A single-step procedure for the isolation of individual mRNA species from crude lysates of *Physarum polycephalum*

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An individual mRNA species was isolated directly from lysates of *Physarum polycephalum* by hybridization to immobilised complementary DNA. The solvent for lysis was a 6 M-guanidinium salt solution, and the hybridization reaction was carried out at 42°C in 4 M guanidinium salt/33% (v/v) formamide buffer. The mRNA species isolated by this procedure was active in cell-free protein synthesis and directed the synthesis of a polypeptide of M_r 25 500 ± 750.

Physarum *Plasmoidal lysate* *Hybrid selection* *Messenger RNA* *Purification* *Poly(A)$^+$-messenger RNA*

1. INTRODUCTION

Messenger RNA fulfils a pivotal role in the expression of genetic information [1–3]. The unique properties of mRNA are of great value because, on the one hand, mRNA may be translated to yield proteins and, on the other hand, mRNA may be used to isolate its gene. We sought to exploit these properties of mRNA to study gene expression during the mitotic cycle of *Physarum polycephalum*. During the course of this work we developed a simplified method for the isolation of particular mRNA species. The new feature of the procedure is that individual mRNA species are isolated directly from crude lysates, in a single step, by hybridisation to immobilised DNA.

The method is based on lysing cells in a solvent which has the following properties:

(i) The solvent must inactivate RNase:
(ii) It must disrupt cellular and subcellular structures;
(iii) It must dissociate nucleoproteins;
(iv) Its pH and ionic strength should favour the native form of DNA and also the formation of a stable complex between RNA and denatured DNA;
(v) It must effectively solubilise components of the lysate other than the mRNA fraction so that they neither interfere with the interaction between RNA and immobilised DNA, nor bind to, nor clog, the solid support to which the DNA is attached.

Solvent containing high concentrations ($\geq$4 M) of guanidinium salts, such as guanidinium chloride [4,5] or guanidinium thiocyanate [6,7] were shown to meet our requirements.

The principle of the method was established in [8] when the poly(A)$^+$-mRNA fraction of *Physarum* was isolated from lysates made by dissolving the slime mould in 6 M guanidinium salts (chloride or isothiocyanate). The poly-(A)$^+$-mRNA fraction was then isolated directly from the lysate by means of the affinity of the poly(A)-tails for poly(U) immobilised on a solid support. Essentially the same procedure, except that higher temperatures are needed for the hybridisation reaction, can be used to isolate mRNA by hybrid selection directly from lysates prepared by dissolving *P. polycephalum* in 6 M guanidinium salts.

The method is simple and effective because it minimises the risk of damage to mRNA through the effects of RNases. The mRNA species isolated by this novel procedure from crude lysates of *P. polycephalum* was found to be functional in directing protein synthesis in the nuclease-treated rabbit reticulocyte lysate system.

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2. EXPERIMENTAL

2.1. *Buffers*

Lysis buffer for lysing *Physarum* was either 6 M guanidinium chloride/1 mM-β-mercaptoethanol/ 15 mM Tris-HCl (pH 7.5) or 6 M guanidinium isothiocyanate/1 mM β-mercaptoethanol/10 mM Tris-HCl (pH 7.5).

Eluting buffer for recovering poly(A)$^+$-mRNA from poly(U)-Sepharose 4B or individual poly-(A)$^+$-mRNA from hybrids was 90% (v/v) formamide/1 mM EDTA/ 15 mM Tris-HCl (pH 7.5).

Wash buffer for purifying the poly(A)$^+$-mRNA·poly(U)-Sepharose 4B complex was 4 M guanidinium chloride/1 mM mercaptoethanol/ 15 mM Tris-HCl (pH 7.5).

Storage buffer for poly(A)$^+$-mRNA was 10 mM Tris-HCl (pH 7.0). This buffer was also used to wash the poly(A)$^+$-mRNA·poly(U)-Sepharose 4B complex immediately before treatment with eluting buffer.

Lysis/formamide buffer was made by mixing 2 vol. lysis buffer (or of lysate) with 1 vol. AR-formamide. SSC (standard saline citrate) was 0.15 M NaCl/0.015 M Na-citrate (pH 7.0).

0.01 × SSC, 0.1 × SSC etc., refer to solutions whose composition is related to SSC by the factor shown.

Denhardt's solution was 0.02% (w/v) Ficoll/0.02% (w/v) polyvinylpyrrolidone/ 0.02% (w/v) bovine serum albumin.

G50 buffer was 0.5% (w/v) SDS/200 mM NaCl/1 mM EDTA/10 mM Tris-HCl (pH 7.5).

2.2. *General precautions*

All glassware was siliconised with dimethyldichlorosilane solution and autoclaved at 15 lb.in^{-2} at 120°C for 15 min. All buffers and plastic-ware were also autoclaved as above. The precautions in [10] are recommended.

2.3. *Growth of microplasmodia of* P. polycephalum

Microplasmodia (strain M_3 C VIII) were grown at 26°C in the semi-defined medium of [11], except that chick embryo extract was replaced with 1 ml of a haematin solution (0.05% (w/v) haemin in 1% (w/v) NaOH) for each 100 ml of medium. Microplasmodia were grown in 125 ml medium contained in 500 ml flasks which were placed on a reciprocal shaker moving at 80 osc./min with a displacement of 10 cm/osc. and subcultured every 2–3 days to maintain log-phase growth. The microplasmodia were harvested in log-phase ~2 days after inoculation.

2.4. *Isolation of the poly(A)$^+$-mRNA fraction from crude lysates of microplasmodia*

The method used was described in [8]. Poly-(U)-Sepharose was treated with 1 M NaCl to swell the gel, thoroughly washed with eluting buffer and equilibrated with wash buffer. Microplasmodia were harvested by centrifugation (3000 rev./min for 2 min at 4°C) and lysed in pre-cooled lysis buffer (10 ml/g wet wt microplasmodia). The lysate was clarified by centrifugation (3000 rev./min for 10 min) and shaken for 2–3 h at 4°C with 50 mg (dry wt) of prepared poly(U)-Sepharose/g wet wt microplasmodia.

The poly(U)-Sepharose was recovered by centrifugation (3000 rev./min for 10 min), washed extensively with wash buffer, then packed into a column and washed again with wash buffer and storage buffer until no more UV absorbing material was eluted (i.e., A_{260} of eluate <0.002 units). The poly(A)$^+$-mRNA was then eluted from the column by treatment with eluting buffer and precipitated from the eluate at -20°C by the addition of 0.01 vol. 5 M potassium acetate and 2.5 vol. absolute ethanol/vol. eluate. The poly-(A)$^+$-mRNA was recovered by centrifugation in a microfuge, dissolved in storage buffer and reprecipitated and redissolved twice more. The poly(A)$^+$-mRNA was stored at -20°C in storage buffer.

2.5. *Isolation of poly(A)$^+$-mRNA from mouse 3T6 cells*

The procedure in [12] was used. Cells were lysed with Nonidet P40 and RNA was isolated by extraction with phenol. Poly(A)$^+$-mRNA was then isolated from total cellular RNA by affinity chromatography using poly(U)-Sepharose 4B.

2.6. *Translation of poly(A)$^+$-mRNA in the rabbit reticulocyte lysate system*

The assay was done as in [13]. Nuclease-treated rabbit reticulocyte lysate (25 μl) containing 5 μCi [^{35}S]methionine and 19 non-radioactive amino acids was mixed with poly(A)$^+$-mRNA (up to 5 μl mRNA in storage buffer) and brought to 30 μl

with the addition of water. Poly(A)$^+$-mRNA was tested at 3 different concentrations. The reaction mixtures were incubated at 34°C for 90 min then cooled on ice. A (2 μl) sample was withdrawn from each assay, mixed with 0.1 M KOH (150 μl), a drop of H_2O_2 was added, and samples were incubated at 34°C for 10 min. An excess of ice-cold 10% (v/v) Trichloroacetic acid was added and samples were kept at 0°C for 15 min to precipitate the protein. The precipitated protein was recovered by filtration using Oxoid cellulose acetate filters (grade 0.45 μm). Assay tubes and filters were washed with 5% (v/v) Trichloroacetic acid. Each filter was washed once in distilled water, then with ethanol, then with ethanol/ether (1 : 1), and finally with ether. Radioactivity of the filters was measured in a Beckman LS7500 scintillation counter using a Packard toluene-based scintillant.

2.7. *Separation of the products of cell-free synthesis*

The radioactive proteins synthesised in the cell-free system were separated by electrophoresis through 1% (w/v) SDS–15% (w/v) polyacrylamide gels as in [14]. The gel was dried and the radioactive proteins were detected by fluorography [15].

2.8. *Iodination of RNA*

The method in [16] was used. The procedure followed was that in [17]. ^{125}I (1 mCi, in 0.1 M NaOH) was acidified by the addition of 0.7 M Na-acetate (pH 4.6) (4 μl), RNA solution (up to 20 μl solution of 1 μg RNA/μl storage buffer) was added, followed by 12 mM thallium III trichloride/0.2 M Na-acetate (pH 4.6) (8 μl). The solution was incubated at 60°C for 20 min, cooled, then neutralised by 1 M Na-phosphate, (pH 7.0) (100 μl) and 0.1 M Na-sulphite (30 μl) was also added. The solution was then incubated at 60°C for 1 h.

^{125}I-labelled RNA was purified by gel filtration through Sephadex G50 (medium grade). The column of gel was contained in a siliconised Pasteur pipette, plugged with glass wool. The column was first loaded with yeast tRNA (25 μg) which was washed through with samples (200 μl) of G50 buffer. The reaction mixture was then loaded and washed through the column with G50 buffer. Fractions (200 μl) were collected. The fractions comprising the first peak of eluted radioactivity were pooled, RNA was precipitated by the addition of 2.5 vol. ethanol. The precipitate was dissolved in lysis/formamide buffer (1 ml). The specific activity was usually 1–2 $\times$ 10^7 cpm/μg RNA.

2.9. *Isolation of p5-13 DNA*

Escherichia coli strain FMA-10 carrying plasmid p5-13, which contains a 5 kilobasepair *Hin*dIII – *Bam*Hl fragment of *Physarum* DNA inserted into pAC 184, was grown in the presence of chloramphenicol, and p5-13 DNA was isolated as in [20].

2.10. *Binding of DNA to aminothiophenol-paper*

Aminothiophenol-paper, which binds DNA covalently, is similar to DBM-paper [18]. Aminothiophenol-paper, which has a higher capacity for binding DNA than DBM-paper, was prepared by a procedure developed by Seed as in [19]. Sheets of Whatman 540 paper were shaken for 16 h in a sealed bag with 350 mM NaOH/30% (v/v) 1,4-butanediol diglyceryl ether, (2 mg/ml) Na-borohydride. The paper was then washed for 1 h with 0.25 M NaOH/50% (v/v) ethanol, then incubated with shaking for 2 h in 1 vol. 5% (w/v) 2-aminothiopenol in ethanol and 1 vol. 0.5 M NaOH. The paper was washed twice in absolute ethanol, and then with 0.1 M HCl. The washing procedure was repeated twice more, the paper was washed in distilled water, dried in air, and stored in the dark at 20°C.

Aminothiophenol-paper was diazotised by treatment with 1.2 M HCl/0.03% (w/v) $NaNO_2$ at 4°C for 30 min. Immediately before use, the paper was washed twice with ice-cold distilled water, twice with ice-cold 50 mM Na-phosphate buffer (pH 6.5) then dried by blotting.

Plasmid DNA, made linear by treatment with restriction endonucleases *Bam*Hl and *Hin*dIII, was denatured by treatment with 80% (v/v)-dimethyl-sulphoxide/50 mM Na-phosphate (pH 6.5) at 80°C for 10 min, then cooled quickly on ice. Denatured DNA (10 –15 μl) was loaded onto a disc (7 mm diam.) of freshly diazotised aminothiophenol-paper at 0°C, then left at 4°C for $\geqslant$4 h to allow the DNA to bind. Before use, DNA/aminothiophenol-paper discs were washed in 80% (v/v) DMSO/50 mM Na-phosphate (pH 6.5) at 4°C for 30 min and in 0.1 $\times$ SSC at 95°C for 15 min to remove non-covalently bound DNA.

2.11. *Materials*

Pancreatic RNase ribonuclease was purchased from Cabiochem; ^{125}I was purchased from Amersham International (Bucks). Guanidinium chloride was either prepared from Analar guanidinium carbonate obtained from BDH (London) or was bought from Sigma (St. Louis MO). Poly-(U)-Sepharose 4B was bought from Pharmacia (Uppsala). Agarose (type 1) was purchased from Sigma. AR formamide, bought from Fisons, was deionised before use with a mixed-bed resin AG-501 × 8 from Biorad. All other chemicals were of reagent grade or better.

3. RESULTS

We describe below the development of a method for isolating a particular RNA species directly from crude lysates of *P. polycephalum* by hybridisation to complementary immobilised DNA. To investigate the optimum conditions for hybridisation in crude lysates, preliminary experiments were carried out with p5-13 DNA, which comprises a 5000 basepair *Hind*III–*Bam*HI fragment of *P. polycephalum* genomic DNA inserted into plasmid pAC 184, and the ^{125}I-labelled poly(A)$^+$-mRNA fraction of *P. polycephalum*.

3.1. *Isolation of poly(A)$^+$-mRNA fraction*

The isolation of the poly(A)$^+$-mRNA fraction by hybridisation to immobilised poly(U) in crude lysates of *Physarum* was described in detail previously. Plasmodia or microplasmodia of *P. polycephalum* were lysed in lysis buffer (e.g., 6 M guanidinium chloride) poly(U)-Sepharose 4B was added to the lysate and the mixture was gently agitated for 2 h at 4°C. The gel was recovered by low-speed centrifugation, washed to remove contaminants and the poly(A)$^+$-mRNA fraction was recovered by washing the gel with eluting buffer.

The poly(A)$^+$-mRNA fraction was iodinated (as in section 2.8) for use as a radioactive probe. The specific radioactivity was $1–2 \times 10^7$ cpm/μg poly(A)$^+$-mRNA.

3.2. *Hybridisation in 4 M guanidinium chloride/formamide solutions*

The principle of the above method for the isolation of the poly(A)$^+$-mRNA fraction also applies to the isolation of RNA by hybridisation to immobilised denatured DNA. However, the detailed conditions for the hybridisation step were adjusted to take into account both the lower concentrations of the reactants (viz., individual mRNA species and immobilised DNA), and the need to diminish the partly bi-helical secondary structure of both mRNA and denatured DNA. The adjustment required is a rise in the temperature of the hybridisation reaction.

Hybridisation between poly(A)-tails and immobilised poly(U) in lysis buffer takes place readily at 4°C because the secondary structure of the reactants is single-stranded in character and there is no impediment to poly(A)·poly(U) complex formation. In contrast, the optimum temperature for DNA·RNA and DNA·DNA hybrid formation is regarded as 25°C below the melting temperature (T_m) of the bi-helical product [21]. At T_m–25°C hybridisation proceeds at an acceptable rate and the partly bi-helical structure of RNA and denatured DNA is diminished and so is no longer a major barrier to hybrid formation. The temperature of the reaction is kept low in order to minimise thermal hydrolysis of internucleotide bonds.

Lysis/formamide buffer (e.g., 4 M guanidinium chloride 33% (v/v) formamide) at 35–42°C was found to be suitable for RNA·DNA hybrid formation. In practice, 1 vol. AR formamide was added to 2 vol. lysate. The addition of formamide to 4 M guanidinium chloride solutions reduced the T_m of salmon sperm DNA from 75–60°C (table 1); i.e. T_m–25°C was reduced from 50–35°C.

The hybridisation of *Physarum* ^{125}I-labelled poly(A)$^+$-mRNA fraction with p5-13 DNA was examined both at 65°C in a conventional solvent (e.g., 3 × SSC) and also at 37°C and at 42°C in lysis/formamide buffer (fig. 1). The extent (and hence the rate) of hybridisation was the same in lysis/formamide buffer at 42°C as in an equivalent volume of 3 × SSC buffer at 65°C. The results show (fig. 1) that lysis/formamide buffer at 37–42°C is suitable for hybrid-selection experiments.

3.3. *Properties of p5-13 DNA·^{125}I-labelled poly(A)$^+$-mRNA hybrid*

The properties of the hybrid formed in experiments such as those described in fig. 1 are summarised in table 2. The T_m of the p5-13 DNA·^{125}I-

Table 1

Thermal stability of salmon sperm DNA in solution of 4 M guanidinium chloride buffer in formamide

Solvent	T_m(°C)[a]
4 M Guanidinium chloride buffer	75[b]
4 M Guanidinium chloride buffer/ 20% (v/v)-formamide	68
4 M Guanidinium chloride buffer/ 33% (v/v)-formamide	60

[a] T_m is defined as the temperature at which 50% of the increase in absorbance (at 280 nm), owing to the thermal denaturation of DNA, was found to have taken place. Measurements were not made at 260 nm because of the high absorbance of formamide at this wavelength.

[b] For rat liver DNA in 4 M guanidinium chloride buffer, T_m 75°C [22]

labelled poly(A)$^+$-mRNA hybrid was 75°C in 0.1 × SSC and was 65°C in 0.01 × SSC (table 2). The shift of 10°C for a 10-fold decrease in the buffer concentration is in accord with a 12°C shift noted for rat liver DNA [22] and also with a shift of 13°C noted for double-stranded RNA isolated from virus-like particles of *Penicillium chrysogenum* [23]. The high resistance of the RNA·DNA hybrid to RNase treatment (table 2) reveals the absence of long tails of non-hybridised mRNA. We infer that most, if not all, of a particular mRNA species hybridises with the *Hin*dIII–*Bam*HI fragment inserted into plasmid p5-13.

Previously, similar methods were used to measure the T_m in 0.5 × SSC buffer of hybrids formed between ^{125}I-labelled rRNA and its gene. The values obtained were 78°C for the *Neurospora crassa* DNA·^{125}I-labelled *N. crassa* rRNA hybrid, and 77°C for the *Xenopus laevis* DNA·^{125}I-labelled *X. laevis* rRNA hybrid [17]. The slightly lower value of 75°C in 0.1 × SSC found for the T_m of p5-13 DNA. ^{125}I-labelled poly(A)$^+$-mRNA hybrid is accounted for by the 5-fold decrease in buffer concentration and it is equivalent to T_m 82°C in 0.5 × SSC.

3.4. *Isolation of p5-13 DNA-selected-mRNA directly from crude cell lysates*

Microplasmodia of *Physarum* (10 g) were lysed at −20°C in Lysis buffer (30 ml). The lysate was clarified by low-speed centrifugation, then for-

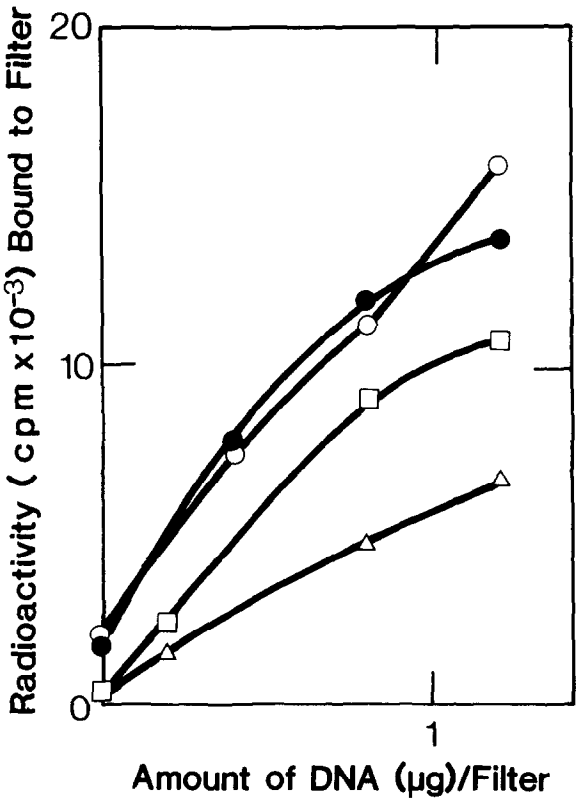

Fig. 1. Hybridisation of p5-13 DNA and ^{125}I-labelled poly(A)$^+$-mRNA fraction of *Physarum* in lysis-formamide buffer. p5-13 DNA was made linear by treatment with restriction endonucleases and bound to aminothiophenol-paper as in section 2.10. The paper discs were placed in the appropriate hybridisation buffer, which also contained 20 µg rabbit reticulocyte rRNA/ml, and incubated under hybridisation conditions for 2 h. The radioactive probe (~2 µg RNA of ~1 × 10^7 cpm/µg RNA) was then added. The incubation was continued for 16 h. The paper discs were then washed extensively as in section 2 and the radioactivity released into 0.1 × SSC between 65–95°C was then measured. The radioactivity released by this treatment accounted for ⩾80% of the total radioactivity complexed with the immobilised DNA. To establish a frame of reference, the hybridisation reaction was also carried out in a conventional buffer, viz. 3 × SSC/10 × Denhardt's solution/0.5% (w/v) SDS/10% (w/v)-dextran. (△) Lysis/formamide buffer (20 ml) at 37°C; (○) lysis/formamide buffer (10 ml) at 37°C; (●) lysis/formamide buffer (20 ml) at 42°C; (□) 3 × SSC/10 × Denhardt's solution/0.5% (w/v) SDS/10% (w/v)-dextran (20 ml) at 65°C.

Table 2

Thermal stability of p5-13 DNA·^{125}I-labelled-mRNA hybrids and its resistance to RNase

Solvent	T_m(°C)[a]	Resistance RNase[b]
0.1 × SSC	75	-
0.01 × SSC	65	-
90% (v/v)-formamide (eluting buffer)	>25 <42	-
2 × SSC	-	>90%

[a] The thermal stability of the DNA·RNA hybrid was measured by following the release of ^{125}I-label into solution from the immobilised complex, on heating for 5 min at a specified temperature. The buffer was replaced by a fresh sample and the temperature was raised by 5°C, and so on.

[b] Resistance to RNase is defined as the % of radioactivity remaining in the DNA·RNA hybrid after the filter was treated with 1.3 μg pancreatic RNase/ml of 2 × SSC at 20°C for 40 min as in [17]. The radioactivity released into solution (0.1 × SSC) on heating from 65–90°C was used to measure the amount of hybrid bound to control and to RNase treated filters. The % resistance to RNase is defined as 'radioactivity eluted between 65–95°C in 0.1 × SSC after RNase treatment/radioactivity eluted between 65–95°C in 0.1 × SSC without RNase treatment of hybrid' × 100

mamide (20 ml) was added. Six discs of ATP-paper, 1 cm diam., each carrying 10 μg immobilised p5-13 DNA (section 2.9) were added to the lysis/formamide buffer solution, which was then gently shaken at 42°C for 40 h. The paper discs were removed and washed at 42°C with lysis/formamide buffer (200 ml), then at 20°C with Eluting buffer (2 ml). The bound mRNA was recovered by washing the discs with Eluting buffer at 42°C. Yeast tRNA (25 μg) was added as carrier and RNA was precipitated at −20°C by the addition of K-acetate buffer (final conc. 1%) and 2.5 vol. ethanol. The RNA was redissolved in storage buffer and precipitated twice more, then redissolved in sterile, distilled water (10 μl).

3.5. *Translation of p5-13 DNA-selected mRNA in the rabbit reticulocyte lysate system*

The purified mRNA was found to direct the synthesis of polypeptides in the nuclease-treated rabbit reticulocyte lysate system of [13] (table 3). The products of cell-free translation were analysed by electrophoresis in SDS-15% polyacrylamide gels (fig. 2). A protein of M_r 25 000 ± 750 was detected only in samples to which p5-13 DNA-selected mRNA was added (fig. 2). The minimum size of an mRNA species coding for a protein of this size is ~750 nucleotides excluding non-coding sequences. An mRNA species of this size is substantially smaller than the ~5000 basepair *Hin*dIII-*Bam*HI fragment which is the *Physarum* component of p5-13 DNA.

Table 3

Activity of p5-13 DNA-selected mRNA in cell-free protein synthesis

Additions to nuclease-treated rabbit reticulocyte lysate	Radioactivity of ^{35}S-labelled acid-insoluble protein (cpm)
None	990
Poly(A)$^+$-mRNA isolated from mouse 3T6 cells (1 μg)	9035
p5-13 DNA-selected mRNA (1 μl)	2060
p5-13 DNA-selected mRNA (3 μl)	1690
p5-13 DNA-selected mRNA (5 μl)	1150

The nuclease-treated rabbit reticulocyte lysate system of [13] was used as in section 2.6

4. DISCUSSION

The principle of performing hybridisation reactions in crude lysates, by using a buffer for lysis that inhibits RNase action, was established when the poly(A)$^+$-mRNA fraction of *P. polycephalum* was isolated by interaction with immobilised poly(U) [8]. The above experiments show that the same principle applies to the isolation of a particular mRNA species by hybridisation to immobilised DNA. The reaction conditions were modified by the introduction of 33% (v/v) formamide and by raising the temperature of hybridisation from 4°C, which is suitable for the interaction between poly(A)-tails and immobilised poly(U), to 42°C for the interaction between mRNA and immobilised DNA.

The advantages of carrying out hybrid selection experiments directly in crude lysates include not

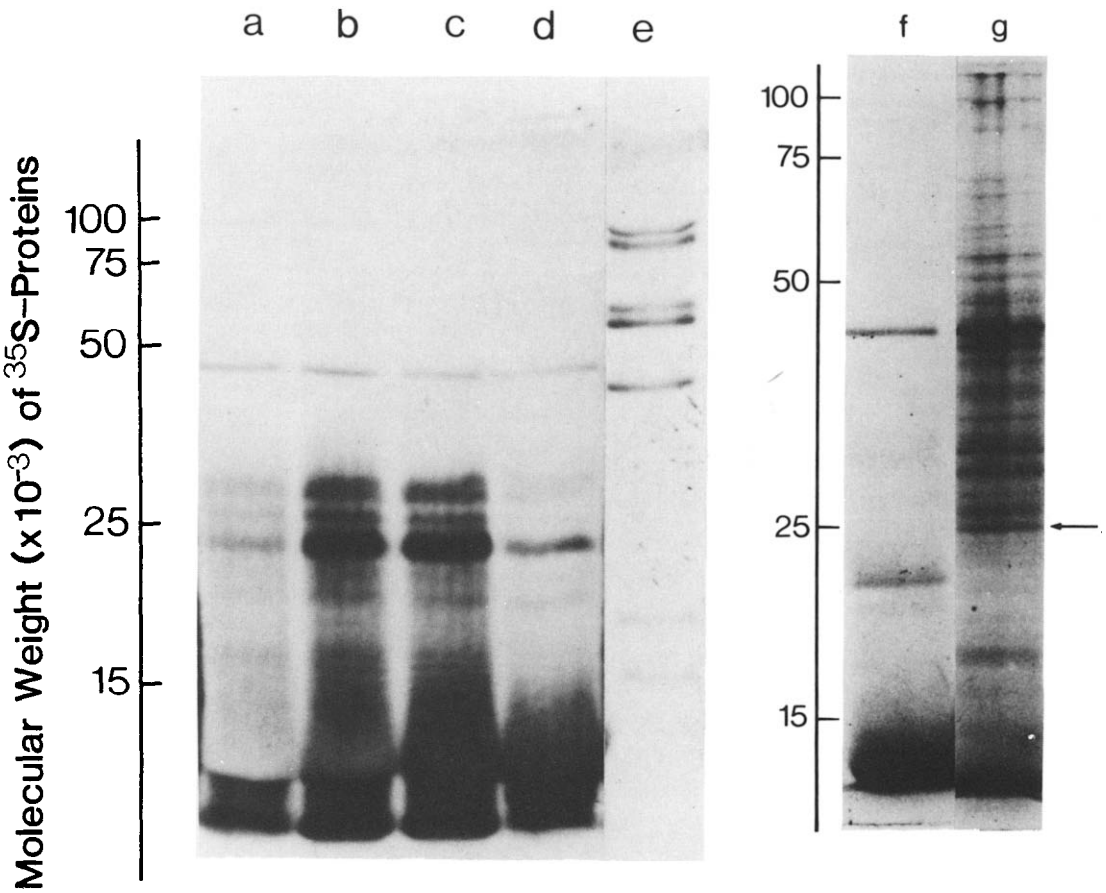

Fig. 2. Radioautograph of polyacrylamide gel after the separation of [^{35}S]methionine-labelled protein products of p5-13 DNA-selected RNA in cell-free protein synthesis. p5-13 DNA-selected RNA was translated in the nuclease-treated rabbit reticulocyte system using [^{35}S]methionine as a radioactive tracer (table 3). After incubation, samples of the reaction mixture were heated with SDS, then separated on 10% (w/v) polyacrylamide gels as in [14]: (a) p5-13 DNA-selected RNA (5 μl); (b) p5-13 DNA-selected RNA (3 μl); (c) p5-13 DNA-selected mRNA (1 μl); (d) control, without added RNA; (e) M_r markers, lysozyme (14 000), trypsin inhibitor (19 000), creatine kinase (40 000), glutamate dehydrogenase (53 000), catalase (60 000), fructose-6-phosphate kinase (81 000) and phosphorylase *a* (94 000). (f) control, without added RNA; (g) *P. polycephalum* poly(A)$^+$ mRNA fraction (1 μg). The M_r scale for tracks (f) and (g) is given on the leftside of (f). The arrow (track (g)) denotes the product which corresponds in size with the principal product of p5-13 selected RNA (see tracks (a)–(c)).

only the simplicity of the procedures, but also the reduction in the risk of degrading the desired mRNA species. Moreover, many different mRNA species may be isolated in the same experiment and the DNA immobilised on aminothiophenol-paper may be used repeatedly. The method is also suitable for use on both a small and a large scale because the requirements are so simple.

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