

Translation of Two Messenger RNAs from Lens in a Cell Free System from Krebs II Ascites Cells

SPECIALIZED tissues which synthesize only a small number of different proteins offer obvious advantages as sources of specific and pure messenger RNAs (mRNAs). Presumptive mRNAs have been isolated and partially characterized from reticulocytes^{1,2}, muscle³, myelomas⁴ and eye lens⁵. The messenger role of 9S RNA from reticulocytes has been established by its ability to direct the synthesis of haemoglobin in cell-free systems from reticulocytes² and Krebs II ascites tumour cells⁶, and in *Xenopus* oocytes⁷. We show here that 10S and 14S RNA fractions from calf lens direct the synthesis of different polypeptide components of lens crystallin in the ascites cell-free system. These results complement and extend the demonstration, in the accompanying article⁸, that 14S lens RNA is translated in the reticulocyte lysate.

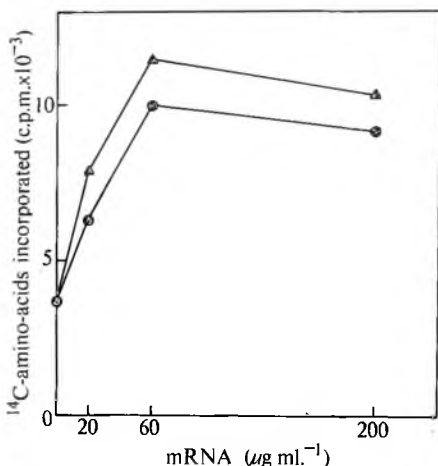


Fig. 1 Stimulation of protein synthesis by lens mRNAs. Reactions contained in 25 μl . : 10 μl . preincubated ascites S-30, 2.5 mM Mg acetate, 100 mM KCl, 25 mM Tris-HCl (pH 7.5), 6 mM 2-mercaptoethanol, 1 mM ATP, 0.1 mM GTP, 5 mM creatine phosphate, creatine phosphokinase (0.2 mg ml.⁻¹) and 5 $\mu\text{Ci ml}^{-1}$ of a mixture of ¹⁴C-labelled amino-acids (54 mCi matom⁻¹, Radiochemical Centre, Amersham) supplemented with the six missing amino-acids (asparagine, cysteine, glutamine, histidine, methionine and tryptophan), at 20 μM each. Lens mRNA was added as indicated. After incubation at 37° C for 60 min, incorporation into hot TCA precipitable material was assayed as before⁹. \blacktriangle , 10S mRNA; \odot , 14S mRNA.

Messenger RNA fractions were isolated by zonal centrifugation through a sucrose gradient of lens polysomes dissociated by sodium dodecylsulphate (SDS)⁵. Ascites S-30 was prepared and preincubated as described previously⁹, and conditions for protein synthesis were those used for translation of globin mRNA⁶. In contrast to the non-preincubated reticulocyte system in which total incorporation is reduced by added mRNA,^{2,8} the ascites S-30 was stimulated by 10S or 14S lens RNA (Fig. 1). As with globin mRNA, the lens messengers are translated about once on average. The products have been identified by co-electrophoresis with added marker crystallins both on SDS gels, which separate polypeptides according to molecular weight^{10,11}, and on acidic and basic urea gels which separate according to charge.

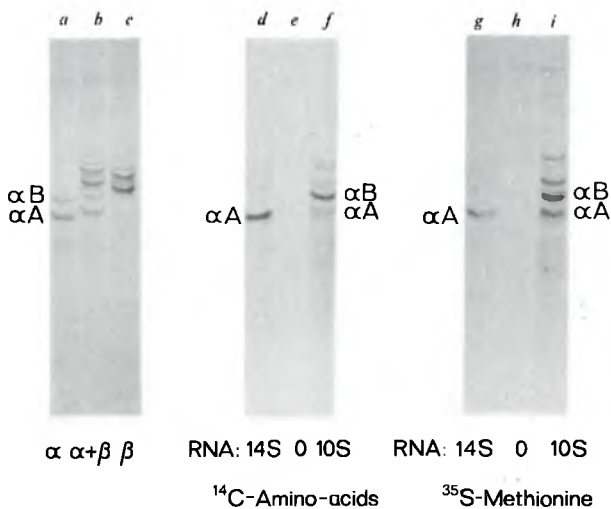


Fig. 2 SDS gel analysis of the cell-free product. Protein synthesis reactions (50 μl), performed as in Fig. 1, used either ^{14}C -amino-acid mixture (see Fig. 1), or ^{35}S -methionine (10 μM , 10 Ci mmol^{-1}) plus the remaining nineteen unlabelled amino-acids (20 μM each). Lens mRNA was added at 60 $\mu\text{g ml}^{-1}$ where shown. After 60 min at 37° C reactions were terminated by incubation for 15 min at 37° C with pancreatic RNase (20 $\mu\text{g ml}^{-1}$) and 10 mM EDTA. SDS and 2-mercaptoethanol were added to 2% and 1% respectively, together with 5–10 μg of unlabelled α or β crystallin markers, and the entire reaction mixture was heated to 100° C for 2 min. Gels contained 0.1% SDS, 15% acrylamide and 0.4% methylenebisacrylamide, and were 9 cm long. A stacking gel was used, and gels were prepared and run as in ref. 26, except that the ionic strength of the running buffer was doubled. The gels were stained and destained¹¹, and longitudinal slices were dried before autoradiography. SDS gels of 5–10 μg of α , $\alpha+\beta$ and β crystallins respectively, stained with Coomassie brilliant blue are shown in a-c. Autoradiographs (4 days) of ^{14}C -labelled cell-free product with added 14S mRNA, 10S mRNA and no added mRNA are shown in d, f and e respectively. Autoradiographs of ^{35}S -methionine labelled cell-free product with added 14S mRNA, 10S mRNA and no mRNA are shown in g, i and h respectively. The labels α A and α B on the autoradiographs indicate that the labelled bands were exactly superimposable on the stained bands of added α crystallin marker which were clearly distinguishable against the background of the proteins of the ascites cell-free system.

Water soluble lens proteins can be fractionated into α , β and γ crystallins, each containing several polypeptide species^{12–14}. SDS gel electrophoresis of purified α crystallin reveals two fractions, α A and α B, with apparent molecular

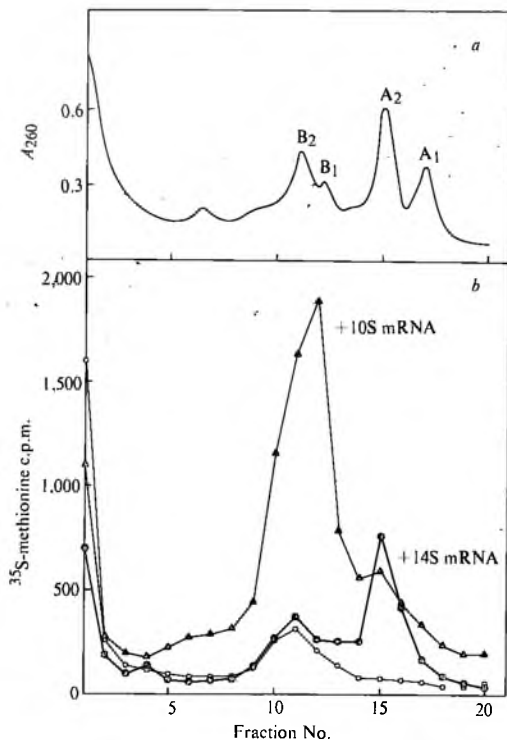


Fig. 3 Analysis of cell-free product on alkaline urea gels. Protein synthesis reactions (20 μ l.), and RNAase treatment were carried out as in Fig. 2. The proteins were then precipitated with 5% TCA and washed (5% TCA twice, ethanol, ethanol : ether (1 : 1), and ether once each). The labelled sample together with 100 μ g α crystallin was applied to polyacrylamide gels containing 6 M urea (pH 8.9) and electrophoresed as previously described. Subsequently the gels were stained with amido black, monitored for absorbance at 540 nm and cut into 1.5 mm slices which were solubilized in NCS and assayed for radioactivity by scintillation counting.

weights of 19,000 and 22,000, present in the ratio of 2 : 1 (Fig. 2a and refs. 15 and 16). Similarly, β crystallin contains several polypeptides with molecular weights between 23,000 and 27,000 (Fig. 2c). When α and β crystallins are run together the individual polypeptide chains can be resolved clearly (Fig. 2b).

The products of cell-free protein synthesis were mixed with either unlabelled α or β crystallin and electrophoresed on SDS gels, which were subsequently stained for protein, sliced longitudinally and dried for autoradiography. The polypeptide chains of the added crystallin marker were clearly identifiable on the stained gels against the background of ascites cell proteins. Autoradiographs of endogenous and mRNA-stimulated products are shown in Fig. 2*d-i*. The endogenous ascites products, labelled with either ^{35}S -methionine (Fig. 2*h*) or ^{14}C -amino-acid mixture (Fig. 2*e*) in the absence of added mRNA, showed no radioactive bands in the crystallin region. In contrast, the cell-free product made with added 14S mRNA exhibited a single radioactive band which corresponded exactly with unlabelled crystallin αA marker (molecular weight 19,000). This was so when either ^{35}S -methionine (Fig. 2*g*) or ^{14}C -amino-acid mixture was used (Fig. 2*d*), and there was little contamination with other radioactive products.

The 10S mRNA directed the synthesis of material migrating with crystallin αB (molecular weight 22,000), and lesser amounts of material which migrated with crystallin αA and with β (or possibly γ) chains (Fig. 2*f*, ^{14}C -amino-acid mixture). The apparent proportions of β and γ crystallins in the 10S mRNA product is exaggerated when ^{35}S -methionine is used (Fig. 2*i*), presumably because of their higher content of this amino-acid¹³. Furthermore, comparison of Fig. 2*d* and Fig. 2*g* shows that this label leads to an underestimate of the amount of crystallin αA synthesized under the direction of 14S mRNA relative to the amount of αB in the 10S product. This is unexpected as the methionine contents of αA and αB crystallins are similar¹⁷.

Gels run in urea at pH 3.0 (in the absence of SDS) which separate the A and B chains of α crystallin¹⁶ support these conclusions but do not allow further identification of the cell-free products. Urea gels run at pH 8.9 (in the absence of SDS) provide further identification, since the αA and αB fractions are resolved into two components each, namely αA_1 , αA_2 , αB_1 and αB_2 (Fig. 3*a* and refs. 8 and 18). The ^{35}S -methionine labelled cell-free products gave the profiles shown in Fig. 3*b*. The 14S mRNA gave rise almost exclusively to crystallin αA_2 , which may be a precursor of αA_1 (ref. 19). The 10S mRNA product seems to contain both αB_2 and αB_1 , though unequivocal identifications cannot be made because some β crystallins also migrate in this position (Herbrik and Bloemendal, unpublished results).

These results indicate that the two lens mRNAs are active in the Krébs II ascites cell system and direct the synthesis of at least two identifiable lens polypeptide chains. It is surprising that the mRNA coding for crystallin αA_2 (molecular

weight 19,000) sediments as rapidly as 14S. By contrast the messengers for globin (molecular weight 16,000) and crystallin α B (molecular weight 22,000) sediment at 9S^{1,2} and 10S⁵ respectively. This disparity might be explained in several ways. (1) The sedimentation coefficient of the 14S mRNA might be anomalously high giving a spurious estimate of its molecular weight. Measurements of the sizes of the RNAs in denaturing conditions do not support this possibility. (2) The 14S mRNA might contain very long untranslated regions. Untranslated regions exist in bacteriophage RNA²⁰ and mammalian mRNA²¹⁻²³. The low proportion of adenylate residues in lens mRNA⁵ makes it unlikely that A-rich sequences are responsible. (3) The 14S RNA might be bicistronic, coding for two polypeptide chains. (4) The initial product might be a longer precursor molecule which is proteolytically trimmed to yield crystallin α A. Such post-translational modification has a precedent in the processing of virus coded proteins in picornavirus-infected cells²⁵.

The lens mRNAs are translated in the Krebs II ascites cell system with no requirement for additional lens components. Similarly lens⁸ and myeloma⁴ mRNAs have been translated in the reticulocyte cell-free system, and globin mRNA in *Xenopus* oocytes⁷ and in cell-free extracts from Krebs II⁶ and Landschutz ascites cells, and rat and mouse liver (manuscript in preparation). These results suggest that neither tissue-specific nor species-specific initiation factors are obligatory, at least in these instances.

Finally, this work, and our work with globin mRNA⁶, show that the ascites system combined with SDS gel electrophoresis gives a rapid and sensitive assay for messenger activity.

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