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Duck-Haemoglobin Synthesis in Frog Cells

The Translation and Assay of Reticulocyte 9-S RNA in Oocytes of *Xenopus laevis*

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9-S RNA was prepared from duck reticulocytes by dodecylsulfate treatment of the ribonucleoprotein particle derived from EDTA-treated reticulocyte polyribosomes. The messenger content of this RNA was assayed by microinjection into oocytes of the frog *Xenopus laevis*. The 9-S RNA was found to direct the synthesis of a molecular species that eluted from Sephadex G-100 with added duck reticulocyte haemoglobin. When analysed on a cation-exchange resin this material was found to contain the three globin-chain species characteristic of duck reticulocyte haemoglobin. Moreover, paper electrophoresis at pH 6.5 and pH 3.5, and paper chromatography showed that these globin-like species made in the oocyte were virtually indistinguishable, in terms of their histidine-containing tryptic peptides, from the corresponding globin chains derived from duck reticulocyte haemoglobin.

A comparison between the products made following the injection into oocytes of reticulocyte 9-S RNA from rabbits, mice or ducks proved that it is the exogenous messenger that gives rise to the information content of the proteins whose synthesis is elicited by the injected RNA.

The results also showed that the oocyte contains translational systems that are neither cell-type nor species specific, and that duck globin messenger RNAs require no reticulocyte-specific factors for their translation in oocytes.

The construction of a saturation curve showed that there is a linear relationship between the amount of messenger injected and the amount of haemoglobin synthesized, up to the point at which haemoglobin synthesis constitutes over 35% of the total protein being made. Consequently the frog oocyte provides a convenient assay system for small quantities of duck globin mRNAs, and the messenger activity of as little as 1 ng of 9-S RNA can be detected very simply. Thus a combination of the duck reticulocyte and frog oocyte may provide a useful system for studying post-transcriptional control, and the formation of mRNA, in a eukaryotic cell.

The duck reticulocyte system has much to recommend it as a model system for studying the formation of mRNA in eukaryotic cells [1]. However, the products of translation of purified 9-S mRNA from duck reticulocytes have not been well characterized, although it is clear, by analogy with reticulocyte systems from other species [2,3] and from our own results [27] and those of Pemberton *et al.* [4] that the 9-S RNA fraction from duck reticulocytes does contain some globin messenger activity.

The oocyte of the frog *Xenopus laevis* provides a sensitive system for assaying the messenger content of RNA fractions extracted from eukaryotic cells: for messenger RNAs, when injected into oocytes, are translated faithfully and efficiently [3,5—8] (and unpublished results of Stavnezar, Mitch-Smith, Gurdon, Huang and Lane).

The present paper outlines various methods for preparing biologically active 9-S duck globin messenger RNA, and also describes the translation of this RNA in frog oocytes. The 9-S RNA fraction is shown to elicit the synthesis of three molecular species that closely resemble the three major duck reticulocyte globin chains.

The oocyte is also shown to be a convenient system for assaying nanogram quantities of duck globin mRNA. Furthermore, it is argued that this whole cell assay system may be useful for studying the properties of molecular species involved in the

process of messenger formation, as well as for the study of the messenger itself.

A comparison between the globin chain species synthesized in response to injected duck, mouse or rabbit 9-S reticulocyte RNA proves conclusively that genuine translation of the exogenous messenger occurs in the oocyte. Moreover, the results described also confirm that translation of globin messengers requires no reticulocyte-specific factors, and that the oocyte contains translational systems that are neither cell-type nor species specific.

MATERIAL AND METHODS

Handling of Oocytes

The injection procedure and culture medium were as described by Gurdon *et al.* [5] and by Lane *et al.* [3].

Preparation of Duck-Reticulocyte RNA Fractions

Duck reticulocytes were produced by the method of Scherrer *et al.* [10]. Polyribosomes were prepared as described by Morel *et al.* [11]. 9-S RNA was prepared by three slightly different methods. The first of these involved dodecylsulfate-deoxycholate treatment of the mRNA protein particles released from EDTA-treated polysomes, and differed from the method described by Morel *et al.* [11] only in that the 9-S RNA fraction obtained from the final sucrose gradient was precipitated four times with ethanol, dried, dissolved in water at a concentration of 1 mg/ml and stored at −70 °C.

The second method of preparation involved dodecylsulfate treatment of whole polyribosomes and followed the procedure of Lingrel [12].

The third method used as starting material the 9-S RNA product obtained by the second method. The 9-S RNA was then purified by hot-phenol extraction employing a method similar to that described by Scherrer [13]. Thus to 400 μg reticulocyte 9-S RNA, dissolved in 1 ml water, was added 1 ml of 25% sodium dodecylsulfate solution, 19 ml 10 mM sodium acetate pH 5.1 and 20 ml 90% phenol. The solutions used were pre-warmed to 60 °C, and the above mixture was agitated for 3 min at 60 °C before being brought to 0 °C by immersion in a cooling bath. The phenolic phase was discarded and two more 60 °C extractions were performed using first 15 ml and then 10 ml 90% phenol. A fourth extraction using 10 ml phenol solution was performed at room temperature. The RNA was then precipitated three times with ethanol (16 h at −20 °C) before being dissolved in water. More than 80% of the starting material was recovered after this final stage. The hot-phenol method described by Scherrer [13] was also used to prepare total polyribosomal RNA.

Injection Mixture

The injection medium (88 mM NaCl, 1.0 mM KCl, 15 mM Tris, 0.05% diethyl pyrocarbonate, pH 7.6) was boiled, and cooled, before use. RNA preparations were dissolved in this medium and were then stored at −70 °C in sealed RNAase-free capillary tubes.

Homogenization of Oocytes

Thawed samples of oocytes were homogenized in a solution containing 52.2 mM Tris, 52.5 mM glycine, 5 mM histidine monohydrochloride and 1.1 M 2-mercaptoethanol, to which duck haemoglobin (prepared by lysing reticulocytes with an equal volume of distilled water) had been added to give a final concentration of 10 mg/ml; the final pH was 8.9. Homogenates were centrifuged at 75000 × *g* for 30 min at 4 °C, and the resulting supernatant applied directly to a Sephadex G-100 column (50 × 1.2 cm^2) equilibrated with buffer (pH 8.9) containing 52.2 mM Tris, 52.5 mM glycine and 7 mM 2-mercaptoethanol.

Carboxymethyl-Cellulose Chromatography

Globin samples were prepared by the method of Schapira *et al.* [14] except that the acid-acetone used contained 0.1% (v/v) 2-mercaptoethanol and 1.2% (v/v) 12 N HCl. A few drops of 2-mercaptoethanol were added to samples prior to the acid-acetone extraction step.

Globin was analysed by the method of Lingrel [12] modified in that columns (18 × 1.5 cm^2) of Whatman CM-52 were used, and that these were eluted with a 2 × 335 ml sodium phosphate gradient (8 mM Na_2HPO_4 pH 6.7 — 80 mM Na_2HPO_4 pH 6.7) made up in 8 M urea.

Paper Chromatography and Paper Electrophoresis of Tryptic Peptides

After carboxymethyl-cellulose chromatography the fractions corresponding to the separated duck globin chains were pooled and the three samples so obtained were dialysed exhaustively against deionized water. The globin chain solutions were then lyophillized. After adding 5 ml 0.1 M NH_4HCO_3, triphenylchloroketone-treated trypsin was added in an amount equal to approximately $^1/_{50}$th of the amount of substrate present. The solutions were shaken at 37 °C for 6 h, and were then lyophillized. The peptide mixtures were dissolved in deionized water and were lyophillized: this cycle was repeated three more times before the mixtures were finally dissolved in electrophoresis buffer at pH 6.5. After centrifugation each supernatant was spotted onto Whatman No. 1 paper as a 4 to 8-cm track

(0.5 mg/cm²): papers were either electrophoresed at pH 6.5 (pyridine—acetic acid—water, 25:1:225, v/v/v) or pH 3.5 (pyridine—acetic acid—water, 1:10:89, v/v/v), or they were submitted to descending chromatography (butanol—acetic acid—water—pyridine, 30:6:24:20, v/v/v/v). Marker dyes mixture (containing ε-dinitrophenyl-lysine, methyl green, acid fuchsin, xylene cyanol and orange G) was run on either side of the central track. After separation of the peptides the paper track was cut into three strips. The central strip was cut into 0.5-cm sections and these were eluted by shaking overnight in scintillation vials containing 1 ml 0.1 N ammonia solution. The vials were counted, permitting localization of the oocyte-derived histidine-containing tryptic peptides. One lateral strip was stained with the Pauly reagent [15], which identifies a peptide as containing either histidine or tyrosine or both. It is not always possible to distinguish between the different colours given by tyrosine and histidine-containing peptides, and no attempt was made to do so. The other lateral strip (in the case of electrophoresis at pH 6.5) was stained with α-nitroso β-naphthol [15], which stains tyrosine-containing peptides red. In other words, the combination of stains used enabled one to determine which reticulocyte-derived peptides contained histidine but not tyrosine. This combination of stains does not tell one which peptides contain both these amino acids but as will become apparent there appears to be at the most only two tyrosine-containing duck-globin tryptic peptides that do not also contain histidine.

Scintillation Counting

Samples were counted using an ethoxyethanol-based scintillation fluid [3]; for counting aliquots from the carboxymethyl-cellulose column effluent or for counting paper strips this scintillation fluid was diluted by 20% with Triton X-100.

RESULTS AND DISCUSSION

The Translation of Duck-Reticulocyte 9-S RNA

9-S reticulocyte RNA was prepared by dodecyl-sulfate treatment of the 20-S mRNA protein that is released by EDTA treatment of duck reticulocyte polysomes [11, 16]. The RNA so obtained was dissolved in injection medium and 20 frog oocytes were injected, individually, with 50 nl of this solution. The oocytes were then incubated in [³H]histidine-containing culture medium for 8 h. Fig. 1(A) shows the Sephadex G-100 profile of histidine-labelled supernatant proteins obtained from a batch of oocytes that had been injected with 9-S RNA. Fig. 1(B) shows the elution profile obtained

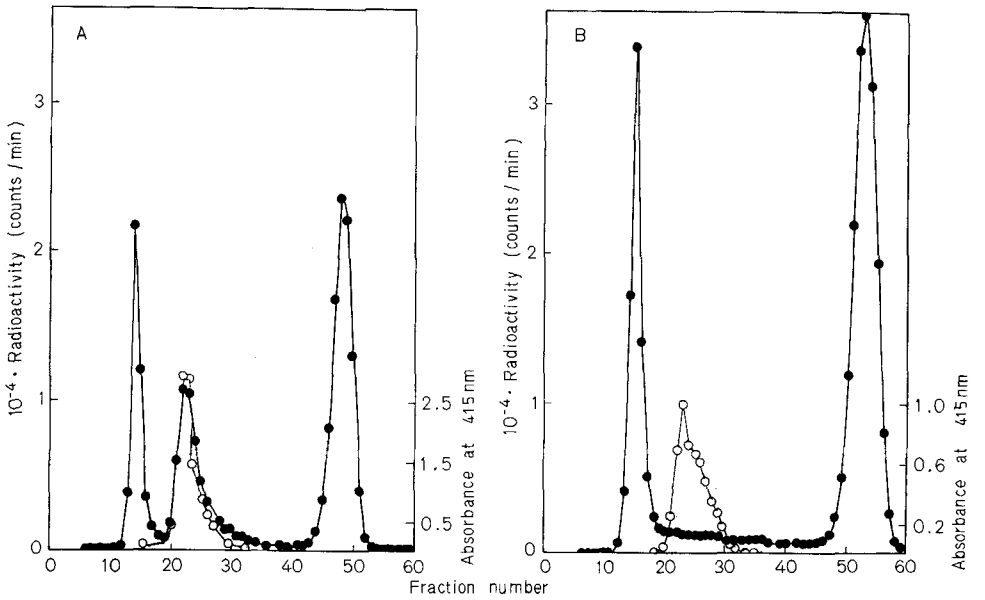

Fig. 1. *Sephadex G-100 profile of [³H]histidine labelled supernatant proteins from oocytes injected (A) with 9-S RNA and (B) with no RNA.* Oocytes were injected, and then homogenized with marker duck haemoglobin, as described in Materials and Methods; the resulting supernatant was applied to a Sephadex G-100 column (1.2×50 cm) equilibriated in 52.2 mM Tris—52.2 mM glycine pH 8.9. (A) Material derived from 20 oocytes which had been injected with 9-S duck reticulocyte RNA dissolved in injection medium (at 5 mg/ml yielding an intracellular concentration of approximately 250 μg/ml). The oocytes had been cultured for 8 h in medium containing [³H]histidine at 0.1 mCi/ml (52 Ci/mmol). (B) Material from 20 oocytes treated similarly except the injection mixture used contained no RNA. (○) Absorbance 415 nm; (●) ³H radioactivity per oocyte

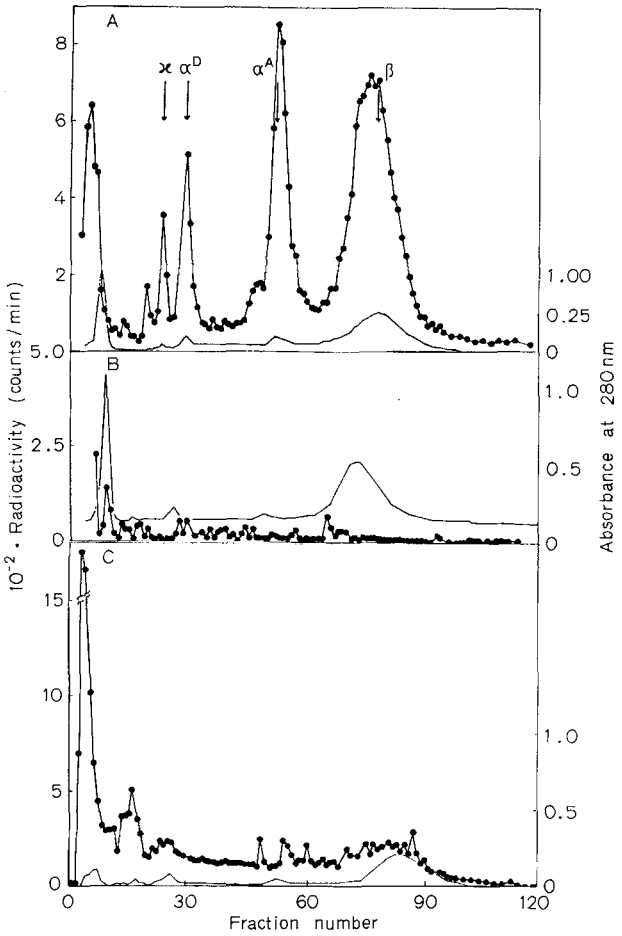

Fig. 2. *CM-cellulose chromatography of acid-acetone insoluble material from haemoglobin region of Sephadex elution profile.* The haemoglobin region from the Sephadex elution profile of supernatant proteins derived from injected oocytes was (after addition of further marker haemoglobin) subjected to acid-acetone treatment [14]. The resulting globin was chromatographed on a Whatman CM-52 carboxymethyl-cellulose column eluted with a 2×335 ml 8–80 mM Na_2HPO gradient, pH 6.7 [12]. The column plus sample was washed with the starting buffer, until 10 fractions had been collected, before the gradient was started. The nomenclature used for the three duck globin chains is analogous to that used for chicken haemoglobins, which are electrophoretically similar [17]. (A) Globin-like material from a batch of 20 oocytes. The cells had been injected with duck reticulocyte 9-S RNA (dissolved in injection medium at a concentration of 1.25 mg/ml), and had been incubated for 8 h in medium containing [^{3}H]histidine (at 1 mCi/ml, 52 Ci/mmol). (B) Material from a batch of 20 oocytes which had been treated with injection medium alone; the cells had been incubated in [^{3}H]histidine-containing medium (1 mCi/ml, 52 Ci/mmol) for 8 h. (C) CM-cellulose chromatography of the front peak material from the Sephadex elution profile shown in Fig. 1(A). (●) Radioactivity; (——) absorbance at 280 nm

from a batch of oocytes treated identically except that 9-S RNA was omitted from the injection medium. The results show that the presence of 9-S RNA in the injection mixture is associated with the formation of a labelled species that co-elutes with added marker duck haemoglobin.

The nature of the molecular species synthesized under the direction of duck reticulocyte 9-S RNA was investigated further by treating the separately pooled haemoglobin regions of the two Sephadex elution profiles with acid-acetone (see Material and Methods). The material insoluble in the acid-acetone was collected and then chromatographed on Whatman CM-52 columns eluted with a sodium phosphate gradient, as described by Lingrel [12]. Fig. 2(A) shows the results obtained with the haemoglobin-region material derived from 9-S RNA-injected oocytes. Fig. 2(B) shows the profile obtained with material from oocytes treated with injection medium alone. The results show that the presence of 9-S RNA in the injection mixture is associated with the formation of three chromatographically distinct labelled species each of which co-elutes with one of the three globin chains derived from added duck marker globin.

The 9-S RNA may also elicit the synthesis of a minor species (marked × in Fig. 2A) that is not clearly represented in the added marker duck reticulocyte globin and it not necessarily another species of globin polypeptide. It is interesting to note that a minor species, eluting in the same position from a CM-cellulose column, was made under the direction of duck 9-S RNA in a mouse cell-free system [26]. Radioactive globin produced by incubating duck reticulocytes *in vitro* contains only three labelled globin chains [27] (and A. Stewart, personal communication). It must also be noted that in Fig. 2(A) the β-chains from oocytes elute a few tubes earlier than do the marker β-chains: this is not invariably the case, and the elution profiles of some freshly prepared samples show perfect coincidence in the β-chain region.

The definitive identification of the molecular species made under the direction of duck reticulocyte 9-S RNA was achieved by comparing the histidine-containing tryptic peptides present in oocyte-derived and reticulocyte-derived globin chains by paper electrophoresis (at pH 6.5 and pH 3.5) and by paper chromatography.

Tryptic peptides were prepared from the mixed oocyte-derived and reticulocyte-derived globin chains (see Material and Methods) and the peptide mixture obtained was then applied as a wide band to Whatman No. 1 paper. After electrophoresis at pH 6.5 or pH 3.5, or after descending chromatography, the paper was cut into three strips before being stained and counted as described in the Methods section. From the results shown in Fig. 4 it can be seen that, as judged by electrophoresis at pH 3.5 or pH 6.5 and by descending chromatography in butanol—acetic acid—pyridine—water, the

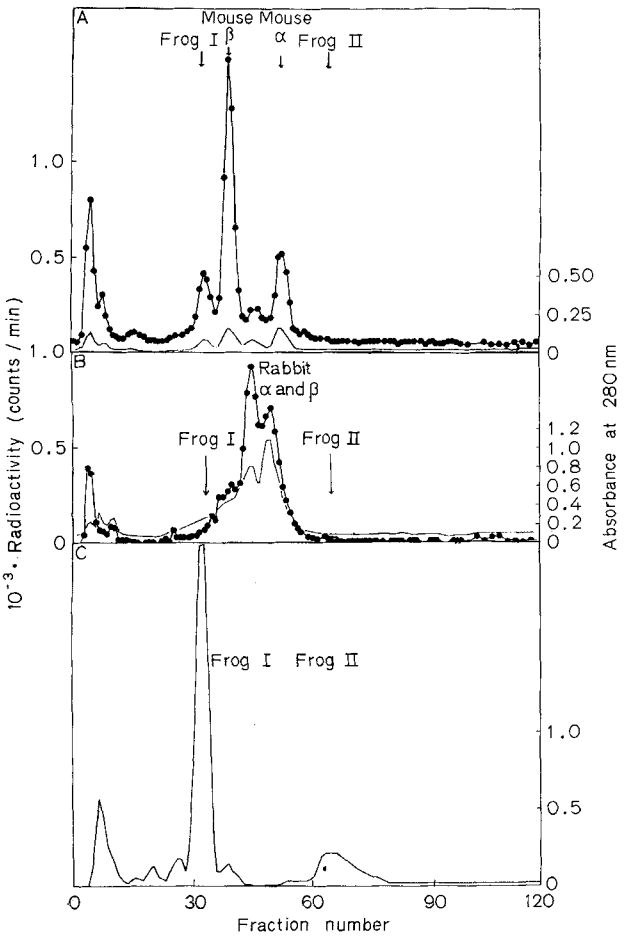

Fig. 3. *CM-cellulose chromatography of globins from RNA-injected oocytes and from frog red-blood cells.* As described in Fig. 2, globin was prepared from RNA-injected oocytes. The globin was then chromatographed as described in Material and Methods. (A) Globin-like material from a batch of 20 oocytes which had been injected with rabbit reticulocyte 9-S RNA (dissolved in injection medium at approximately 0.5 mg/ml); the cells had been incubated in [^{3}H]histidine-containing medium (0.5 mCi/ml, 52 Ci/mmol) for 18 h. Commercial as opposed to fresh haemoglobin was used to provide the carrier globin in this instance. (B) Material from a batch of 20 oocytes which had been injected with mouse reticulocyte 9-S RNA (approximately 1 mg/ml). After injection the cells had been incubated in medium containing [^{3}H]histidine (1 mCi/ml, 52 Ci/mmol) for about 12 h. A reticulocyte lysate, which had been stored for several days at 4 °C, was used as the source of carrier globin. (C) CM-cellulose elution profile obtained with globin prepared from a 3% (w/v) saponin lysate of washed frog red-blood cells. (——) Absorbance at 280 nm; (●) radioactivity

histidine-containing tryptic peptides of oocyte-derived and reticulocyte-derived β-chains are identical. The same is true of oocyte and reticulocyte α^A-chains. The α^D-chains from these two sources have not been compared very rigorously but, as can be seen from Fig. 4, there is complete coincidence in the staining and radioactivity patterns, except in one region. This discrepancy may well reflect the presence of a peptide that contains tyrosine but not histidine. Marbaix and Lane [8] discuss some of the many other alternative explanations. Moreover the $\alpha^{D}{}_1$chain material pooled for tryptic digestion was by no means pure: it included appreciable quantities of ^{3}H-labelled frog proteins as well as the mRNA-associated species designated "x" in Fig. 2(A). The analytical methods used are nonetheless quite capable of revealing differences between peptide mixtures: for, as can be seen from Fig. 4 the α^D, α^A and β globin chains are shown to be polypeptides having quite different tryptic digestion products.

It is probable that it is the exogenous messenger that gives rise to the information content of the new proteins that are made following microinjection of a foreign messenger RNA [3, 8, 18]. However, this point is by no means proven. Fig. 2(A), 3(A) and 3(B) show a comparison between the globin-like materials made in oocytes injected with 9-S RNA from duck, rabbit or mouse reticulocytes. It can be seen that the RNA from a given species gives rise to globin-like molecules that co-chromatograph with globin chains derived from the haemoglobin of that species. However, the globin chains of the three species mentioned above do not behave similarly on columns of CM-cellulose CM-52, as can be judged from their elution volumes relative to two markers, namely the two major globin chains of adult frog globin. Since the chromatographic system used has been found to yield reproducible results, one can conclude that the messenger RNA from a particular species directs the synthesis of globin chains characteristic of that species. Thus, the injected messenger does seem to give rise to the information content of the proteins whose synthesis it elicits. The fact that the major frog globin chains are chromatographically distinct from the globin chains made under the direction of the messenger [6] does not in itself prove this point: for it is conceivable that the foreign RNA merely elicited the synthesis of some minor frog globin species that happened to co-chromatograph with the added marker. However, the species-specific response noted above rules out even this remote possibility.

Reticulocyte-derived and oocyte-derived mouse globin both give rise to four chromatographically distinct species: this is perhaps the result of incomplete haem removal for, as the arrows on Fig. 3(A) show, if haemoglobin is obtained from freshly lysed mouse reticulocytes it yields globin that contains only two chromatographically distinguishable species. This phenomenon, which has been discussed by Lane [6], does not, of course, bear upon

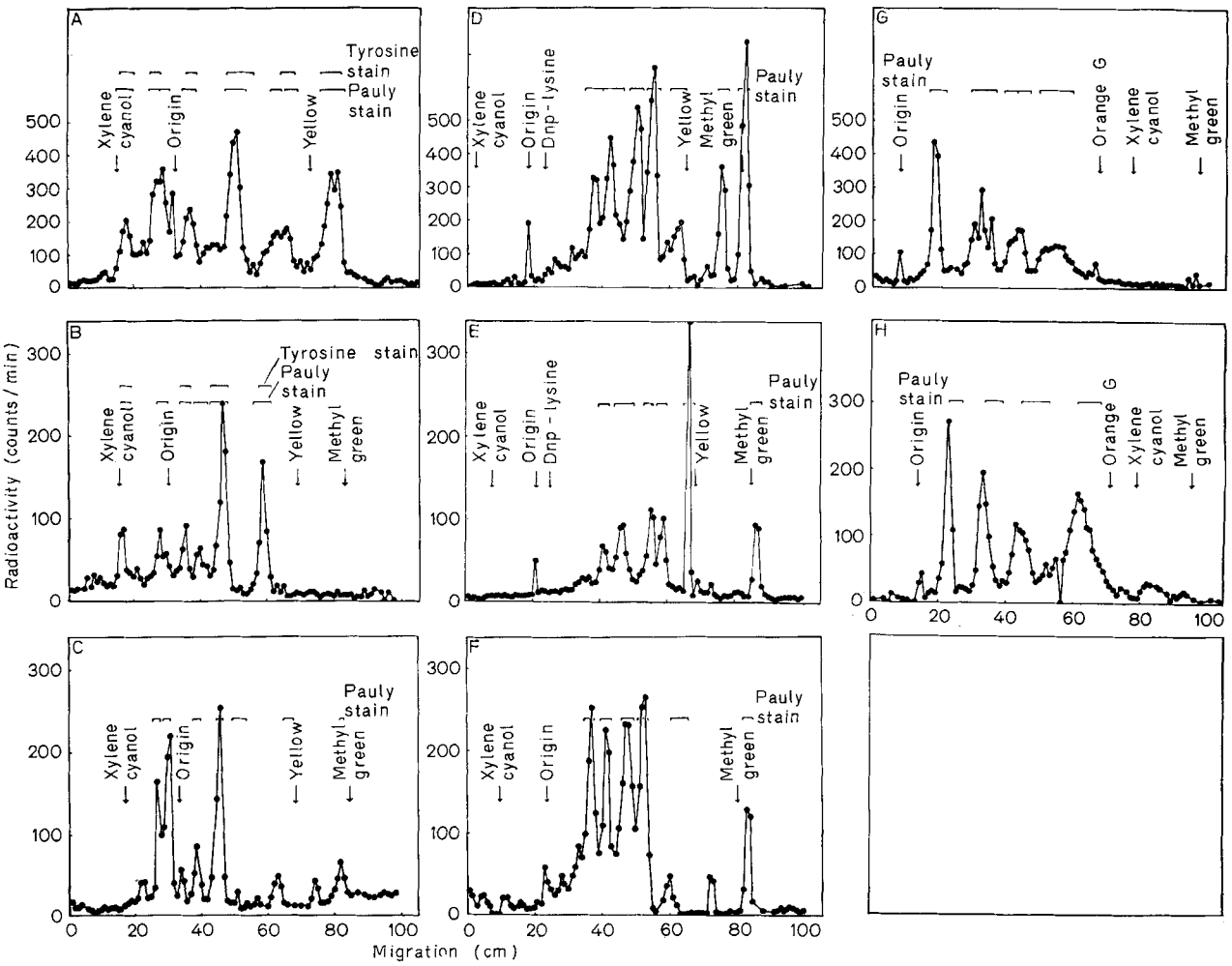

Fig. 4. *Electrophoresis and chromatography of tryptic peptides prepared from the mixed oocyte-derived and reticulocyte-derived* α^D, α^A, *and* β *duck-globin chains.* The duck globin chains had been separated by CM-cellulose chromatography (see Fig. 2A add Methods section). The peptide mixtures from β-chain (A, D, G), α^A-chain (B, E, H) and α^D-chain (C, F) were analysed by paper electrophoresis at pH 6.5 (A, B, C) and pH 3.5 (D, E, F) and by paper chromatography (G, H). Oocyte-derived histidine peptides were identified by their content of [^{3}H]histidine, whilst histidine-containing reticulocyte-derived peptides were partially identified by staining with the Pauly reagent, or in the case of papers electrophoresed at pH 6.5, by staining in addition with α-nitroso-β-naphthol (see Material and Methods). Arrows denote the position of marker dyes: the yellow marker is probably a derivative of methyl green

the main conclusions that can be drawn from this type of experiment, namely that there is genuine translation of the injected mRNA. One can conclude therefore that the 9-S RNA fraction from duck reticulocytes contains at least three species of globin mRNA, and that these messengers can be translated, with a high degree of fidelity, within the oocyte.

The results described also show that some at least of the translational systems within the oocyte are not species specific since the duck, rabbit and mouse messengers are all capable of being translated in these frog cells. One can also argue that not all the translational systems within the frog cell are cell-type specific, for the results show that globin messengers from reticulocytes of three species of animal can be translated in oocytes. However, this argument is not complete for it is possible that the messenger RNA of another species in some way circumvents the cell-type specific nature of the translational machinery of the oocyte. One can also deduce that if there is a translational requirement for messenger-specific factors, then the factors needed for translation of globin mRNAs must already be present in oocytes. All these findings are in exact agreement with those reported by Lane *et al.* [3].

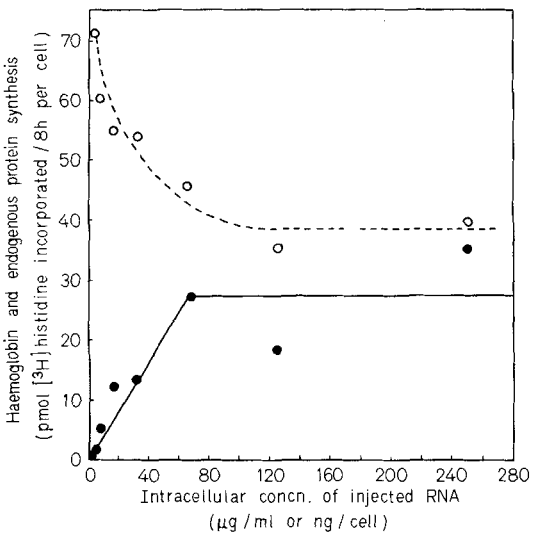

Fig. 5. *Saturation curve for oocytes injected with duck-reticulocyte 9-S RNA.* Each point is based on the Sephadex G-100 analysis of approximately 20 oocytes. The cells were incubated for 8 h in medium containing [^{3}H]histidine (52 Ci/mmol) at 1 mCi/ml. Values for haemoglobin and endogenous protein radioactivity were calculated using the method described by Moar *et al.* [19]. To convert radioactivity, measured as counts/min, to pmoles of histidine incorporated into protein, the free histidine pool of the oocyte was measured (using an amino-acid analyser) as was the amount of free [^{3}H]histidine per oocyte (using a Sephadex G-100 column). The resulting figure for the specific activity of the precursor pool enabled the data to be expressed as pmoles of histidine incorporated per cell per 8 h. The intracellular concentration of RNA has been calculated assuming a 20-fold dilution of RNA due to injection, but ignoring leakage. The oocytes were obtained from a female frog that had ovulated about 2 months previously. (●——●) Duck haemoglobin synthesis; (O----O) endogenous frog-protein synthesis

The Translational Capacity of Oocytes Injected with Duck 9-S RNA

The construction of a curve relating the intracellular concentration of injected duck messenger to the amount of haemoglobin synthesized permits further characterization of the messenger preparation used and also yields additional information about the translational systems within the oocyte.

Duck globin mRNA solutions at concentrations of from 5 to 0.08 mg/ml were injected (separately) into batches of oocytes. A comparison between Fig. 2(A) and 2(C) shows that it is only the haemoglobin region of the Sephadex elution profile that contains duck globin chains; consequently, the globin made under the direction of the added messenger can be quantitated using the method described by Moar *et al.* [19] for estimating the size of the Sephadex haemoglobin peak. Fig. 5 shows the results obtained plotted as a saturation curve. There is an approximately linear relationship between the amount of messenger injected and the amount of haemoglobin synthesized, up to the point at which haemoglobin synthesis constitutes over 35% of the total protein synthesis. This saturation level is reached when each oocyte contains about 50 ng of 9-S RNA. Thus the oocyte system can be used to detect minute amounts of duck globin messenger. Using just one separation method (Sephadex G-100) one can detect the messenger activity in as little as 1 ng of 9-S RNA. A second analytical step would increase the sensitivity of the assay system still further; however the type of CM-cellulose column used for globin chain separations (see Fig. 2 and 3) is not ideal in that one of the newly synthesized oocyte proteins elutes just before the major globin chain.

DISCUSSION

The saturation curve shown in Fig. 5 enables one to calculate the capacity of the oocyte for the translation of exogenous mRNA. Fig. 5 also shows the competitive effect that increasing amounts of haemoglobin messenger RNA have on endogenous protein synthesis. Moar *et al.* [19], using similar amounts of rabbit mRNA, also noted competitive effects: however, at lower concentrations of injected messenger, these authors found no competition and were able to show that oocytes possess a spare translational capacity. In the experiments shown in Fig. 5, duck messenger injection caused total protein synthesis to rise by 20% at the most, whereas Moar *et al.* [19] noted increases of greater than 100% with rabbit mRNA. It is possible to explain all these differences in terms of the toxic effects of the much greater amounts of RNA required to reach saturation when injecting this particular batch of duck messenger. (One should note that unless great care is taken during preparation of both duck and rabbit mRNA it is not even possible to reach the saturation level.) However, different species of globin mRNA may not have the same intrinsic capabilities for supporting protein synthesis (*cf.* [26, 27]).

Amino-acid analysis showed that the oocytes used contained a free histidine pool of approximately 71 pmoles per cell. Moreover, it seems likely that the functional histidine pool of the oocyte is equal to the free histidine pool (Lane, Woodland and Gregory, unpublished results); consequently it is possible to calculate a minimum value for the time taken to translate each globin mRNA (see Table 1). After microinjection, each duck globin mRNA can be read at least once every 30 min: a similar calculation enables one to deduce that the rabbit globin mRNA used by Gurdon *et al.* [5] was read, on average, once every 10 min. This difference may be the result of differences in the purity of the two

Table 1. *Efficiency of Hb mRNA translation in injected oocytes of measured histidine pool size*

Hb mRNA in samples for injection	Hb mRNA Injected per cell		Cells per sample	[^{3}H]-Histidine incorporated into Hb[c]	Pool size of histidine	Specific activity of intracellular histidine pool[d]	Globin chain synthesis[e]	Globin molecules synthetized per molecule of 9-S RNA
(1)	(2)[a]	(3)[b]	(4)	(5)	(6)	(7)	(8)	(8)/(3)
µg/ml	ng	pmol		counts × min^{-1} × cell^{-1} × h^{-1}	pmol/cell	counts × min^{-1} × pmol^{-1}	pmol × cell^{-1} × h^{-1}	h^{-1}
5000	250.0	1.25	15	4948	71	1125	0.463	0.370
2500	125.0	0.625	20	4987	71	2160	0.243	0.400
1250	62.5	0.318	19	5058	71	1485	0.359	1.13
675	31.8	0.159	26	2593	71	1592	0.172	1.08
317.5	15.9	0.009	24	2806	71	1845	0.160	2.02
158.8	7.9	0.040	30	1009	71	1597	0.0665	1.68

[a] These values disregard leakage from injected oocytes, through this is believed to occur (see text).
[b] Assumes that the molecular weight of 9-S mRNA is 200000. Various published and unpublished measurements by different methods have estimated it to be between 175000 and 225000.
[c] All samples were labelled by incubation for 8 h at 20 °C in ^{3}H-histidine of specific activity 50 Ci/mmol.
[d] Calculated from the histidine pool size and the acid soluble c.p.m./cell recovered from columns of 'Sephadex'.
[e] Assumes 9.5 histidine residues per average globin chain.

messenger preparations: however, one must also consider the possibility that duck and rabbit globin mRNAs are qualitatively different and that they are translated at intrinsically different rates in oocytes and in reticulocyte lysates [26, 27].

Avian red blood cells are nucleated and duck immature erythrocytes will synthesize RNA *in vitro* [10, 22, 23]. The characterization by translation of the mRNA from such cells (which is described in the present paper) means that it is now possible to obtained a radioactively labelled RNA preparation that contains mRNAs coding for particular polypeptides, and which very probably contains labelled mRNA molecules amongst the radioactive species present. This extends the results obtained by Pemberton *et al.* [4]. The duck reticulocyte has much to recommend it as a model system for studying post-transcriptional control and the formation of mRNA in eukaryotic cells [1]: it is hoped that the oocyte system will facilitate such studies. The oocyte has advantages over and above its purely passive use as a sensitive microassay. Results obtained with a normal living cell may well be of greater physiological significance than are those obtained with cell-free systems. In particular, the use of a whole cell has many advantages when studying molecular species believed to be involved in intracellular regulatory mechanisms. For example the presence of translatable globin sequences in putative messenger precursors, such as the high-molecular-weight pre-messenger RNAs [24, 25, and Imaizumi, T., Diggelmann, H. & Scherrer, K. unpublished results], could be detected directly with an assay system of sufficient sensitivity.

It is relevant, therefore, to note that hot-phenol-extracted whole polysomal RNA (see Material and Methods) elicits haemoglobin synthesis when injected into oocytes. This demonstrates that the biological activity of the mRNA is not obscured by an excess of other classes of RNA and it also shows that the hot-phenol extraction procedure which is widely used for preparing nuclear RNA [24] does not destroy mRNA activity. This has been confirmed by extracting 9-S mRNA prepared from dodecylsulfate-treated whole polysomes (see Materials and Methods), which is in itself biologically active, with hot phenol. The resulting phenol-extracted 9-S RNA causes haemoglobin synthesis when injected into oocytes. The haemoglobin-forming activity per µg of this RNA is similar to that of mRNA prepared from 20-S mRNA · protein particles.

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