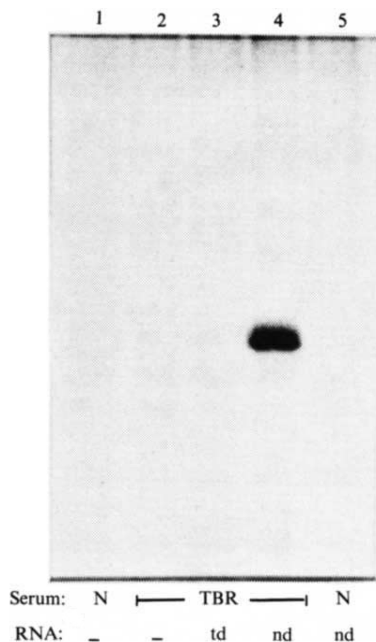

Fig. 3 Autoradiogram of SDS–polyacrylamide gel analysis of phosphorylated peptides. A portion of the bacteria-bound immune complexes obtained after protein synthesis and immunoprecipitation was resuspended directly in 25 μl of reaction buffer containing 20 mM Tris-HCl (*p*H 7.2), 5 mM $MgCl_2$ and 1 μM [γ-^{32}P] ATP (1,000 Ci $mmol^{-1}$) as previously described[5]. After 10 min at 30 °C the bacteria-bound immune complexes and any phosphorylated polypeptides were washed and then resuspended in sample buffer and boiled for 1 min to release any polypeptides bound to the bacteria. After electrophoresis the gel was fixed in 10% acetic acid–5% methanol and washed overnight. The phosphorylated polypeptides were detected by exposure of the dried gel for 1 h to Kodak-X-Omat film at −70 °C using a DuPont Cronex Hi-Plus intensifying screen to enhance detection.

sufficient to initiate and maintain neoplastic transformation. Identification of the target(s) of $p60^{src}$ phosphorylation and the further characterisation of the ASV *src* gene product should permit resolution of the apparent discrepancies.

The Schmidt–Ruppin strain, subgroup D, was originally obtained from John Wyke, and the td strain from Peter Vogt. This research was supported by grants CA-2117, CA-15823 and CA-21326 from NIH, and grant VC-243 from the American Cancer Society. M.S.C. is supported by grant DRG-181-F from the Damon Runyon–Walter Winchell Cancer Fund.

Note added in proof: The enzymatic activity observed here cannot be ascribed to the translation of virus-specific mRNA from cells sequestered by virions because identical results were obtained when these experiments were repeated with the 3′ third of the viral RNA selected, as described previously[4], from T1 RNAse-digested full-length (39 S) genome RNA.

ELEANOR ERIKSON
MARC S. COLLETT
R. L. ERIKSON

Department of Pathology, University of Colorado Medical Center, Denver, Colorado 80262

Received 22 May; accepted 30 June 1978.

1. Hanafusa, H. in *Comprehensive Virology* Vol. 10 (eds Fraenkel-Conrat, H. & Wagner, R. R.) 401–483 (1977).
2. Brugge, J. S. & Erikson, R. L. *Nature* **269**, 346–348 (1977).
3. Purchio, A. F., Erikson, E. & Erikson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4661–4665 (1977).
4. Purchio, A. F., Erikson, E., Brugge, J. S. & Erikson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1567–1571 (1978).
5. Collett, M. S. & Erikson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2021–2024 (1978).
6. Pelham, H. R. B. & Jackson, R. J. *Eur. J. Biochem.* **67**, 247–256 (1976).
7. Kessler, S. W. *J. Immun.* **115**, 1617–1624 (1975).
8. Laemmeli, U. K. *Nature* **277**, 680–685 (1970).
9. Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83–88 (1974).
10. Kamine, J. & Buchanan, J. M. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2011–2015 (1977).
11. Beemon, K. & Hunter, T. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3302–3306 (1977).
12. Kamine, J., Burr, J. G. & Buchanan, J. M. *Proc. natn. Acad. Sci. U.S.A.* **75**, 366–370 (1978).

Evidence for translation of rabbit globin mRNA after liposome-mediated insertion into a human cell line

THE ability to insert messenger RNA selectively into large numbers of differentiated eukaryotic cells and thereby alter the expression of the eukaryotic genome would provide a new and direct approach to the study of the molecular mechanisms involved in protein synthesis. Several procedures have been used to facilitate the cellular incorporation of administered RNA, including microinjection of RNA into *Xenopus* oocytes[1–6] and into fully differentiated eukaryotic cells[7,8], direct co-cultivation of cells with naked RNA (refs 9–12), and encapsulation of mRNA within erythrocyte ghosts followed by Sendai virus induced fusion of the ghosts to recipient cells in culture[13]. We describe here the use of liposomes for the selective insertion of functional mRNA into differentiated eukaryotic cells *in vitro*. We provide evidence which indicates that cultured human epithelial carcinoma cells (HEp-2) treated with liposomally encapsulated rabbit globin mRNA are stimulated to produce a globin-like protein.

Globin mRNA was isolated from the reticulocytes of rabbits suffering from phenylhydrazine-induced anaemia[14] by the method of Aviv and Leder[15] and sequestered within liposomes[16] by the technique of Ostro *et al.*[17] The liposomes were mixed with HEp-2 cells in the presence of ^{3}H-amino acids either with or without transcription inhibitors (actinomycin D or α-amanitin). The cells were lysed and the soluble proteins purified by ammonium sulphate precipitation followed by passage through an immunoadsorbent column consisting of Sepharose 4B covalently linked to the IgG fraction of goat anti-rabbit globin antiserum[18].

The proteins eluted from the immunoadsorbent columns were analysed by electrophoresis on SDS polyacrylamide gels. The distribution of radioactivity of the proteins isolated from cells which had been incubated with liposomally sequestered globin mRNA was compared to that of the proteins derived from untreated cells (Fig. 1*a*). All liposome-treated preparations had three major peaks of radioactivity which occurred in fractions 15, 20 and 24 with R_f values of 0.38, 0.50 and 0.75, respectively. The latter value corresponded to the R_f of the rabbit globin standard. Although the first two peaks (R_f 0.38, 0.50) occurred in proteins isolated from non-liposome-treated control cells, a peak co-electrophoresing with the globin standard (R_f 0.75) was completely absent (Fig. 1*a*). When HEp-2 cells treated with liposomally sequestered globin mRNA were grown in the presence of actinomycin D, the peak of radioactivity corresponding to the globin standard was enhanced whereas the two main contaminating protein peaks were eliminated (Fig. 1*b*). Also, peptides migrating further than the globin subunit were noted which were obvious only in the lysates obtained from cells treated with liposome-encapsulated mRNA and grown in the presence of actinomycin D. These smaller peptides may possibly represent either incomplete translation products or partially degraded globin. When HEp-2 cells treated with empty liposomes and exogenously added globin mRNA were grown in the absence of actinomycin D, the two major protein contaminants were present but no peak of radioactivity co-migrated with the globin standard (Fig. 1*b*). Additional controls consisted of protein isolates obtained from cells grown in the presence of actinomycin D which were either liposome-untreated or incubated with empty liposomes and exogenously added globin mRNA. In both instances (data not shown), all the major peaks of radioactivity were absent.

Proteins obtained from HEp-2 cells grown in the presence of α-amanitin and treated with either liposomally sequestered globin mRNA, or empty liposomes and exogenously added mRNA, were analysed on 2–20% sucrose gradients alone, in the presence of monospecific goat anti-rabbit globin antibody, or combined with antibody and excess nonradioactive globin

0028–0836/78/0274—0921$01.00

© Macmillan Journals Ltd 1978

(Fig. 2). The sucrose gradient profiles show that although other proteins appear, isolates obtained from cells treated with liposomally sequestered mRNA exhibit a peak of radioactivity co-sedimenting with the globin subunit standard. This peak is absent in control cell lysates (Fig. 2*a*). When purified monospecific antibody was mixed with the experimental cell protein isolates, the peak of radioactivity corresponding to the globin subunit standard was shifted to the bottom of the gradient

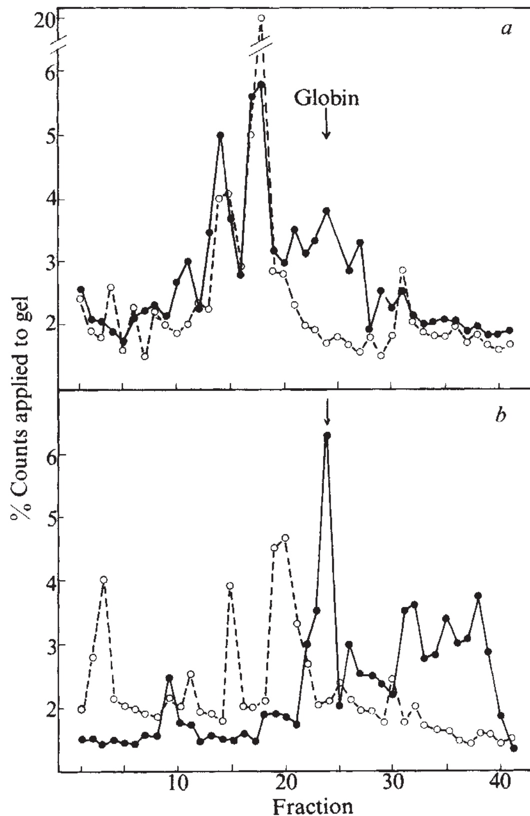

Fig. 1 SDS polyacrylamide gel electrophoresis of a mixture of radioactive HEp-2 cell protein isolates (previously purified by immunoadsorbent chromatography) and rabbit globin standard. Ether infusion liposomes, consisting of lecithin and dicetylphosphate in an 8:2 molar ratio and containing rabbit globin mRNA, were prepared by the method of Deamer and Bangham[16] as adapted by Ostro *et al.*[17] for the sequestration of RNA. HEp-2 cells were mixed with 1 ml of liposomes on a tumbledrum apparatus for 1 h at 37 °C in the presence of 250 μCi of ^{3}H-amino acids and 5 ml of serum-supplemented minimal essential medium. Subsequently, the liposome–cell mixture was added to 30 ml of RPMI-1640 medium containing 100 μCi of ^{3}H-amino acids and incubated at 37 °C overnight. To inhibit endogenous protein synthesis, actinomycin D (5 μg ml^{-1}) or α-amanitin (2 μg ml^{-1}) was mixed with the HEp-2 cells either 3 h (actinomycin D) or 6 h (α-amanitin) before the addition of liposomes. These levels of the transcription inhibitors were maintained during the 1 h of liposome–cell co-cultivation as well as during a subsequent 5 h incubation period. The cells were then lysed and the soluble proteins isolated as described in the text. Lyophilised protein samples obtained from cells treated with liposomally sequestered globin mRNA were analysed on 10% SDS polyacrylamide gels by the method of Weber and Osborn[24]. Samples containing between 5×10^3 and 2×10^4 counts were applied to the SDS gels in the presence of 100 μg of rabbit globin standard and electrophoresed for 5 h. The gels were then fixed in methanol, water, acetic acid (227:227:46) overnight and stained in 12.5% trichloroacetic acid (TCA) containing 0.1% Coomassie blue. After the gels had been analysed for the presence of protein bands by scanning at 600 nm, they were cut into 2-mm slices, solubilised at 60 °C in a 2:1 mixture of 30% hydrogen peroxide and 60% perchloric acid, and counted. *a*, ●, Radioactivity profile of proteins from HEp-2 cells treated with liposome-sequestered globin mRNA; ○, radioactivity profile of proteins from untreated HEp-2 cells. *b*, ●, Radioactivity profile of proteins from HEp-2 cells grown in the presence of 5 μg ml^{-1} actinomycin D and treated with liposome-sequestered globin mRNA; ○, radioactivity profile of HEp-2 cells treated with empty liposomes and 100 μg of exogenously added globin mRNA. Peaks at fractions 15, 20 and 24 have R_f values of 0.38, 0.50 and 0.75, respectively.

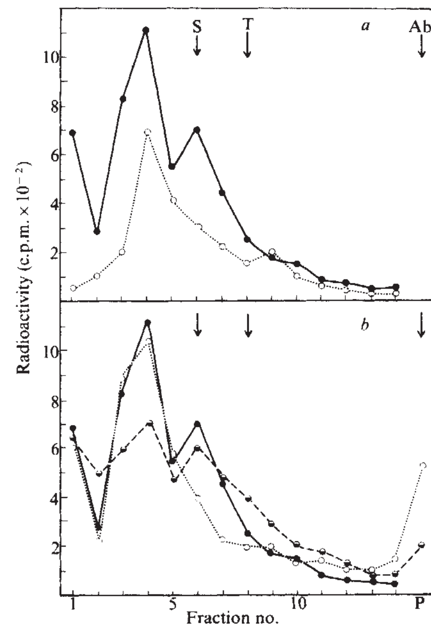

Fig. 2 Sedimentation analysis on 2–20% sucrose gradients of HEp-2 cell protein isolates (previously purified by immunoadsorbent chromatography) in the presence of monospecific goat anti-rabbit globin antibody. A goat was immunised with pure rabbit globin in complete Freund's adjuvant. The antiserum obtained gave a single precipitin arc with rabbit globin by immunoelectrophoresis. To remove non-antibody proteins, the antiserum was passed through an immunoadsorbent column consisting of Sepharose 4B covalently linked to rabbit globin[18]. The purified antibody was eluted with 0.2 M glycine sulphate, *p*H 2.3, and concentrated by ultrafiltration. The resulting antibody preparation also gave a single arc with rabbit globin by immunoelectrophoresis and did not react with a concentrated protein isolate (30 mg of protein per ml) from untreated HEp-2 cells. Protein isolates obtained from experiments using α-amanitin (2 μg ml^{-1}) were analysed on 2–20% sucrose gradients in the presence of monospecific goat anti-rabbit globin antibody. Gradients were prepared in 0.05 M Tris buffer, *p*H 7.5, 0.05 M KCl, 5 mM $MgCl_2$ and were centrifuged in an SW41 rotor at 40,000 r.p.m. for 30 h. The following samples were analysed: protein derived from cells treated with liposomally sequestered mRNA (experimental lysate); protein derived from cells treated with empty liposomes and exogenously added mRNA (control lysate); separate experimental and control lysates plus 1 mg of monospecific goat anti-rabbit globin antibody; separate experimental and control lysates and antibody plus 1.5 mg of pure globin; globin subunit, globin tetramer and antibody standards. All gradients were fractionated into 0.6-ml aliquots on an Isco density gradient fractionator. Fractions derived from gradients containing globin and antibody standards were monitored at 280 nm, and fractions from gradients containing radioactive protein isolates were TCA precipitated onto Millipore filters and counted. *a*, ●, Radioactivity profile of proteins from HEp-2 cells treated with liposome-sequestered globin mRNA; ○, radioactivity profile of proteins from HEp-2 cells treated with empty liposomes and exogenously added globin mRNA. *b*, ●, Radioactivity profile of proteins from HEp-2 cells treated with liposome-sequestered globin mRNA; ○, radioactivity profile of proteins from HEp-2 cells treated with liposome-sequestered globin mRNA and mixed with antibody before centrifugation; ⊖, radioactivity profile of proteins from HEp-2 cells treated with liposome-sequestered globin mRNA and mixed sequentially with excess nonradioactive globin and antibody before centrifugation. S, subunit standard; T, tetramer standard; Ab, antibody marker; P, pellet.

© 1978 Nature Publishing Group

coincident with the antibody marker. The addition of excess nonradioactive globin to the experimental cell lysate before the addition of antibody inhibited this shift (Fig. 2*b*).

We have demonstrated by SDS gel electrophoresis and sucrose density gradient analysis that HEp-2 cells, treated with liposomally encapsulated globin mRNA, are stimulated to synthesise a previously nonexistent globin-like protein product which reacts with monospecific goat anti-rabbit globin antibody. Sucrose density gradient analysis of the HEp-2 cell translation products revealed the presence of a globin subunit-like protein molecule. However, the assembled globin tetramer ($\alpha_2\beta_2$) is not seen, perhaps because of the lack of haemin in the recipient HEp-2 cell or the selective binding of β-chain message to specific protein synthesis initiation factors[19]. Whether these or other factors affect globin assembly in our system remains to be determined. Although the data presented strongly indicate that globin subunits are produced by HEp-2 cells treated with liposomally sequestered globin mRNA, further chemical analysis of the purified product, such as peptide mapping and isoelectric focusing, must be carried out to prove unequivocally that the protein product is in fact globin.

Multilamellar liposomes (500 Å diameter) and small unilamellar liposomes (200 Å diameter) have been used previously to entrap and deliver into cells a wide variety of macromolecules[20–23]. However, until recently the ability of liposomes to sequester high molecular weight RNA had not been demonstrated[17], possibly due to the limited trapping efficiency of the standard small liposomes. By using large unilamellar liposomes produced by the ether infusion technique of Deamer and Bangham[16], steric problems can be overcome and large molecules such as mRNA can be efficiently sequestered.

The transfer of mRNA into eukaryotic cells by the techniques which have been previously used either involve the tedious manipulation of direct microinjection[1–8] or fail to protect the administered message from external nuclease degradation[9–12]. Furthermore, entrapment of mRNA within erythrocyte ghosts, although overcoming the above mentioned problems, requires an external fusogen to be added to insert sequestered message into the cell. The use of liposomes enables encapsulated mRNA to be inserted into large numbers of cells, as well as cell types, without the addition of an added fusogen. Although the need for fusing agents may not be important when RNA is delivered to cells *in vitro*,it would preclude any potential *in vivo* application.

We thank Dr Gerald Weissman for his advice during these experiments, and Ms Alice Gilman-Sachs and Jeanette Wallman for their technical assistance. This work was supported by grants PHS AI-04073 and PHS CA-05843 of the NIH.

MARC J. OSTRO,
DARIO GIACOMONI,
DON LAVELLE,
WILLIAM PAXTON,
SHELDON DRAY

Department of Microbiology and Immunology,
University of Illinois at the Medical Center,
Chicago, Illinois 60612

Received 7 April; accepted 7 July 1978.

1. Lane, C. D., Marabaix, G. & Gurdon, J. B. *J. molec. Biol.* **61**, 73–91 (1971).
2. Chan, L., Kohler, P. B. & O'Malley, B. W. *J. clin. Invest.* **57**, 576–585 (1976).
3. Cavalieri, R. L., Havell, E. A., Vilcek, J. & Pestka, S. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4415–4419 (1977).
4. Faust, C. F., Vassalli, P. & Rungger, D. *Molec. Biol. Rep.* **1**, 3–8 (1973).
5. Labarca, C. & Paigen, K. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4462–4465 (1977).
6. DeMaeyer-Guignard, J., DeMayer, E. & Montagnier, L. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1203–1207 (1972).
7. Graessmann, A., Graessmann, M. & Mueller, C. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4831–4834 (1977).
8. Stacey, D. W. & Allfrey, V. G. *Cell* **9**, 725–732 (1976).
9. Dray, S. in *Immune RNA in Neoplasia*, (ed. Fink, M.) 61–73 (Academic, New York, 1976).
10. Adler, F. L., Fishman, M. J. & Dray, S. *J. Immun.* **97**, 554–558 (1966).
11. Wang, S. R., Giacomoni, D. & Dray, S. *Exp. Cell Res.* **78**, 15–24 (1973).
12. Giacomoni, D. *et al. Cell Immun.* **11**, 389–400 (1974).
13. Anderson, W. F. & Krueger, L. *J. Cell Biol.* **70**, 185a (1976).
14. Evans, M. J. & Lingrel, J. B. *Biochemistry* **8**, 3000–3005 (1969).
15. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412 (1972).
16. Deamer, D. & Bangham, A. D. *Biochim. biophys. Acta* **443**, 629–634 (1976).
17. Ostro, M. J., Giacomoni, D. & Dray, S. *Biochem. biophys. Res. Commun.* **76**, 836–842 (1977).
18. Wofsy, L. & Burr, B. *J. Immun.* **103**, 380–382 (1969).
19. Kabat, D. & Chappell, M. R. *J. biol. Chem.* **252**, 2684–2690 (1977).
20. Cohen, G. M., Weissmann, G., Hoffstein, S., Awasthi, Y. C. & Srivastava, S. K. *Biochemistry* **15**, 452–460 (1976).
21. Magee, W. E., Talcott, M. L., Straub, S. X. & Vriend, C. Y. *Biochim. biophys. Acta* **451**, 610–618 (1976).
22. Braidman, I. P. & Gregoriadis, G. *Biochem. J.* **164**, 439–445 (1977).
23. Colley, M. C. & Ryman, B. E. *Biochim. biophys. Acta* **451**, 417–425 (1976).
24. Weber, K. & Osborn, M. *J. biol. Chem.* **244**, 4406–4412 (1969).

Translation of rabbit globin mRNA introduced by liposomes into mouse lymphocytes

SEVERAL interesting questions in developmental biology can be studied by the combination of messenger RNA from one kind of cell with the translational apparatus of another cell type. Such experiments can provide proof of the identity of a mRNA and can yield information regarding the species and cell-type specificities of translation systems. The first successful approach to this problem was that taken by Lane *et al.*[1] who injected globin mRNA into frog oocytes. However, results obtained using the oocyte, which is an unspecialised cell, cannot necessarily be extended to the translational apparatus of more specialised cells. Many workers have tried to introduce informational macromolecules directly into differentiated cells, using a variety of methods[2–6]. Stacey *et al.* have translated duck globin mRNA after injection into HeLa cells[7]. We have

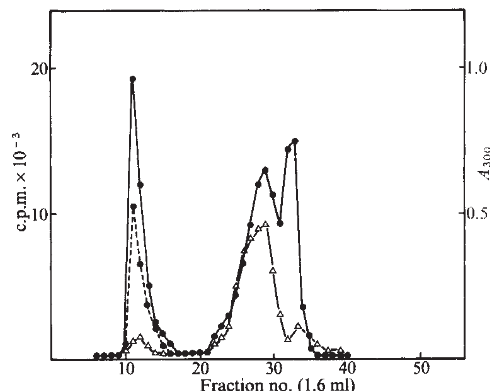

Fig. 1 Sepharose-4B chromatography of liposomes containing ^{125}I-globin mRNA. 10 μmol of beef brain phosphatidylserine in chloroform solution was evaporated to dryness under vacuum and suspended in NHTE buffer (0.1 M NaCl, 2 mM histidine, 2 mM triethylamino-ethanesulphonic acid, 0.4 mM EDTA, *p*H 7.4). The suspension was vortexed and subsequently sonicated under nitrogen in a bath-type sonicator at 30 °C for 30 min. 0.02 mmol of Ca^{2+} was added and the mixture was then incubated for 1 h at 37 °C after which the preparation was centrifuged at 2,500*g* for 10 min. The resulting pellet was suspended in 0.1 ml of a solution of globin mRNA (1 mg ml^{-1}) which had previously been dialysed against NHTE buffer, *p*H 7.4. In the experiment concerned with sequestering RNA into liposomes, trace amounts of ^{125}I-globin mRNA were added. The mixture of RNA and liposomes was vortexed, 0.2 mmol EDTA was added and the preparation was then incubated for 30 min at 37 °C. The liposomes were recovered by centrifugation (30,000*g*, 20 min at 20 °C) and washed with Ca^{2+}-, Mg^{2+}-free PBS. Washed liposomes were resuspended in 1 ml of PBS, equilibrated for 30 min at room temperature and applied to a 1.2×42 cm Sepharose-4B column which had been equilibrated with the same buffer. Fractions (1.6 ml) were collected and A_{300} (•- - -•) and radioactivity (•—•) measured. The liposome fractions (void volume) were pooled and centrifuged at 30,000*g* for 20 min at 20 °C. The resulting pellet was suspended in 1 ml PBS containing 1% Triton X-100 and incubated for 1 h at 37 °C. After incubation the whole mixture was passed through the same column. △—△, radioactivity; •- - -•, A_{300} after treatment with Triton X-100. Isolation and iodination of rabbit globin mRNA was carried out as described elsewhere[16,17].

0028-0836/78/0274—0923$01.00

© Macmillan Journals Ltd 1978

demonstrated[8,9] the entrapment of ribonucleic acids of different sizes (28S, 18S, 9S and 4S) in large unilamellar liposomes. Such entrapped macromolecules are protected from ribonucleases and can be isolated both intact and biologically active[8]. Moreover, fusion of these liposomes yields RNA-filled cells[9]. We present here evidence that rabbit reticulocyte 9S mRNA introduced into mouse spleen lymphocytes directs the synthesis of globin.

We used large unilamellar liposomes, first prepared by Papahadjopoulos[11], which entrap high molecular weight molecules with high efficiency[8–10]. The process for the preparation of liposomes is described in the legend to Fig. 1. To separate vesicles containing RNA from untrapped RNA, the mixture of liposomes and RNA was passed through a Sepharose-4B column (Fig. 1). The fractions comprising the void volume were pooled and centrifuged at 30,000*g* for 20 min at 20 °C; the resulting pellet was resuspended in 2 ml of phosphate-buffered saline (PBS) and used for the translation experiments. To prove that the globin mRNA was really sequestered in the liposomal aqueous space, we incubated liposomes containing ^{125}I-globin mRNA in a solution of 1% Triton X-100 for 1 h at 37 °C (ref. 12). As shown in Fig. 1, after detergent treatment the ^{125}I-globin mRNA is retarded by the gel filtration column to the same extent as free globin mRNA.

For the translation experiments, purified mouse spleen lymphocytes were used. These cells were incubated for 2 h with liposomes containing globin mRNA and, after removal of liposomes, were incubated for a further 12 h with histidine-deficient Eagle's medium supplemented with 100 μCi of ^{3}H-histidine. The lymphocytes were then washed three times with Hanks' balanced salt solution, lysed with 1% Triton X-100 and then sonicated for 10 min. The cell debris was removed by centrifugation at 10,000*g* for 10 min, and 100 μl of the supernatant was removed for indirect immunoprecipitation[18]. Purified monospecific guinea pig anti-rabbit globin antibodies and goat anti-guinea pig IgG antibodies were used. The resulting immunoprecipitate was solubilised in SDS sample buffer and electrophoresed on a 12.5% polyacrylamide gel[19]. Control experiments were run exactly as described above, except that the lymphocytes were incubated with empty liposomes and free rabbit globin mRNA (20 μg ml^{-1}). Figure 2 shows the results of such an experiment: gel slot *b* shows that in cells incubated with liposomes containing rabbit globin mRNA, there is a band which co-migrates with globin; no such band of radioactivity is found in cells incubated with empty liposomes and free rabbit globin mRNA (slot *a*).

Fig. 2 SDS–polyacrylamide gel electrophoresis of the immunoprecipitate from cells incubated with empty liposomes and free rabbit globin mRNA (*a*) and with liposomes containing rabbit globin mRNA (*b*). Spleen lymphocytes from BALB/c mouse were purified by using Ficoll–Hypaque[20]. Antibodies against rabbit globin were prepared by immunisation of guinea pigs with purified rabbit globin (1 mg ml^{-1}) mixed with an equal volume of Freund's complete adjuvant. The immunisation procedures described by Avrameas were followed[21]. γ-globulin fractions were prepared from antisera by 40% ammonium sulphate precipitation and further purified by using immunoadsorbent of globin prepared with glutaraldehyde[22]. The purified antibodies gave a single arc in immunoelectrophoresis analysis and did not react with lysates prepared from mouse spleen lymphocytes. For the fusion of cells with liposomes, about 4×10^6 mouse spleen lymphocytes were incubated with 2 ml liposomes (2 μmol lipid per ml) as described in the text. For immunoprecipitation, 100 μl of cell lysates were used, following the method described by Craig *et al.*[18]. The immunoprecipitates were solubilised in SDS sample buffer and electrophoresed on a 12.5% polyacrylamide slab gel[19]. The gel was stained, dried and fluorographed.[23]

Our results show that mRNA for rabbit globin introduced into mouse spleen lymphocytes can be translated, giving rise to material closely related to rabbit globin. This finding contributes to the question of the cell-type and species specificities of the translation systems within the living cell. As rabbit globin mRNA is successfully translated in mouse spleen lymphocytes, we can conclude that all the components required to translate it are present in differentiated cells as different as rabbit reticulocytes and mouse lymphocytes. Thus, if messenger-specific components are required for the translation of globin messenger, then such components are present and available in mouse lymphocytes. If it is assumed that such factors do exist in mouse lymphocytes, then it is clear that their presence cannot be the only phenomenon determining the appearance of cell-type specific proteins during cell differentiation. Evidence for tissue specificity has only been found with purified cell-free extracts derived from terminally differentiated tissues[13,14]. Of course, our results do not rule out tissue specificity for the different factors (such as initiation factors) because it can be argued that lymphocytes and reticulocytes have a common stem cell[15]. We believe that our system of introducing foreign mRNAs into differentiated cells by means of liposomes[8,9] will give answers to such problems and will facilitate studies related to the synthesis and breakdown of mRNA in the host cell.

I thank Dr J. R. Tata for the freedom to pursue my own research goals, Dr C. D. Lane for constructive suggestions, Dr D. B. Thomas for mouse lymphocytes, Dr I. Kerr for discussion, and Ms R. Harris, M. Layton and S. Shannon for technical assistance. G.J.D. is in receipt of an EMBO long-term fellowship.

GIORGOS J. DIMITRIADIS

National Institute for Medical Research,
The Ridgeway,
Mill Hill, London NW7, UK

Received 26 April; accepted 30 June 1978.

1. Lane, C. D., Marbaix, G. & Gurdon, J. B. *J. molec. Biol.* **61**, 73–91 (1971).
2. Hill, M. & Huppert, J. *Biochim. biophys. Acta* **312**, 26–35 (1970).
3. Furusawa, M., Nishimura, T., Yamaizumi, M. & Okada, Y. *Nature* **249**, 449–450 (1974).
4. Loyter, A., Zakai, N. & Kulka, R. G. *J. Cell Biol.* **66**, 292–304 (1975).
5. Maeyer-Guignard, J., Maeyer, E. & Montagnier, L. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1203–1207 (1972).
6. Graessmann, A. *Expl Cell Res.* **60**, 373–382 (1970).
7. Stacey, D. W. & Allfrey, V. G. *Cell* **9**, 725–732 (1976).
8. Dimitriadis, G. J. *FEBS Lett.* **86**, 289–293 (1978).
9. Dimitriadis, G. J. *Nucleic Acids Res.* **5**, 1381–1386 (1978).
10. Mayhew, E., Papahadjopoulos, D., O'Malley, J. A., Carter, W. A. & Vail, W. J. *Molec. Pharmac.* **13**, 488–495 (1977).
11. Papahadjopoulos, D., Vail, W. J., Jacobson, K. & Poste, G. *Biochim. biophys. Acta* **394**, 483–491 (1975).
12. Weissmann, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 88–92 (1975).
13. Heywood, S. M. *Proc. natn. Acad. Sci. U.S.A.* **67**, 1782–1788 (1970).
14. Prichard, P. M., Picciano, D. G., Laycock, D. G. & Anderson, W. F. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2752–2756 (1971).
15. Stavnezer, J. & Huang, R. C. C. *Nature new Biol.* **230**, 172 (1971).
16. Dimitriadis, G. J. & Georgatsos, J. G. *FEBS Lett.* **46**, 96–100 (1974).
17. Dimitriadis, G. J. & Georgatsos, J. G. *Nucleic Acids Res.* **2**, 1719–1726 (1975).
18. Craig, R. K., Brown, P. A., Harrison, O. S., McIlreavy, D. & Campbell, P. N. *Biochem. J.* **160**, 57–74 (1976).
19. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
20. Harris, R. & Ukaejiofo, E. O. *Br. J. Haemat.* **18**, 229–235 (1970).
21. Avrameas, S. *Immunochemistry* **6**, 43–52 (1969).
22. Ternynck, T. & Avrameas, S. *FEBS Lett.* **23**, 24–28 (1972).
23. Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83–88 (1974).

© 1978 Nature Publishing Group