# TRANSLATION OF MAMMALIAN mRNAs IN CELL-FREE EXTRACTS OF ASCITES CELLS AND WHEAT GERM

Michael B. Mathews
Department of Biochemistry and Biophysics
University of California, San Francisco
San Francisco, California 94143

and
Bryan E. Roberts
Department of Biophysics
Weizmann Institute of Science
Rehovot, Israel

Translation constitutes an important and reliable means, though not the only means, for the identification and assay of mRNAs. The value of well-characterized systems capable of translating a variety of heterologous mRNAs has become increasingly apparent over the last 3–4 years. Currently systems of several sorts are available, ranging from highly fractionated cell-free extracts to the unrefined reticulocyte lysate and intact *Xenopus* oocytes. In the present article, we review some properties and applications of systems of an intermediate level of complexity. These systems, preincubated S-30 extracts (post-mitochondrial supernatants) of eucaryotic cells, offer the advantage of a low endogenous background together with the convenience of relatively simple preparation and use. In this regard they resemble the analogous extracts of bacterial cells which have played a major role in investigations of the metabolism of procaryotic mRNAs. These principles are illustrated by reference to S-30 extracts of Krebs II ascites cells and of wheat germ.

## Ascites cell system

The Krebs II tumour originated as a spontaneous mouse carcinoma and was passed into the ascitic form in which state it can be conveniently propagated. One mouse can yield some 5 ml packed cells (about $10^9$ cells) from an inoculum of $1–2 \times 10^7$ cells given one week previously. It is this fecundity, rather than any unique properties of these cells, which has led to the widespread reliance on this system. Comparable extracts have been prepared from a variety of other cell types, such as rat and mouse liver, Landschutz ascites cells, and several lines of cultured cells (1, 2). These extracts exhibit similar properties to those of the Krebs ascites system.

The key step in the preparation of the ascites system is preincubation of the S-30 under conditions of protein synthesis. This exhausts the endogenous protein synthesizing capacity of the system without destroying its ability to read added mRNAs (3). As a result the response to the addition of exogenous mRNA can be

measured simply in terms of the incorporation of labelled amino acids into acid-insoluble material, and product analysis is simplified. Other steps in the preparative procedure, such as the removal of inhibitory substances by gel filtration, are also important but the distinguishing feature of systems of this type is the means by which a low endogenous background is obtained.

When presented with purified mRNAs such as rabbit globin mRNA, calf lens crystallin mRNA or encephalomyocarditis (EMC) virus RNA, incorporation is stimulated by up to 20-fold. The characteristic products of these messengers are clearly visible over the faint endogenous background (4). Several other cellular and viral mRNAs such as those for histones (5), reovirus proteins (2) and tryptophan oxygenase (6) have also been translated in this system, and at present there is no indication that there are mRNAs which the system is incapable of translating. Whether this truly represents the omnipotence of ascites cells or is an artifact of the conditions of preparation or use of the system is debatable. Messenger purity does not seem to be a major problem in that a preparation containing as little as 0.1 % of the mRNA for tryptophan oxygenase produced detectable amounts of product. Within limits, rRNA contamination seems not to interfere either (3, 7).

As normally prepared, the system appears to be partially deficient in initiation factors (4). When supplemented with a crude initiation factor preparation from reticulocytes there is a several-fold increase in the efficiency of translation of added globin or crystallin mRNAs. EMC RNA is affected less or not at all. The endogenous background is also enhanced suggesting that preincubation does not result in the destruction or permanent inactivation of the endogenous messengers. For many purposes it may be advantageous to augment the system with reticulocyte factors. Some authors have also reported that the system exhibits a stimulation of or dependence on added tRNA (7): most users do not observe this phenomenon, however, and its origin is unclear.

It would be unrealistic not to mention that the ascites system does have some limitations. The period of active chain initiation on an added mRNA is rather short, only about 10 min (3). On the other hand, amino acid incorporation proceeds for a considerable length of time, up to 90 min using EMC RNA as the message. This is a result of the slow rate of chain elongation, which proceeds at about 25 residues/min under typical incubation conditions (M. B. M. and M. Osborn, in preparation). It is not known whether this rate is constant along a message, or whether the ribosomes travel faster along most of the mRNA but become stalled at certain critical points. The latter might account for the phenomenon termed "premature termination" whereby translation of some mRNAs, notably EMC RNA and possibly immunoglobulin light chain mRNA, also tends to cease at certain points (8, 9). In view of the extraordinary length of this mRNA (about $2.6 \times 10^6$ daltons) it might be that it is cleaved before it can be fully read. The S-30 contains RNases capable of digesting both single- and double-stranded RNA (10).

Despite these apparent flaws, the ascites system has played an invaluable role in mRNA identification and in studies of the mechanism of protein synthesis in mammals. It will surely continue to render service in these and other directions in the future.

Wheat system

Cell-free protein synthesizing systems from wheat, which can be prepared from either the embryo or the germ, have recently acquired a wholly new significance. Embryos can be isolated from commercial grain by simple physical separation procedures capable of yielding large quantities of material. Alternatively, wheat germ may be obtained commercially. In either case, the material is disrupted by grinding with sand in a suitable aqueous medium, filtered, centrifuged and preincubated under conditions of protein synthesis. This results in a system with a very low endogenous background and an ability to efficiently translate both plant and animal mRNAs. Tobacco mosaic virus (TMV) RNA stimulates incorporation by over 100-fold, and mammalian globin mRNA elicits a 30-fold response (11).

As was the case with the ascites system, the wheat embryo cell-free system was originally developed to translate viral RNA (12). The mRNAs from two plant viruses, satellite tobacco necrosis virus (STNV) and brome mosaic virus (BMV), behave straightforwardly and give rise to virus coat proteins which can be easily identified (13, 14). TMV RNA, the polycistronic mRNA which served as template in the initial studies of the system, presents a more complex case. In the wheat germ system it directs the synthesis of a large number of polypeptides ranging from 10,000–140,000 daltons, but little or no coat protein is detectable on SDS-polyacrylamide gels. Nevertheless we have demonstrated that a portion (at least) of the viral coat protein is translated faithfully (15). For this purpose we exploited a virus mutant which differs from wild-type TMV in possessing a methionine residue in its coat protein. RNA from this mutant directs the synthesis of a methionine-containing tryptic peptide which is absent from digests of wild-type RNA-directed products. This novel peptide has been very thoroughly characterized and shown to correspond to the mutant coat peptide by electrophoretic and partial sequence analysis. The detailed mechanism of TMV RNA translation is far from clear, but there is reason to believe that further cell-free studies may help to elucidate the processes occurring *in vivo*.

The scope of the wheat system has been dramatically widened with the demonstration that it can read various mammalian mRNAs, including those for rabbit globin (both $\alpha$ and $\beta$ chains) (11) and immunoglobulin heavy and light chains (16). Preliminary evidence indicates that these are not isolated cases, and that the system is capable of faithfully translating several other mammalian cellular and viral mRNAs. The efficiency of the system is high, especially when it is prepared according to the modified procedures (11), and is comparable to the ascites system in this regard.

The advantages of the wheat system are similar to those of the ascites system: large quantities of extract can be easily prepared; the low endogenous background of the preincubated S-30 facilitates messenger testing and product analysis; the system is efficient; and it is capable of synthesizing large polypeptides (up to 1,400 residues in the TMV RNA-directed product). There is no doubt that this system will assume a growing importance in the areas of mRNA assay and identification.

## Conclusion.

Both the wheat and the ascites preincubated S-30s can translate a variety of mammalian mRNAs. Both systems are easily prepared from readily available starting materials, perhaps slightly more conveniently from wheat. If it is an advantage to use a system as distantly related as possible, to minimize the chance that the extract contains interfering components, the wheat system might have some advantage for mammalian mRNAs. On the other hand it is conceivable that the wheat system might lack some essential components required for certain mammalian mRNAs, though there is no sign of this at present.

Proteins which are synthesized in the form of longer precursor polypeptides requiring proteolytic action to convert them to the mature form seem to prevent special problems in cell-free systems. The proteolytic enzymes responsible for such cleavages appear to be deficient in cell-free extracts. Thus TMV and EMC coat proteins of the correct sizes are not formed in significant quantities in wheat or ascites S-30s primed by the appropriate viral RNA, though tryptic digests show that these sequences are translated. Crude extracts of poliovirus-infected HeLa cells also fail to cleave the precursor polypeptides which they synthesize (17), but the *Xenopus* oocyte system has at least some ability to carry out cleavages of this sort (18). That this defect of the cell-free systems may be remediable is suggested by recent successes with the wheat system. Incubation of the TMV RNA-directed polypeptides with a tobacco leaf extract results in the accumulation of coat protein which can be incorporated into intact virus particles in a reconstitution experiment (B. E. R., unpublished data).

At the present the wheat system seems to be a very satisfactory substitute for the ascites system: it remains to be seen whether it suffers limitations similar to those of the latter, and the comparative virtues of the two systems remain to be assessed.

## Acknowledgements

During the course of this work MBM was supported by a Beit Memorial Fellowship for Medical Research and BER by a Postgraduate Studentship from the Ministry of Agriculture, Fisheries and Food followed by a Royal Society Research Fellowship.

## References

1. Sampson, J., Mathews, M. B., Osborn, M. and Borghetti, A. F. (1972) *Biochemistry 11*, 3636–3640.
2. McDowell, M. J., Joklik, W. K., Villa-Komaroff, L. and Lodish, H. F. (1972) *Proc. Nat. Acad. Sci. USA 69*, 2649–2653.
3. Mathews, M. B. and Korner, A. (1970) *Eur. J. Biochem. 17*, 328–338.
4. Mathews, M. B., Pragnell, I. B., Osborn, M. and Arnstein, H. R. V. (1972) *Biochim. Biophys. Acta, 287*, 113–123.
5. Jacobs-Lorena, M., Baglioni, C. and Borun, T. W. (1972). *Proc. Nat. Acad. Sci. USA 69*, 2095–2099.
6. Schutz, G., Beato, M. and Feigelson, P. (1973) *Proc. Nat. Acad. Sci. USA 70*, 1218–1221.

7. Aviv, H. and Leder, P. (1972) *Proc. Nat. Acad. Sci. USA 69*, 1408–1412.
8. Kerr, I. M., Brown, R. E. and Tovell, D. R. (1972) *J. Virol. 10*, 73–81.
9. Swan, D., Aviv, H. and Leder, P. (1972) *Proc. Nat. Acad. Sci. USA 69*, 1967–1971.
10. Robertson, H. D. and Mathews, M. B. (1973) *Proc. Nat. Acad. Sci. USA 70*, 225–229.
11. Roberts, B. E. and Paterson, B. M. (1973) *Proc. Nat. Acad. Sci. USA* in press.
12. Marcus, A., Luginbill, B., and Feeley, J. (1968) *Proc. Nat. Acad. Sci. USA 59*, 1243–1250.
13. Klein, W. H., Nolan, C., Lazar, J. M. and Clark, J. M. (1972) *Biochemistry 11*, 2009–2014.
14. Shih, D. S. and Kaesberg, P. (1973) in press.
15. Roberts, B. E., Mathews, M. B. and Bruton, C. J., submitted for publication.
16. Helman, M., Roberts, B. E. and Givol, D. (1973) abstract, in press.
17. Roumiantzeff, M., Summers, D. F. and Maizel, J. V. (1971) *Virology 44*, 249–258.
18. Laskey, R. A., Gurdon, J. B. and Crawford, L. V. (1972) *Proc. Nat. Acad. Sci. USA 69*, 3665–3669.