptides were labeled with [^{35}S]methionine, and were separated by sodium dodecyl sulfate-urea-polyacrylamide (18) slab gel electrophoresis (19).
(a) Polypeptides synthesized in wheat germ extract (10) Conditions of electrophoresis: 8% acrylamide gel; 16 hr at 35 V (50 ma) per slab at room temperature; direction from top to bottom. (A) wheat extract, endogenous synthesis; (B) wheat extract with BMV RNA 1-4; (C) BMV coat protein synthesized *in vivo*; (D) wheat extract with reovirus ssRNA; (E) reovirion polypeptides synthesized *in vivo*.
(b) Polypeptides synthesized in ascites cell-free extract (19). Conditions of electrophoresis as in (a), except for 20 hr. (A) ascites extract, reovirus ssRNA added; (B) reovirion polypeptides synthesized *in vivo*.

---

a considerable number of intermediate sized products present, which may be incomplete nascent polypeptides. Similarly, translation of unfractionated BMV RNAs yields no large products, but does produce a series of small polypeptides in addition to the major coat protein product. The major reovirus-specific polypeptides synthesized in the wheat germ extracts appear to be σ_1 (42,000 molecular weight) and σ_3 (34,000 molecular weight), with smaller amounts of σ_2 (38,000 molecular weight) and μ_1 (80,000 molecular weight) as identified by electrophoretic mobility. The fidelity of translation was investigated by

tryptic digest peptide analysis of the *in vitro* products; the peptide patterns yielded by the synthesized products *in vitro* were very similar to authentic virion polypeptides synthesized *in vivo*.

In wheat germ cell-free extracts there is a general tendency for translation of small messenger RNAs to be more efficient (11). In the case of BMV, the small monocistronic RNA4 prevents the translation of the larger RNAs present in an unfractionated mixture; however, large BMV RNAs 1 and 2 are efficiently translated into large polypeptides when examined in the absence of the small RNAs (16). This could be a regulatory function in the process of BMV message translation (6,16). In this respect, it is interesting that there appears to be a similarity between *in vitro* translation characteristics of unfractionated BMV and reovirus RNAs. In the absence of the smaller ssRNAs, we might expect therefore that λ RNAs can be translated. Both *et al.* (21) find that wheat germ extracts can translate a mixture of λ RNAs. It remains to be seen if purified individual λ RNAs can be translated *in vitro*, and if other ssRNAs or their products suppress the translation. It is clear, however, that caution should be taken in interpreting *in vitro* results since results with extracts from different organisms, including wheat germ, may differ, particularly in heterologous systems. For example, σ_1 and σ_3 are the major small reovirus polypeptides synthesized in response to unfractionated reovirus mRNA in the wheat germ system (Fig. 2a), while in contrast, σ_{2a} and σ_3 are the major small polypeptides synthesized in the ascites cell-free system (Fig. 2b). In addition, significant amounts of μ_0 and μ_1 are also formed in response to exogenously added unfractionated reovirus mRNA in the ascites system (3) as shown in Fig. 2b. When translation is initiated *in vivo*, extracts from reovirus-infected mouse L cells can synthesize λ polypeptides *in vitro* (17). It is feasible that, *in vivo*, the ssRNAs undergo some modification, allowing the large RNAs to be initiated. Both, Lavi and Shatkin (21) also find that with the unfractionated reovirus ssRNA, the large RNAs are less efficient, although in their system, the λ products are synthesized.

This apparent discrepancy may be due to the different source of wheat germ, or to the fact that under their conditions, *in vitro* synthesis continues for about two hours, and in this time enough λ polypeptides are synthesized to be detected. Also, the appearance of minor products depends on the quantity of product loaded onto the gel. Using high specific activity ^{3}H amino acid labeling of reovirus polypeptides in our system, we have found very small amounts of products co-electrophoresing with authentic λ polypeptides when the total reaction mixture was fractionated on gels and analyzed by high efficiency scintillation counting. Autoradiographic analysis of ^{35}S labeled products on slab gels, however, under the conditions described here, does not detect these polypeptides (Fig. 2a, D).

Polyacrylamide gel electrophoresis of polypeptide products synthesized *in vitro* in the wheat germ system in response to reovirus or BMV ssRNAs reveals significant amounts of radioactively-labeled product migrating faster than the smallest complete respective virus-specific polypeptide (Fig. 2a). In the case of unfractionated BMV RNA where the coat protein is the only major polypeptide synthesized, the molecular weight of the additional components, n_1,

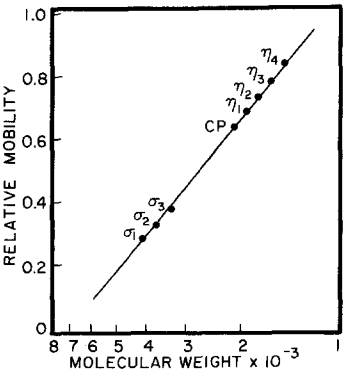

Fig. 3. Determination of the molecular weight values of the BMV-specified polypeptide components (n_1, n_2, n_3 and n_4--see Fig. 2a) synthesized *in vitro* in wheat germ extract (10,20). The four marker proteins used were reovirus capsid polypeptides σ_1 (42,000), σ_2 (38,000) and σ_3 (34,000), and BMV coat protein CP (20,000).

n_2, n_3 and n_4 (Fig. 2a), progressively decreases by a relatively constant value of about 1500 to 1800 daltons (Fig. 3), approximately the amount of protein that would be coded for by the genetic information covered by a ribosome on a messenger RNA. It appears, therefore, that several of the low molecular weight polypeptides formed in response to exogenously added mRNA in the wheat system represent incomplete nascent chains which are formed by defective termination as a result of a "ribosome-jam" near the 3' end of the mRNA. This phenomenon may account for polypeptides translated from reovirus ssRNA which do not correspond to viral polypeptides (Fig. 2a, D).

ACKNOWLEDGMENTS: This work was supported in part, by Public Health Service research grants AI-08909 and AI-01466 from the National Institutes of Health, and by special grant 759 from the American Cancer Society. The authors gratefully acknowledge the helpful discussions and laboratory hospitality of Drs. W. K. Joklik and Paul Kaesberg.

REFERENCES:

1. Joklik, W. K. (1973) in Viral Replication and Cancer, eds., J. L. Melnick, S. Ochoa, and J. Oro, pp. 123-151, Editorial labor, S. A. Barcelona.
2. Skehel, J. J., and Joklik, W. K. (1969) Virology 39, 822-831.
3. Smith, R. E., Zweerink, H. J., and Joklik, W. K. (1969) Virology 39, 791-810.
4. Zweerink, H. J., McDowell, M. J., and Joklik, W. K. (1971) Virology 45, 716-723.
5. Lane, L. C., and Kaesberg, P. (1971) Nature New Biol. 232, 40-43.
6. Shih, D. S., and Kaesberg, P. (1973) Proc. Nat. Acad. Sci. U.S.A. 70, 1799-1803.
7. Lodish, H. F. (1974) Nature 251, 385-388.
8. McDowell, M. J., Joklik, W. K., Villa-Komaroff, L., and Lodish, H. F. (1972) Proc. Nat. Acad. Sci. U.S.A. 69, 2649-2653.
9. Graziadei, W. D., and Lengyel, P. (1972) Biochem. Biophys. Res. Commun. 46, 1816-1823.
10. Davies, J. W., and Kaesberg, P. (1973) J. Virol. 12, 1434-1441.
11. Klein, W. H., Nolan, C., Lazar, J. M., and Clark, J. M. (1972) Biochemistry 11, 2009-2014.
12. Davies, J. W., and Kaesberg, P. (1974) J. Gen. Virol. 25, 11-20.
13. Öberg, B., Saborio, J., Persson, T., Everitt, E., and Philipson, L. (1975) J. Virol. 15, 199-207.

14. Prives, C. L., Aviv, H., Paterson, B. M., Roberts, B. E., Rozenblatt, S., Revel, M., and Winocour, E. (1974) Proc. Nat. Acad. Sci. U.S.A. 71, 302-306.
15. Both, G. W., Moyer, S. A., and Banerjee, A. K. (1975) Proc. Nat. Acad. Sci. U.S.A. 72, 274-278.
16. Shih, D. S., Davies, J. W., and Kaesberg, P. (1975) Proc. First Intersect. Congr. Internat. Assoc. Microbiol. Soc. (Tokyo). In press.
17. McDowell, M. J., and Joklik, W. K. (1971) Virology 45, 724-733.
18. Zweerink, H. J., and Joklik, W. K. (1970) Virology 41, 501-518.
19. Samuel, C. E., and Joklik, W. K. (1974) Virology 58, 476-491.
20. Weber, K., and Osborn, M. (1969) J. Biol. Chem. 244, 4406-4412.
21. Both, G. W., Lavi, S., and Shatkin, A. J. (1975) Cell 4, 173-180.