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ISOLATION AND IN VITRO TRANSLATION OF LEGHAEMOGLOBIN mRNA FROM YELLOW LUPIN ROOT NODULES*  

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Messenger RNA for leghaemoglobin, extracted from polysomes of yellow lupin root nodules, was purified by chromatography on oligo(dT)-cellulose followed by sucrose-gradient centrifugation. Leghaemoglobin mRNA activity was assayed in a cell-free protein synthesizing system derived from wheat germ. The purified mRNA was sedimented in sucrose gradient in the 9S region which corresponds to a molecular weight of about 225,000. The poly(A) segment was estimated to be 66 nucleotides in length, based on hybridization with $[^3H]$poly(U). Analysis of the translation product using immunoprecipitation and polyacrylamide-gel electrophoresis in denaturing conditions revealed that the product made in vitro was identical with authentic leghaemoglobin.

Leghaemoglobin, the myoglobin-like haemoprotein, occurs in the nitrogen-fixing legume root nodules (for review see Bergersen, 1971). This protein is an obligate partner in the symbiosis between plants of the family Leguminosae and the bacterium — Rhizobium. Although so far the biological role of leghaemoglobin is not fully understood, it appears that it maintains an optimal partial pressure of oxygen within the nodule, facilitating oxygen diffusion to the bacteroid surface during the fixation of atmospheric nitrogen (Wittenberg et al., 1974). According to recent findings, leghaemoglobin is localized in the host cell cytoplasm and does not penetrate the membrane sacs (Verma & Bal, 1976).

It is now established that apoleghaemoglobin is a plant gene product (Dilworth, 1969; Cutting & Schulman, 1971; Verma et al., 1974). The most conclusive evidence has been provided by the last group of authors who isolated soybean messenger RNA for the above protein and identified the product of its translation as an authentic apoleghaemoglobin. Soybean leghaemoglobin mRNA was the first plant template RNA species isolated in the highly purified state and producing a defined protein in cell-free systems.

* This investigation was supported by the Polish Academy of Sciences within the Project No. 09.7.1.
Nitrogen-fixing root nodules seem to be a very interesting biological model for studying complex relations between the legume plants and bacteroids (Rhizobia species adapted for symbiosis). Leghaemoglobin is considered a representative example of the genetic and cellular regulation of eukaryote-prokaryote symbiotic system since the synthesis of apoprotein is under the control of the plant partner, whereas haem (prosthetic group of leghaemoglobin) is of bacterial origin (Cutting & Schultman, 1971). A detailed analysis and characterization of gene products expressed as a direct consequence of symbiosis may lead to better understanding of the mechanism of enzyme induction in legume plants.

In this paper we report on successful isolation and translation in vitro of the mRNA for leghaemoglobin from yellow lupin root nodules, and compare its properties with mRNA from soybean tissue (Verma et al., 1974; Sidloï-Lumbroso & Schultman, 1977). A preliminary account of this study was presented at the symposium on Translation of Natural and Synthetic Polynucleotides in Poznań (Koniczny et al., 1977).

MATERIALS AND METHODS

Reagents. 4S RNA and wheat germ ribosomes were obtained according to the procedure of Golińska & Legocki (1973). Other RNA standards were prepared from purified wheat germ ribosomes by phenol extraction and separation by sucrose gradient centrifugation. TMV-RNA was isolated according to Marcus et al. (1974).

Plants. Root nodules were collected from field-grown yellow lupin plants var. Baltyk, 8 - 10 weeks after germination. The material collected was frozen at −20°C until further use.

Isolation of polysomes. All glassware used for the isolation or storage of polysomes and RNA preparations was heated for 3 h at 200°C. Plastic surfaces were treated with 0.2% diethylpyrocarbonate to destroy ribonucleolytic activity. All operations prior to phenol extraction were carried out at 0 - 4°C. Frozen root nodules (100 g) were ground to a powder and mixed with 250 ml of buffer containing 150 mM-Tris/acetate, 20 mM-KCl, 5 mM-magnesium acetate, 5 mM-2-mercaptoethanol, 0.2 M-sucrose and 0.4% Nonidet P-40, adjusted to pH 8.5, and stirred in a cold bath for 5 min; the resulting slurry was centrifuged at 20000 g for 10 min at 4°C. The postmitochondrial supernatant was layered over 2 ml of 1.5 M-sucrose cushion in 50 mM-Tris/acetate buffer containing 20 mM-KCl, and 5 mM-Mg-acetate, pH 8.5. The polysomes were pelleted by centrifuging at 128000 g for 4 h at 4°C in a Beckman 42.1 rotor. For preparation of mRNA, crude polysomes were suspended in the buffer composed of 0.1 M-Tris/acetate, 0.1 M-NaCl, 2 mM-EDTA and 1% SDS; final pH 9.0.

Preparation of messenger RNA for leghaemoglobin. Crude polyosomal RNA was isolated from resuspended polysomes by double extraction each time with an equal volume of phenol-chloroform-isoamyl alcohol (50: 49: 1, by vol.), followed by precipitation at −20°C with two volumes of ethanol. Polyosomal RNA dissolved in 0.01 M-Tris/HCl buffer, pH 7.4, containing 0.5 M-NaCl and 0.5% SDS was then
applied on oligo(dT)-cellulose column. Poly(A)-containing RNA was isolated essentially according to the procedure described by Aviv & Leder (1972). Routinely, 100 A₂₆₀ units in 5 ml were applied onto an oligo(dT)-cellulose column (0.8 x 1.5 cm) previously equilibrated with the same buffer. The column was washed with the same buffer until the absorbance at 260 nm of the eluate was below 0.05, and poly(A)-containing RNA was eluted with water. To this fraction (usually 1.6 A₂₆₀ units in 2-3 ml), potassium acetate was added to a final concentration of 0.2 M and RNA was recovered by precipitation with ethanol. In preliminary experiments, poly(A)-containing RNA was also isolated by direct chromatography of polysomes on oligo(dT)-cellulose in the presence of 0.5% SDS. In each case the poly(A)-containing fraction from either polysomal RNA or polysomes constituted 1.5 to 2% of the A₂₆₀ units applied to the column.

mRNA for leghaemoglobin was purified from poly(A)-containing RNA by sucrose-gradient centrifugation. Linear gradients of 10-30% sucrose in 50 mM-Tris/acetate, 0.1 M-NaCl and 0.1 mM-Na/acetate at a final pH of 8.5 were prepared in Beckman SW 40 cellulose nitrate tubes. RNA samples (15-20 A₂₆₀ units in 0.25 ml of the above buffer) were heated to 35°C for 30 min, loaded on tubes and centrifuged at 40,000 r.p.m. (280,000 g) for 14 h at 15°C. The absorbance at 260 nm of the fractions collected from the gradients was monitored and recorded on ISCO UA-5 absorbance monitor. The fractions were pooled as indicated under Results, precipitated with ethanol in the presence of 0.2 M potassium acetate, pH 5.5, at −20°C, dried, dissolved in sterile water and stored in liquid nitrogen until further use.

Cell-free translation of leghaemoglobin mRNA. A standard cell-free amino acid incorporating system was prepared from wheat germ (General Mills, Vallejo, Calif.) by the method of Marcus et al. (1974). The complete system contained in a final volume of 25 μl: 8 μl of wheat germ extract (fraction S23), 20 mM-Hepes, pH 7.6, 2 mM-dithiothreitol, 2 mM-ATP, 0.4 mM-GTP, 60 μg/ml creatine phosphokinase, 16 mM-creatine phosphate, 100 mM-K-acetate, 3.4 mM-Mg-acetate, variable amounts of mRNA (usually 10 μg), 0.2 μCi of [¹⁴C]leucine (200 mCi/mmol), and each of 19 unlabelled amino acids was added at a concentration of 25 μM. The mixtures were incubated for 120 min at 30°C. To determine the radioactivity incorporated into total proteins, 10 μl aliquots were withdrawn, placed on Whatman 3 MM filters, immersed in cold 10% trichloroacetic acid for 10 min, heated for 15 min in 5% trichloroacetic acid, washed in ethanol, dried and counted in a toluene scintillator in a Tri-Carb liquid scintillation spectrometer with an efficiency of 70% for ¹⁴C.

Immunoprecipitation of leghaemoglobin and SDS-gel electrophoresis of translation products. The leghaemoglobin synthesized in vitro was detected by immunoprecipitation. Specific antiserum was prepared from rabbits immunized with leghaemoglobin purified to homogeneity from postmitochondrial supernatant of root nodule extract by ammonium-sulphate fractionation, chromatography on DEAE-cellulose and gel filtration on Sephadex G-100 (Broughton et al., 1972). The γ-globulin fraction was prepared from immune sera by double precipitation with
55% ammonium sulphate. The antiserum was analysed by immunoelectrophoresis and immunodiffusion, and each time a single line of precipitation was observed when root nodule protein extract was tested. Usually, immunoprecipitation was performed using 200-400 μl of the protein-synthesizing system, under conditions described in the preceding section. The reaction was terminated by the addition of an equal volume of detergent solution containing: 20 mM-Na-phosphate buffer, pH 7.2, 2% Triton X-100, 1% deoxycholate, and 0.2% SDS. The mixture was incubated for 30 min at room temperature. Then the leghaemoglobin antibody (about 350 μg of γ-globulin fraction) was added, immediately followed by 10 μg of carrier leghaemoglobin. The mixture was then incubated for 90 min at 25°C followed by overnight incubation at 4°C. The immunoprecipitate was sedimented by passage through a 100 μl layer of 1.5 m-sucrose containing 10 mM-Na-phosphate buffer, pH 7.2, 1% Triton X-100, 1% deoxycholate and 1.5 m-NaCl. The supernatant was carefully removed and the sediment was washed twice with the above buffer, but with sucrose omitted. Finally, the immunoprecipitate was dissolved in 0.1 m-NaOH, neutralized with 1 m-HCl, reprecipitated with 10% trichloroacetic acid, then the precipitate was collected on glass fiber discs (Whatman GF/C), and counted in toluene scintillating solution. The antigen-antibody complex prepared and washed in this way was also subjected to gel electrophoresis in denaturing conditions. For this purpose the washed precipitate was dissolved in 62 mM-Tris/HCl buffer, pH 6.8, containing 2% SDS, 0.96 m-2-mercaptoethanol and 10% glycerol, and applied to 12.5% polyacrylamide gels containing 0.1% SDS according to Laemmli (1970). Electrophoresis was performed at room temperature at a constant current of 5 mA/gel using bromophenol blue as a marker. The protein bands were stained with Coomassie brilliant blue R-250 and the gels were destained with 40% methanol containing 7% acetic acid. The gels were sliced into 1 mm fractions, dried and assayed for radioactivity in toluene scintillation fluid.

Hybridization analysis of poly(A)-containing RNA. The presence of poly(A) in the messenger fraction was tested by hybridization with [3H]poly(U) according to Verma et al. (1974). The incubation mixture contained in a final volume of 0.5 ml: mRNA for leghaemoglobin (0.2-0.8 A₂₆₀ units), 5 nCi of [3H]poly(U), (50 mCi /mmole), 50 mM-Tris/acetate, pH 7.4, 0.2 m-NaCl and 5 mM-Mg-acetate. Samples were incubated for 15 min at 25°C and then digested by pancreatic RNase (20 μg/ml) for 30 min. The hybridized poly(A)-containing RNA was precipitated by the addition of 2 ml of cold 5% trichloroacetic acid in the presence of 100 μg of carrier RNA. After 30 min at 0°C, the precipitate was collected by trapping on Whatman GF/C filters and the radioactivity was measured in a scintillation counter.

RESULTS

As we have shown earlier, the polysomes from lupin root nodules contained polyadenylated RNA and supported incorporation of amino acids into proteins in the presence of postribosomal supernatant from wheat germ (Konieczny
et al., 1977). To isolate the leghaemoglobin mRNA we have extracted total polysomal RNA and submitted it to further purification. The first step of fractionation was the isolation of poly(A)-containing RNA fraction. As can be seen from Table 1, about 1.5\% of the applied total RNA was retained on oligo(dT)-cellulose. Since the subsequent washing with 0.1 M NaCl resulted in elution of only a small amount of RNA, poly(A)-containing RNA was recovered from the column by direct elution with water or 10 mM-Tris/acetate buffer, pH 7.2. Both RNA fractions, bound and not bound on oligo(dT)-cellulose, revealed a high level of template activity in the cell-free wheat germ system (Table 2). It is interesting to note from Table 2 that the \( 7\)mGTP, a methylated analogue of the 5'-terminus of eukaryotic mRNA, inhibits the translation of both poly(A)-containing RNA and poly(A)-lacking RNA.

### Table 1

**Binding of polysomal RNA from lupin root nodules to oligo(dT)-cellulose**

Polysomes were prepared from lupin root nodules as described in Materials and Methods. Polysomal RNA was dissolved in 10 mM-Tris/Cl buffer pH 7.4, containing 0.5 M NaCl and 0.5\% SDS, and was applied onto an oligo (dT)-column (0.8 × 1.5 cm) which was equilibrated previously with the same buffer. The poly(A)-containing RNA was eluted from the column with 0.1 M NaCl followed by H\(_2\)O.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Total RNA applied</th>
<th>RNA eluted with</th>
<th>RNA bound (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{A}_{260} ) units</td>
<td>0.1 M NaCl</td>
<td>H(_2)O</td>
</tr>
<tr>
<td>1</td>
<td>80</td>
<td>0.16</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>84</td>
<td>0.12</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0.1</td>
<td>1.5</td>
</tr>
</tbody>
</table>

This may be a preliminary indirect indication that these mRNA species contain the modified ("capped") 5'-terminus. Purification and properties of poly(A)-lacking RNA are being studied separately and the results will be published elsewhere. The presence of poly(A) segment in the RNA bound to oligo(dT)-cellulose was additionally confirmed by the hybridization assay with \( ^{3} \)Hpoly(U), (Fig. 1). The length of the poly(A) region in leghaemoglobin mRNA was estimated from \( ^{3} \)Hpoly(U) hybridization measurements to be about 66 adenosine residues (9.7\% of mRNA).

Poly(A)-containing RNA isolated by oligo(dT)-cellulose chromatography was further fractionated by sucrose-gradient centrifugation in a low ionic strength buffer. The RNA isolated from the separated fractions was tested for template activity in the wheat germ system. Two kinds of measurements were made: total incorporation of \( ^{14} \)Cleucine into the hot trichloroacetic acid-precipitable material (Fig. 2A), and immunoprecipitation with leghaemoglobin antibody. Although the latter activity was distributed in the region between 7 and 12S, the maximum of leghaemoglobin mRNA activity was found to sediment at 9S (Fig. 2B). The specific activity of the leghaemoglobin mRNA recovered from the sucrose gradient was about 15 times higher than that of the oligo(dT)-cellulose filtered RNA.
Table 2
Requirements for amino acid incorporation in wheat germ cell-free system, directed by RNA from lupin root nodules

Polysomal RNA, and RNAs containing and not containing poly(A) were prepared as described under Methods. To the complete system from wheat germ (for details see Methods), 0.2 A₂₆₀ unit of RNA template was added in a volume of 25 µl.

<table>
<thead>
<tr>
<th>Reaction components</th>
<th>[¹⁴C] leucine incorporated at 30°C (pmoles/120 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete postmitochondrial system</td>
<td>3.2</td>
</tr>
<tr>
<td>minus energy generating system</td>
<td>2.5</td>
</tr>
<tr>
<td>minus ATP</td>
<td>4.8</td>
</tr>
<tr>
<td>plus RNA templates:</td>
<td></td>
</tr>
<tr>
<td>TMV RNA</td>
<td>71.9</td>
</tr>
<tr>
<td>total polysomal RNA</td>
<td>30.4</td>
</tr>
<tr>
<td>poly(A)-lacking RNA</td>
<td>32.8</td>
</tr>
<tr>
<td>poly(A)-lacking RNA + 0.1 mm⁻¹mGMP</td>
<td>25.9</td>
</tr>
<tr>
<td>poly(A)-lacking RNA + 0.4 mm⁻¹mGMP</td>
<td>14.8</td>
</tr>
<tr>
<td>poly(A)-containing RNA</td>
<td>21.1</td>
</tr>
<tr>
<td>poly(A)-containing RNA + 0.1 mm⁻¹mGMP</td>
<td>15.8</td>
</tr>
<tr>
<td>9S RNA</td>
<td>36.8</td>
</tr>
</tbody>
</table>

Fig. 1. Oligo(dT)-cellulose chromatography of polysomal RNA. About 100 A₂₆₀ units of RNA were applied to 0.8 × 1.5 cm oligo(dT)-cellulose column as described in text. Poly(A)-containing RNA was eluted with H₂O and the absorbance of the 1 ml fractions was measured at 260 nm. The presence of the poly(A) segment in RNA fractions was detected by hybridization to [³H]poly(U) as described in Materials and Methods.
Fig. 2. Purification of leghaemoglobin mRNA on a low-salt sucrose gradient. Poly(A)-containing RNA (500 µg) from root nodules in a total volume of 300 µl was sedimented in a 10 to 30% linear sucrose gradient as described in Methods. After sedimentation for 14 h at 284,000 g at 14°C, the fractions were collected by pumping from the bottom of the tubes. The material sedimented in the six combined fractions was recovered by ethanol precipitation and assayed for translational activity in the wheat germ system by measuring incorporation of [14C]leucine.

A, Incorporation into total polypeptide products precipitated by hot trichloroacetic acid (open blocks) as described in Methods. The relative migration of RNA standards on parallel gradients is designated by the corresponding S value.

B, Incorporation into the material immunoprecipitated after incubation of the translation product with anti-leghaemoglobin (open blocks). The immunoreactive radioactivity is presented as a percentage of the total RNA activity (shaded blocks).

C, Estimation of molecular weight of leghaemoglobin mRNA. Sedimentation of the leghaemoglobin mRNA was compared with that of the purified RNA standards: 4S RNA (mol. wt. 0.025 × 10^6), 5S RNA (0.038 × 10^6), 18S RNA (0.66 × 10^6) and 25S RNA (1.3 × 10^6).
The procedure for purification of leghaemoglobin mRNA is summarized in Table 3. As can be seen from the specific activities presented, the mRNA was purified about 30-fold with an about 7% recovery. Purification of mRNA on oligo(dT)-cellulose gave a twofold increase of the leghaemoglobin mRNA content in the total mRNA population (as calculated from the percentage of immunoreactive polypeptide in the total translation product). In the final purified RNA preparation, after sucrose-gradient centrifugation, the leghaemoglobin messenger activity constituted about 50% of total mRNA. This value is comparable with that reported for the similar mRNA from soybean (Verma et al., 1974).

Table 3

Purification of leghaemoglobin mRNA

The messenger activity is expressed in pmoles of [14C]leucine incorporated at 30°C during 120 min of incubation with the wheat germ cell-free system (for details see Methods). Total RNA activity was measured as the hot trichloroacetic acid-precipitable activity, and leghaemoglobin (LgHb) mRNA activity as the radioactivity of the material immunoprecipitated with the purified anti-leghaemoglobin.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>RNA (mg)</th>
<th>total mRNA (pmoles/sample)</th>
<th>LgHb mRNA</th>
<th>LgHb mRNA/total mRNA (%)</th>
<th>Purification factor</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total polysomal</td>
<td>32.4</td>
<td>3.5 x 10^4</td>
<td>2.14 x 10^3</td>
<td>5.9</td>
<td>0.07</td>
<td>1</td>
</tr>
<tr>
<td>Oligo(dT)-cellulose</td>
<td>4.5</td>
<td>3.6 x 10^3</td>
<td>5.3 x 10^2</td>
<td>14.9</td>
<td>0.12</td>
<td>1.8</td>
</tr>
<tr>
<td>eluate</td>
<td>0.08</td>
<td>2.95 x 10^2</td>
<td>1.48 x 10^2</td>
<td>49.9</td>
<td>1.84</td>
<td>28.2</td>
</tr>
</tbody>
</table>

In order to test the uniformity of the response of the purified mRNA, a sucrose-gradient fraction of RNA corresponding to 95 was translated in the wheat germ system and the polypeptides synthesized were subjected to immunoprecipitation followed by SDS-acrylamide gel electrophoresis. Under these conditions a single peak of radioactive polypeptide was observed corresponding to the authentic leghaemoglobin coelectrophoresed on the same gel (Fig. 3B). When crude unfractionated RNA preparation was used as a template, several discrete polypeptides differing widely in size were observed (Fig. 3A). Functionality of leghaemoglobin mRNA was also confirmed in the rabbit reticulocyte system where, similarly to the wheat germ system, only one polypeptide product was observed on acrylamide-gel electrophoresis under denaturing conditions (the results not shown).

It is well known that efficient translation of template RNAs requires individually defined incubation conditions; in this respect the concentration of Mg^2+ and K^+ ions seems to be particularly important. Data concerning the effect of these ions
are shown in Fig. 4. It may be seen that the optimum K⁺ concentration for leghaemoglobin synthesis was 100 mM, the Mg²⁺ optimum was 3.5 mM whereas spermine optimum was 200 μM. In general, it may be concluded that the optimal conditions for the apoleghaemoglobin synthesis in vitro are similar to those reported for other mRNAs from eukaryote sources (Verma et al., 1974, Sarkar & Griffith, 1976).

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Fig. 3. Scanning profile of polyacrylamide-gel electrophoresis under denaturing conditions of the polypeptide products synthesized in vitro.

A, Translation products directed by unfractionated RNA preparation.

B, Translation product directed by purified mRNA for leghaemoglobin after immunoprecipitation with anti-leghaemoglobin. Electrophoresis was carried out as described in Materials and Methods. The arrow indicates the position of native leghaemoglobin.
Fig. 4. Effect of Mg$^{2+}$, K$^+$ and spermine on translation of leghaemoglobin mRNA from lupin root nodules. Standard incubation mixtures contained 10 µg of mRNA for leghaemoglobin. Spermine was added together with 3.4 mM-Mg$^{2+}$ and 100 mM-K$^+$. The hot trichloroacetic acid-precipitable radioactivity was measured as described in the text.

DISCUSSION

Purification of mRNA species from eukaryote tissues is accomplished by two useful methods: separation of poly(A)-containing mRNA from rRNA, and mRNA sizing technique. Both these techniques appeared to be very efficient in purification of leghaemoglobin mRNA. In preliminary stages of this work we checked the applicability of several other techniques for fractionation of polysomal RNA, such as chromatography on poly(U)-Sepharose or poly(U)-cellulose and binding to nitrocellulose filters. Since all these techniques allow us to extract poly(A)-containing RNA from the total cellular RNA, the results obtained could be compared with those observed with oligo(dT)-cellulose. It was found that the latter technique resulted in the lowest contamination with rRNA and was the most convenient for isolation of a poly(A)-containing RNA population from lupin polysomes.

It has been known for some time that several viral and eukaryotic mRNAs contain a unique 5'-capped terminus which in many cases is required for efficient translation of these mRNAs (Furuichi & Miura, 1975; Furuichi et al., 1975; Muthukrishnan et al., 1975). One of the methods for studying this requirement is to test the effect of some cap analogues, such as 7-methylguanosine-5'-phosphate, on translation, as it was found that this analogue is a potent inhibitor of translation of capped mRNAs (Hickey et al., 1976). Since translation of poly (A)-containing RNA from lupin root nodules is strongly inhibited by 7-mGMP, it is very likely that mRNA for leghaemoglobin requires a 5'-capped terminus for its efficient translation.

Analysis of the poly(A) content in mRNA by the hybridization assay with $^3$H poly(U) and calculation based on calibrations with poly(A) standards of defined length, showed that the purified leghaemoglobin mRNA contained 9.8% poly(A), which corresponds to 66 nucleotides. Although this may be considered a rough estim-
ate, it indicates that a considerable poly(A) segment is present in the purified mRNA preparation. The size of leghaemoglobin mRNA determined by sucrose-gradient centrifugation corresponds to 95 (Fig. 2C). A similar value was previously calculated for leghaemoglobin mRNA from soybean (Verma et al., 1974; Sidhoo-Lumbroso & Schulman, 1977). From a comparison with sedimentation characteristics of globin mRNA (Morrison & Lingrel, 1976) one may assume that the molecular weight of lupin leghaemoglobin mRNA should be about 225 000 which corresponds to approximately 680 nucleotides including the poly(A) segment. Lupin leghaemoglobin has an apparent molecular weight of 16 000 and contains 145 amino acids (Vainshtein et al., 1975). Since, as it was shown in this work, the mobility of the standard leghaemoglobin corresponds exactly to that of the polypeptide synthesized in vitro, it may be concluded that apoleghaemoglobin is not formed as a larger precursor molecule. Comparison of the determined length of leghaemoglobin mRNA with the theoretically calculated minimal length of the template required for coding 145 amino acids, indicates that only 64% of the coding capacity of this mRNA is used for the direction of apoleghaemoglobin synthesis. In other words, about 36% of this capacity in the mRNA is of an unknown function. Such a larger than expected molecular weight of leghaemoglobin mRNA seems to be a more general feature of eukaryote mRNAs: untranslated regions of different length have been detected in mRNA for ovoalbumin (Rosen et al., 1975), a-crystallin (Bem et al., 1973), vitellogenin (Shapiro & Baker, 1977) and several other mRNA species. Understanding of the function of such regions will require further studies.

We thank Dr. W. Filipowicz for a gift of 7mGMP (P. L. Biochemicals) and for making available to us some of his data prior to publication.

REFERENCES

IZOLACJA I TRANSLACJA IN VITRO mRNA DLA LEGHEMOGLOBINY
Z BRODAWEK KORZENIOWYCH ŁUBINU ŻÓLTEGO

Streszczenie

Z polisomów brodawek korzeniowych łubinu żółtego wyodrębniono mRNA dla leghemoglobininy. W celu oczyszczenia izolowanego mRNA zastosowano chromatografię na oligo(dT)-celulozie oraz wirowanie w gradiencie sacharozowy. Oczyszczyły mRNA wykazywał współczynnik sedimentacji 95, co odpowiada ciężarowi cząsteczkomowemu 225 000. Długość fragmentu poli(A) wyznaczona metodą hybrydyzacji z kwasem [3H]poliuridyłowym wynosiła 66 nukleotydów. Biologiczną aktywność mRNA dla leghemoglobininy badano w bezkomórkowym układzie z frakcji zarodków pszenicy. Siosując metodę immunoprecypitacji oraz elektroforezy na zelu poliakrylamidowym w warunkach denaturujących wykazano, że produkt zsyntetyzowany in vitro jest identyczny z leghemoglobiną.

Received 13 June, 1978.