

# Translation of Ovalbumin mRNA in *Xenopus laevis* Oocytes

## CHARACTERIZATION OF THE SYSTEM AND EFFECTS OF ESTROGEN ON INJECTED mRNA POPULATIONS

LAWRENCE CHAN, PETER O. KOHLER, and BERT W. O'MALLEY

*From the Departments of Cell Biology and Medicine, Baylor College of Medicine, Houston, Texas 77030*

**ABSTRACT** Ovalbumin messenger RNA (mRNA<sub>ov</sub>) purified from hen oviduct was injected into *Xenopus laevis* oocytes. The oocytes were incubated in culture medium containing [<sup>3</sup>H]leucine. Analysis of the oocyte cytosol on Sephadex G-150 columns demonstrated a peak of radioactivity which cochromatographed with authentic ovalbumin. Radioactive protein contained in this peak was precipitated by ovalbumin antiserum, co-electrophoresed with ovalbumin on sodium dodecyl sulfate (SDS) and urea gels at pH 8.7, and eluted with the protein at the same pH (4.8) on CM-cellulose chromatography. Injection of increasing amounts of mRNA<sub>ov</sub> was found to elicit a linear response in terms of ovalbumin synthesis. Moreover, there was linear incorporation of radioactivity into microinjected oocytes over a minimum period of 91 h. Less than 1 ng mRNA<sub>ov</sub> was detected in this system. Ovalbumin mRNA activity was present in RNA preparations from chicks treated with estrogen but was undetectable in animals withdrawn from the hormone. This study constitutes an initial demonstration of a steroid hormone-induced alteration in mRNA population as assayed in intact viable heterologous cells.

### INTRODUCTION

In the chick oviduct, estrogen induces the synthesis of the specific egg white proteins, ovalbumin, conalbumin, lysozyme, and ovomucoid (1, 2). The induction of these proteins is associated with an accumulation of their respective mRNAs (3-5). In the case of ovalbumin, detailed kinetic studies led to the hypothesis that the estrogenic induction of ovalbumin mRNA (mRNA<sub>ov</sub>)<sup>1</sup> was

Dr. Chan is an Established Investigator of the American Heart Association.

*Received for publication 6 August 1975 and in revised form 24 October 1975.*

<sup>1</sup>Abbreviations used in this paper: DES, diethylstilbestrol; mRNA<sub>ov</sub>, ovalbumin mRNA.

a rate-limiting step in steroid hormone-mediated induction of protein synthesis (6, 7). Previous studies performed on the translation of hormone-induced mRNAs also include the glucocorticoid induction of tryptophan oxygenase in the rat liver (8) and the androgenic induction of aldolase in the rat ventral prostate (9) and alpha<sub>2u</sub> globulin in the rat liver (10). In each of these instances the hormone-regulated mRNAs were translated in heterologous cell-free extracts in vitro. While it is unlikely that results obtained from these in vitro translation systems represent biochemical artifacts, it would be desirable also to study such hormone-mediated responses in heterologous living-cell systems. In these systems, specific tissue responses to steroid hormones can be monitored in whole cells under controlled but perhaps more natural conditions. One such living cell system is the *Xenopus laevis* oocyte, first developed by Gurdon et al. for the translation of hemoglobin mRNA (11, 12).

In this communication, we have used and characterized the *Xenopus laevis* oocyte as an extremely sensitive translation system for mRNA<sub>ov</sub>. Using this living-cell system, we have also examined the biological activity of mRNA populations isolated from chick oviducts under differing hormonal states.

### METHODS

*Preparation of recipient oocytes.* Adult female *Xenopus laevis* toads were induced to ovulate by two injections of human chorionic gonadotropin (400 U, Sigma Chemical Co., St. Louis, Mo.). Mature eggs were discarded; the toads were sacrificed 2 wk after induction of ovulation, and stage V oocytes (13) were isolated in Barth's medium (14). These oocytes were used for microinjection experiments.

*Preparation of mRNA<sub>ov</sub>.* mRNA<sub>ov</sub> preparations used for the microinjection were obtained by the sequential application of the following procedures as reported by Rosen et al. (15): (a) total nucleic acid extract; (b) nitrocellulose adsorption; (c) Sepharose 4B chromatography; (d) repeat nitrocellulose adsorption; and (e) preparative agarose gel

electrophoresis. The mRNA<sub>ov</sub> preparation purified by these procedures is better than 95% pure (15).

For induction experiments, groups of 10-day-old white Leghorn chicks were injected with 2.5 mg diethylstilbestrol (DES) in sesame oil subcutaneously daily for 10 days. They were then withdrawn from all hormone for 11 days. On the 12th day of withdrawal, they were treated with a single injection of 2.5 mg DES 18 h before sacrifice. For 0 h controls, the final injection of DES was omitted. Total RNA was isolated from the oviduct of these chicks by extraction in sodium dodecyl sulfate (SDS)-phenol by the method of Rosenfeld et al. (5, 16). Each pooled sample was further purified by affinity chromatography on an oligodeoxythymidylate-cellulose column by the method of Aviv and Leder (17). Samples were applied to the column in 0.5 M KCl 10 mM Tris HCl, pH 7.6. The column was rinsed thoroughly with 0.1 M KCl 10 mM Tris HCl, pH 7.6, and the adsorbed fraction was eluted with 10 mM Tris HCl pH 7.6. This polyadenylate-rich fraction was precipitated in 0.25 M NaCl and 2 vol of ethanol at  $-20^{\circ}\text{C}$  overnight.

**Injection of mRNA<sub>ov</sub> into oocytes.** Unless otherwise specified, each type of mRNA preparation was injected into 15 oocytes with glass micropipettes calibrated to deliver 24 nl of the mRNA<sub>ov</sub> dissolved in water. Control injections of identical volumes of water were administered to oocytes from the same animal. Injected oocytes were incubated at  $19^{\circ}\text{C}$  in sterile Barth's medium (14) containing L-[4,5- $^3\text{H}$ ]-leucine 0.5 mCi/ml (sp act 46 Ci/mmol). Upon termination of the incubations, the oocytes were rinsed three times with buffer A (0.0522 M-glycine 0.0522 M Tris, pH 8.9) and frozen at  $-70^{\circ}\text{C}$ . For analysis, each sample was homogenized in 0.75 ml buffer A with ovalbumin 1 mg/ml added as carrier in a glass Teflon homogenizer (Arthur H. Thomas Co., Philadelphia, Pa.) and centrifuged at 50,000 rpm in a type 65 rotor in a Beckman L3-50 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) at  $4^{\circ}\text{C}$  for 60 min. Aliquots of the supernatant fraction were treated with 10% TCA and heated at  $90^{\circ}\text{C}$  for 10 min, and TCA-precipitable radioactivity was collected on glass fiber filters, which were rinsed with ice cold 5% TCA. The filters were counted in Spectrofluor-Triton X-100 (2:1 in volume).

**Sephadex G-150 column chromatography.** Gel filtration on Sephadex G-150 columns was done at  $23^{\circ}\text{C}$  in buffer A on  $0.9 \times 100\text{-cm}$  columns. 1.2-ml fractions were collected, and 25- $\mu\text{l}$  aliquots were counted in Spectrofluor-Triton X-100 (2:1 in volume).

**SDS-gel electrophoresis.** 10% polyacrylamide discontinuous gel electrophoresis was performed on 12-cm gels in

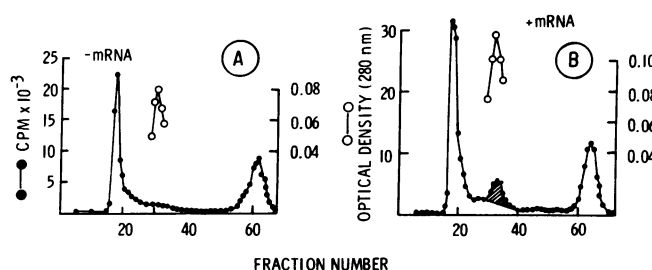


FIGURE 1 Sephadex G-150 analysis. (A) 15 oocytes injected with 24 nl water and (B) 15 oocytes injected with 7.68 ng mRNA<sub>ov</sub> in 24 nl homogenized and centrifuged as described in Methods and analyzed on Sephadex G-150 column 1.2-ml fractions were collected and 25  $\mu\text{l}$  counted in Spectrofluor-Triton X-100 (2:1).

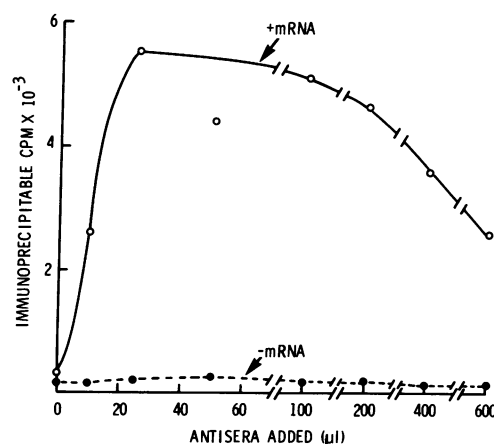


FIGURE 2 Immunoprecipitation of peak<sub>ov</sub>. 50- $\mu\text{l}$  aliquots of peak<sub>ov</sub> from Fig. 1A (water-injected control = 2,400 cpm) and 50- $\mu\text{l}$  aliquots of peak<sub>ov</sub> from Fig. 1B (mRNA<sub>ov</sub> injected = 8,700 cpm) were allowed to react with increasing amounts of ovalbumin antiserum. No attempt was made to use equal counts in the original samples because they both contained equal amounts of carrier ovalbumin, and would have approximately similar equivalence curves.

SDS according to the method of Weber and Osborne (18). Gels were stained with Coomassie Blue and scanned at 600 nm. They were then sliced by a multiple-blade slicer, and 2-cm slices were solubilized in 0.3 ml 30% hydrogen peroxide and counted in Spectrofluor Triton X-100 (2:1).

**Polyacrylamide gel electrophoresis in alkaline urea.** Samples were applied to 10-cm gels containing 7.5% acrylamide and a 0.5-cm stacking gel in 8 M urea, 20 mM Tris HCl, pH 8.7 at room temperature. Electrophoresis was carried out at 1 mA/tube until the marker dye (Bromphenol blue) entered into lower gel and then at 3 mA/tube for about 3 h (19).

**CM-cellulose chromatography.** 2-cm CM-cellulose columns were packed on glass Pasteur pipets and equilibrated with 0.1 M ammonium acetate, pH 3.8. Samples were applied in the same buffer, and the column was successively eluted with 0.1 M ammonium acetate with increasing pH.

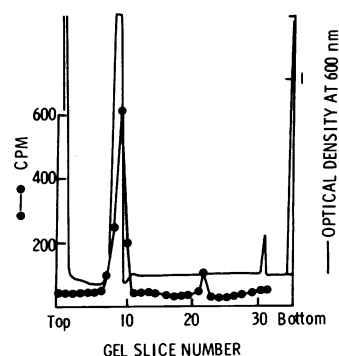


FIGURE 3 SDS-gel electrophoresis. An aliquot from peak<sub>ov</sub> corresponding to approximately 5,000 cpm was run on a 10% SDS acrylamide gel, stained, scanned, sliced, and counted as described in Methods.

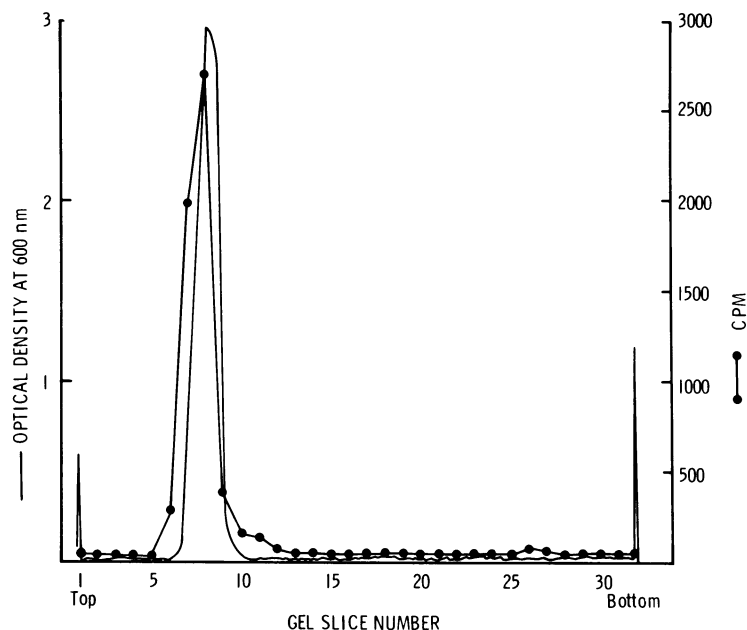


FIGURE 4 Disk-gel electrophoresis in alkaline urea. An aliquot from peak<sub>ov</sub> corresponding to approximately 15,000 cpm was run on a 7.5% acrylamide gel, stained, scanned, sliced, and counted as described in Methods.

1-ml samples were collected, and optical densities of these fractions were read at 280 nm. Aliquots were counted in Spectrofluor-Triton X-100 (2:1).

*Immunoprecipitation with ovalbumin antiserum.* Since carrier ovalbumin (1 mg/ml) was already added to the original sample before G-150 column chromatography, im-

munoprecipitation was carried out simply by the addition of increasing amounts of ovalbumin antiserum. The reaction mixture was incubated at 23°C for 60 min. Under these conditions, 85–90% of authentic [<sup>14</sup>C]ovalbumin is specifically precipitated (15). Immunoprecipitates were pelleted by low-speed centrifugation. Aliquots from the supernatant fraction were counted in Spectrofluor-Triton X-100

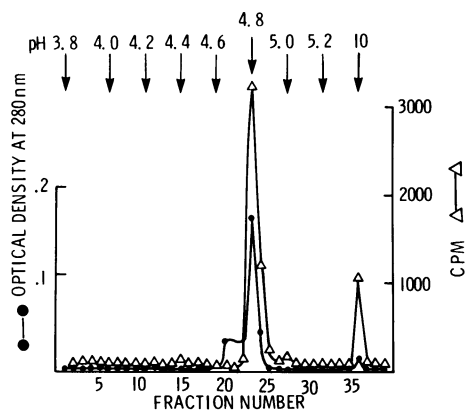


FIGURE 5 CM-cellulose chromatography. A 2.5-cm column of CM-cellulose was set up in a Pasteur pipette in 0.1 M ammonium acetate, pH 3.8. An aliquot of peak<sub>ov</sub> corresponding to 15,000 cpm together with 0.7 mg carrier ovalbumin was loaded on the column in the same buffer. The column was eluted with successive batches of the buffer at increasing pH as shown. 1-ml fractions were collected. Absorbance at 280 nm was determined on whole fractions, before they were counted in Aquasol (New England Nuclear, Boston, Mass.).

TABLE I  
Effect of Microinjection on Protein Synthesis in Oocytes\*

	TCA-precipitable cpm/oocyte $\times 10^{-5}$
Uninjected	2.8
Injected with water†	2.6
Injected with 0.38 ng mRNA <sub>ov</sub> ‡	3.7
Injected with 3.84 ng mRNA <sub>ov</sub> ‡	4.4
Injected with 7.68 ng mRNA <sub>ov</sub> ‡	4.9
Injected with 17.6 ng mRNA <sub>ov</sub> §	4.1
Injected with 0.77 ng mRNA <sub>ov</sub>	1.9

\* Groups of 15 oocytes from the same animal were injected, incubated in Barth's medium containing [<sup>3</sup>H]leucine (0.5 mCi/ml) at 19°C for 20 h. Samples were homogenized in buffer, and TCA-precipitable radioactivity was determined as described in Methods.

† 24 nl per oocyte.

§ 55 nl per oocyte.

|| Oocytes were visibly damaged by the injection process. They were incubated and processed similarly so as to assess the effect of cellular damage to protein synthesis in these oocytes.

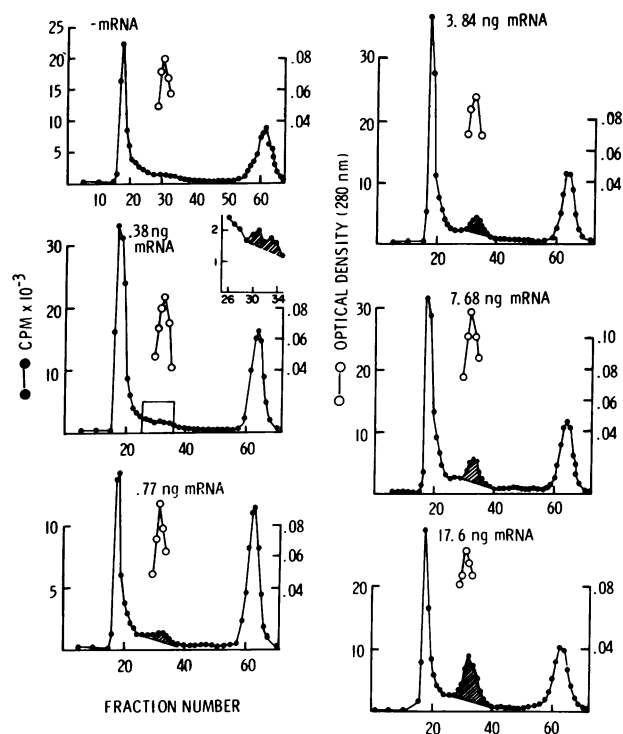


FIGURE 6 Dose-response relationship of mRNA<sub>ov</sub> translation in oocytes: sephadex G-150 analysis. Samples processed as described in Fig. 1 were fractionated on 0.9 × 90-cm Sephadex G-150 columns. 1.2-ml fractions were collected and 25- $\mu$ l aliquots counted in Spectrofluor-Triton × 100.

(2:1). The pellet was washed once in a standard wash buffer (10 mM sodium phosphate pH 7.5, 15 mM sodium chloride, 4 mM L-leucine, 1% sodium deoxycholate, and 1% Triton X-100) and twice in 0.9% saline. The final pellet was dissolved in NCS (Amersham/Searle Corp., Arlington Heights, Ill.) and counted in Spectrofluor (Amersham/Searle Corp.).

## RESULTS

**Ovalbumin synthesis after injections of ovalbumin mRNA<sub>ov</sub>.** When a supernatant fraction of oocytes exposed to [<sup>3</sup>H]amino acids before homogenization was applied to a Sephadex G-150 column, two peaks of radioactivity were routinely observed. The first peak corresponded to nascent radiolabeled oocyte protein which was excluded by the column, and the final peak represented free [<sup>3</sup>H]leucine (Fig. 1A). This pattern was observed when control oocytes injected with water were then incubated in medium containing [<sup>3</sup>H]leucine. An extra peak of radioactivity, however, was observed when mRNA<sub>ov</sub> was injected into the oocytes (Fig. 1B). This extra peak cochromatographed with authentic recrystallized ovalbumin carrier which was monitored by its absorbance at 280 nm.

**Immunoprecipitation of ovalbumin synthesized in *Xenopus* oocytes.** The fractions containing the extra

peak of radioactivity which cochromatographed with authentic ovalbumin were collected and pooled (designated peak<sub>ov</sub>). To test the immunologic activity of the radioactive protein contained in the peak, aliquots were incubated with increasing amounts of ovalbumin antiserum (Fig. 2). The immunoprecipitates were pelleted and washed by repeated centrifugation. The final pellets were solubilized in NCS and counted in Spectrofluor. As shown on Fig. 2, in peak<sub>ov</sub> obtained from oocytes injected with mRNA<sub>ov</sub>, 63% of the radioactivity was precipitated by the antiserum at the equivalence point. Partial solubilization of the immunoprecipitate was observed when excess antiserum was added. In peak<sub>ov</sub> of

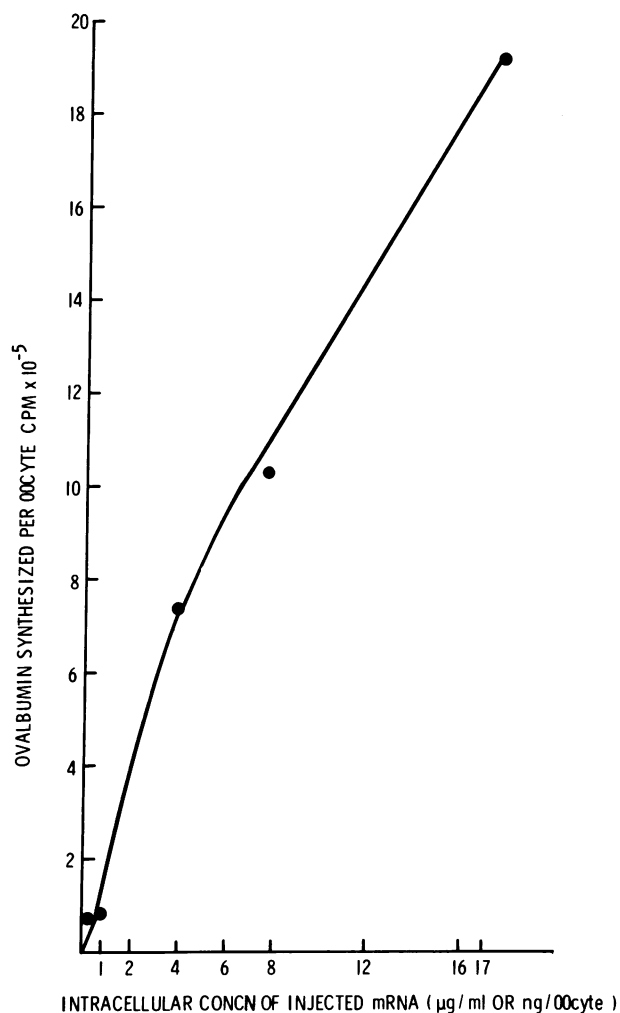


FIGURE 7 Dose-response relationship of mRNA<sub>ov</sub> translation in oocytes: effect on newly synthesized ovalbumin. Peak<sub>ov</sub> from Fig. 6 were collected and counts in ovalbumin determined by immunoprecipitation. Intracellular concentration of injected mRNA is calculated from the amount injected divided by the volume of each oocyte (assumed to be 1  $\mu$ l).

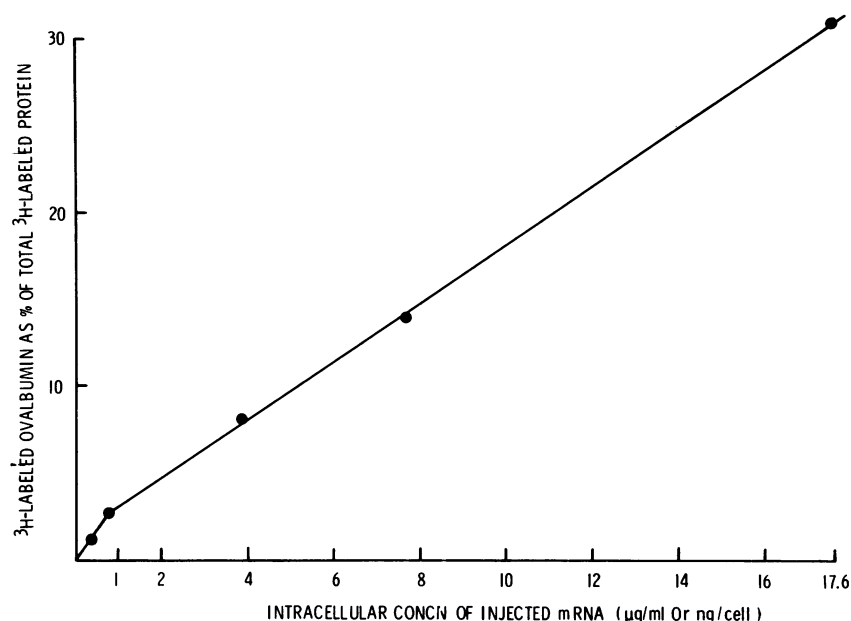


FIGURE 8 Dose-response relationship of mRNA<sub>ov</sub> translation: proportion of newly synthesized ovalbumin as percent of total labeled protein. The counts in total protein were obtained by subtracting counts per minute in ovalbumin determined by immunoprecipitation from total TCA-precipitable counts per minute taken before Sephadex G-150 chromatography.

water-injected samples, only low background radioactivity (0.04%) due to nonspecific coprecipitation was recovered in the precipitate. Since under our conditions of immunoprecipitation about 85-90% of authentic ovalbumin was precipitated, about 75% of the labeled protein under peak<sub>ov</sub> should be considered immunologically similar to ovalbumin.

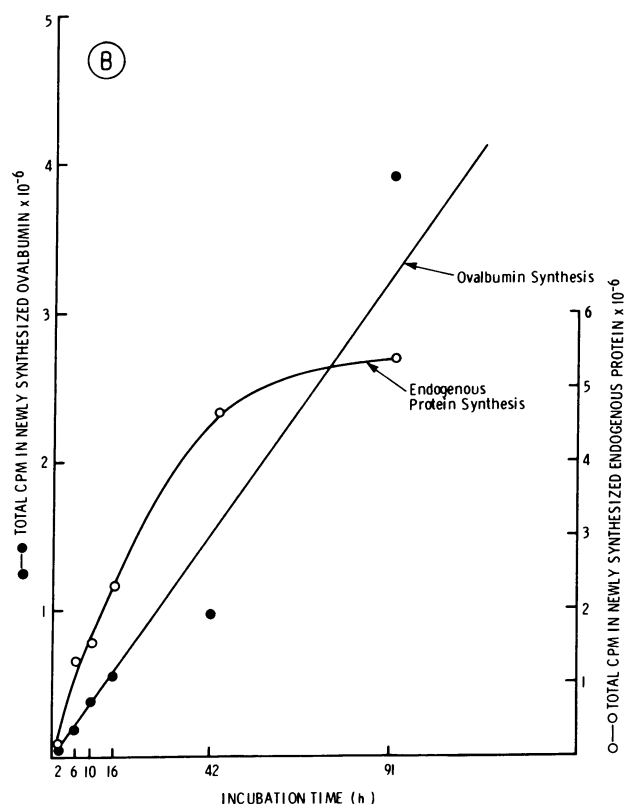
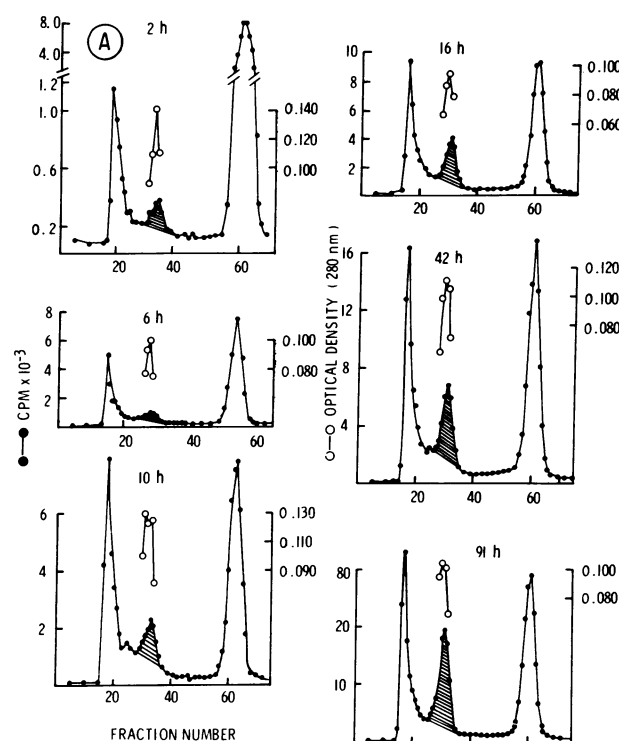
**SDS-gel electrophoresis.** To determine the size of the newly synthesized proteins contained in peak<sub>ov</sub>, the pooled sample was analyzed by gel electrophoresis in 10% polyacrylamide in SDS (Fig. 3). The radioactivity in the gel slices was found to comigrate with authentic ovalbumin added as an internal marker in the same gel. Hence, the major component of the newly synthesized protein under the extra peak of radioactivity had the same molecular weight as native ovalbumin as determined under denaturing conditions.

**Polyacrylamide gel electrophoresis in urea.** To determine the relative net charge of the protein under denaturing conditions, an aliquot of peak<sub>ov</sub> obtained from oocytes injected with mRNA<sub>ov</sub> was run in a 7.5% acrylamide gel in 8 M urea, 20 mM Tris pH 8.7. Peak<sub>ov</sub> was found to migrate to the same region as authentic ovalbumin added to the same gel as an internal marker (Fig. 4).

**CM-cellulose chromatography.** Peak<sub>ov</sub> was analyzed on a 2-cm CM-cellulose column. Both authentic ovalbumin and the majority of the nascent radioactive protein (peak<sub>ov</sub>) were eluted from the column at the identical

pH (4.8). Some residual radioactivity was eluted at pH 10 when no further carrier ovalbumin could be eluted (Fig. 5). These results suggest that the bulk of the radioactive protein in peak<sub>ov</sub> carries the same charge as native ovalbumin. The radioactivity eluted at pH 4.8 but not that eluted at pH 10.0 was found to be totally precipitable in the presence of ovalbumin antiserum.

**Dose-response relationship of injected mRNA<sub>ov</sub>.** To test the dose-response relationship of injected mRNA<sub>ov</sub> in terms of synthesis of ovalbumin in the oocyte, different dilutions of mRNA<sub>ov</sub> were injected into batches of 15 oocytes isolated from the same animal. Incubations were then carried out in the presence of [<sup>3</sup>H]leucine for 20 h. As shown on Table I, total protein synthesis was not affected by the injection procedure itself, since the uninjected and water-injected controls gave the same total counts incorporated into protein. Injection of mRNA<sub>ov</sub>, however, stimulated total protein synthesis. Maximal stimulation was observed when 7.68 ng of mRNA<sub>ov</sub> was injected into each oocyte. One batch of oocytes injected with 0.77 ng mRNA<sub>ov</sub> was visibly damaged but was included to study the effect of partial cellular damage on the response. Total protein synthesis in this instance was below control. All mRNA<sub>ov</sub> samples were injected in a volume of 24 nl except the highest dose used, viz 17.6 ng/oocyte. In this last instance, 55 nl total volume was injected into each oocyte. This large volume may be related to the lower total protein synthesis observed in this group of oocytes.



When the oocyte samples were analyzed on Sephadex G-150 columns, a gradually increasing peak<sub>ov</sub> was observed (Fig. 6). Even at 0.38 ng/oocyte a definite peak<sub>ov</sub> was evident. Individual peaks<sub>ov</sub> were pooled and the radioactivity contained in newly synthesized ovalbumin was determined by immunoprecipitation with ovalbumin antiserum. Fig. 7 shows that the radioactivity incorporated into ovalbumin increases in concert with increasing amounts of injected mRNA<sub>ov</sub>. The radioactivity in the 0.77 ng/oocyte sample was lower than predicted from the curve, probably because of the damage which occurred to the oocytes during the injection (see Table I). However, when [<sup>3</sup>H]labeled ovalbumin was expressed as a percent of the total [<sup>3</sup>H]labeled protein in the oocyte, an essentially linear response was seen, including the damaged sample (Fig. 8). In the oocytes injected with 17.6 ng mRNA<sub>ov</sub>, ovalbumin comprised about 31% of the newly synthesized protein. Saturation was not reached under our experimental conditions.

**Time-course of ovalbumin synthesis.** To determine the time-course of ovalbumin synthesis, batches of oocytes were injected with 3.8 ng/oocyte mRNA<sub>ov</sub> and incubated in the presence of [<sup>3</sup>H]leucine for varying periods of time. Under these conditions, the oocytes were still actively synthesizing new protein at 91 h after the injection (Figs. 9A, 9B). One group of oocytes (data not shown) were incubated in the absence of radioactive amino acids for 8 days and then subsequently incubated in medium containing [<sup>3</sup>H]leucine for 16 h. These oocytes incorporated 70% as much total radioactivity as did the group which was incubated for 16 h in [<sup>3</sup>H]leucine immediately after the injection.

When the samples were analyzed on Sephadex G-150 columns, a peak<sub>ov</sub> was seen at 2-91 h (Fig. 9A). It was also evident in the sample incubated initially in unlabeled medium. In these experiments the [<sup>3</sup>H]leucine-labeled medium was changed every 36 h to ensure an adequate supply of [<sup>3</sup>H]leucine to the oocytes.

The peak<sub>ov</sub> fractions were pooled and analyzed by immunoprecipitation with ovalbumin antiserum. As shown in Fig. 9B, ovalbumin synthesis in the oocytes was linear up to the 91-h sample. Interestingly, endogenous protein synthesis seemed to be inhibited in the samples incubated for long periods of time.

**FIGURE 9** Time-course of mRNA<sub>ov</sub> translation in *Xenopus laevis* oocytes. Oocytes were injected with 3.84 ng mRNA<sub>ov</sub>. They were incubated for varying periods of time and processed as described in Methods. (A) Sephadex G-150 column chromatography was performed as described in legend to Fig. 6. (B) Total new protein synthesis was determined by TCA precipitation in the original samples before Sephadex G-150 chromatography as described in Methods. Newly labeled ovalbumin was determined by immunoprecipitation, and endogenous protein synthesis is obtained by subtraction of immunoprecipitable counts in peak<sub>ov</sub> from total TCA-precipitable counts.

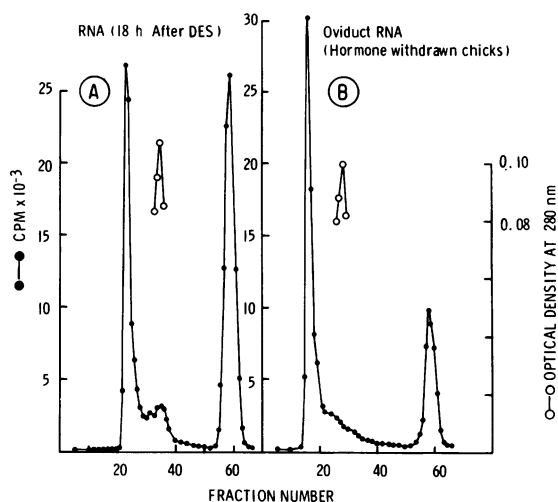


FIGURE 10 Effect of estrogen on mRNA populations in chick oviducts. Groups of chicks were treated with DES 2.5 mg daily for 10 days and then withdrawn from all hormone for 11 days. One group (A) was retreated with a single subcutaneous injection of DES 2.5 mg, 18 h before sacrifice. Control chicks (B) were sacrificed without retreatment with hormone. Total RNA was extracted from oviducts and polyadenylate-containing mRNA fractions were isolated from these preparations by affinity chromatography on oligodeoxythymidylate-cellulose as described in Methods. For the hormone-stimulated sample, 24 nl (35.5 ng) and for the hormone-withdrawn sample, 24 nl (25.5 ng) of Poly A containing RNA was injected. Incubation was at 19°C for 20 h, and the samples were processed and fractionated on Sephadex G-150 column as described in Methods.

**Effect of estrogen on oviduct mRNA.** When partially purified mRNA isolated from oviducts of chicks sacrificed 18 h after receiving DES was injected into oocytes, a definite peak<sub>ov</sub> was observed on the Sephadex G-150 column (Fig. 10). This peak was not readily apparent when a mRNA fraction prepared from oviducts of animals withdrawn from estrogen was injected into the oocyte. Fractions under peak<sub>ov</sub> (fractions 32–38) from each column were then subjected to immunoprecipitation against ovalbumin antiserum (Fig. 11). Whereas the preparation of mRNA from control oviducts did not direct the synthesis of [<sup>3</sup>H]ovalbumin, the mRNA sample prepared from estrogen-stimulated oviducts was shown to contain mRNA<sub>ov</sub> activity.

## DISCUSSION

*Xenopus* oocytes and eggs have been used as efficient translation systems for mRNAs for a number of proteins including rabbit, mouse and duck hemoglobin (11, 12, 20), guinea pig  $\alpha$ -lactalbumin (21), mouse myeloma immunoglobulin light chain (22–24), calf lens crystallin protein (25), encephalomyocarditis virus of mouse (26), honeybee promellitin (27), trout testis protamine

(28), mouse collagen (27, 29), *Rana pipiens* collagen, (30) and beef thyroglobulin (31, 32). We have utilized the same system to translate mRNA<sub>ov</sub>. The ovalbumin synthesized in the oocytes was identified as essentially identical to the authentic protein by the following criteria: (a) it cochromatographed with the native protein on both Sephadex G-150 chromatography and SDS-gel electrophoresis; (b) it carried the same net charge as the native protein on acrylamide-gel electrophoresis in alkaline urea and CM-cellulose chromatography; and (c) it is specifically precipitated with an antiserum against the native protein. In addition, the fraction eluted off the CM-cellulose column at pH 4.8 was also fully precipitated by the antiserum.

It should be noted that even with pure mRNA<sub>ov</sub>, only a proportion of the radioactivity under peak<sub>ov</sub> was precipitable with ovalbumin antiserum. This is explained by the endogenous protein synthesis in the *Xenopus* oocyte. The G-150 column separated ovalbumin from over 90% of the labeled cellular protein, but a significant amount cochromatographed with ovalbumin. The percent precipitable [<sup>3</sup>H]ovalbumin depended on the amount of injected mRNA<sub>ov</sub>; to a certain extent, the more mRNA<sub>ov</sub> injected, the greater was the percent [<sup>3</sup>H]ovalbumin under peak<sub>ov</sub>. The differing amounts of [<sup>3</sup>H]ovalbumin under peak<sub>ov</sub> were not adequately reflected in the SDS and urea gels, possibly because small amounts of protein distributed over the entire gels were lost during the staining and destaining procedures. On the CM-cellulose column, some radioactivity was eluted at pH 10 when no more ovalbumin was eluted. Thus, in this system to quantitate the protein synthesized, it is important to carry out immunoprecipitation with each sample. The usual practice of computing all the radioactivity under the extra peak as a measure of the newly synthe-

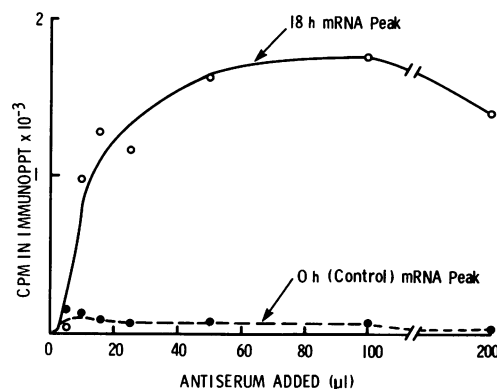


FIGURE 11 Immunoprecipitation of peak<sub>ov</sub> in control RNA and 18-h RNA-injected oocytes. Immunoprecipitation with ovalbumin antiserum was carried out as described in Methods. 50- $\mu$ l aliquots of peak<sub>ov</sub> from 18-h RNA (6,800 cpm) and 0-h control RNA (6,000 cpm) were used.

TABLE II  
Efficiency of mRNA<sub>ov</sub> Translation in *Xenopus laevis* Oocytes

Concentration of mRNA <sub>ov</sub> , ng/ml	320
Injected mRNA <sub>ov</sub> , pg/cell*	7,680
Injected mRNA <sub>ov</sub> , pmol/cell†	0.0118
Number cells/sample	15
cpm [ <sup>3</sup> H]leucine incorporated into ovalbumin/cell/h	3,414.8
Pool size of leucine, pmol/cell§	250.4
Specific activity of intracellular leucine pool, cpm/pmol	828.8
Ovalbumin synthesized, pmol ovalbumin/cell/h¶	0.1287
Number ovalbumin molecules synthesized/h/mRNA <sub>ov</sub> molecule	10.91

\* Assumes no leakage or breakdown of mRNA<sub>ov</sub>.

† Assumes that the molecular weight of mRNA<sub>ov</sub> is 650,000 (15).

§ Eppig and Dumont (35).

|| Calculated from the leucine pool size and the acid-soluble counts per minute per cell recovered from column of Sephadex G-150.

¶ Assumes 32 leucine residues per ovalbumin molecule (36).

sized protein obviously introduces errors in the calculation.

There are certain interesting properties of ovalbumin synthesized in *Xenopus* oocytes as compared to the same protein synthesized in cell-free heterologous translation systems. In both the reticulocyte lysate as well as the wheat germ systems, the translation product identified as ovalbumin migrates as a broad peak on SDS-gel electrophoresis, with an average apparent size distribution slightly smaller than the authentic protein (3, 15). The aberrant mobility of the in vitro product on gels may be related to structural heterogeneity of the carbohydrate chain on the in vitro products as well as to the in vitro synthesis of peptides of differing chain lengths. In the oocyte, the translation product appears to be more homogeneous in size, and to migrate almost identical to the natural protein. This fact and the observation of a similar net charge on CM-cellulose chromatography as well as alkaline urea-gel electrophoresis suggest, but do not prove, that carbohydrate chains are added on to the product synthesized in the oocytes. Preliminary experiments indicate that UDP-*N*-acetylglucosamine may be incorporated into the final ovalbumin molecule in the *Xenopus* oocyte.

We have also presented evidence for the initial demonstration of a steroid hormone-induced alteration in mRNA population as assayed in intact viable heterologous cells. While there was no radioactive immunoprecipitable ovalbumin in samples from oocytes injected with RNA from 3-wk hormone-withdrawn oviducts, it

was clearly shown to be present in samples injected with RNA from oviducts which were stimulated with a single dose of estrogen. The newly synthesized ovalbumin represented about 6.3% of the total labeled protein which would correspond to the injection of 2.8 ng pure mRNA<sub>ov</sub> per oocyte. Since 35.5 ng RNA was injected into each oocyte, it can be calculated that 18 h after a single injection of DES, the restimulated oviduct RNA population contained 7.9% of mRNA<sub>ov</sub> relative to the total poly A-containing mRNA population. This is actually an underestimation since the RNA purified by oligodeoxythymidylate cellulose is still contaminated with at least 50% ribosomal RNA (15). Thus, a better estimate would place the mRNA<sub>ov</sub> content at 15-20% of the total polyadenylate containing RNA pool.

The dose-response curve demonstrates that within the dose range we used for our experiments, the amount of newly synthesized ovalbumin bears roughly a linear relationship to the amount of mRNA<sub>ov</sub> injected. This is true even with oocytes partially damaged by injections if the newly synthesized ovalbumin is expressed as a percent of the total labeled protein. Any damage to the oocytes seemed to impair endogenous mRNA translation to the same extent as translation of the injected mRNA.

It is easily apparent that the *Xenopus* oocyte is an extremely sensitive translation system: the lowest point on Fig. 8 represents an injection of 0.38 ng mRNA<sub>ov</sub> per oocyte to a total of 15 oocytes followed by incubation for 20 h. There were 70,000 cPM incorporated into ovalbumin per oocyte under these conditions. By increasing the specific activity of the incubation medium and prolonging the time of incubation, probably less than 10 pg mRNA can be detected in this translation system.

The efficiency of translation of injected mRNA<sub>ov</sub> molecules compares favorably to that of injected hemoglobin mRNA. As the calculations on Table II show, one molecule of ovalbumin is synthesized from one molecule of mRNA<sub>ov</sub> every 6 min. This is a minimum number since it assumes no leakage, no destruction of injected mRNA or the newly synthesized ovalbumin, and it also assumes absolute purity of the injected mRNA<sub>ov</sub>. This rate of translation also compares favorably to the transit time of 1.3 min calculated for the intact hen oviduct (33). This latter rate was derived at a temperature of 41°C. With the *Q*<sub>10</sub> of 2.2 and an incubation temperature of 19°C, the expected transit time would be approximately 6-7 min. This indicates that the *Xenopus laevis* oocyte is as efficient as the intact hen oviduct in translating ovalbumin mRNA. As reported by Gurdon et al. (34) for hemoglobin mRNA, mRNA<sub>ov</sub> appeared to be remarkably stable up to a period of at least 9 days. During prolonged incubations, mRNA<sub>ov</sub> molecules seemed to compete successfully



against endogenous mRNA for the cellular translation apparatus.

With the development of such an efficient translation assay for a hormone-regulated protein, it would appear feasible to use this system for the detection of biologically intact gene transcripts produced by RNA polymerase from injected samples of chromatin. This is potentially an important biological assay with sensitivities approaching that of hybridization reactions utilizing complementary DNA ( $[^3\text{H}]\text{cDNA}_{\text{ov}}$ ) for ovalbumin mRNA. The oocyte assay for specific mRNA molecules should serve as an important adjunct to  $[^3\text{H}]\text{cDNA}_{\text{ov}}$  hybridization assays since only intact functional molecules of mRNA will be detected in such a system.

#### ACKNOWLEDGMENTS

We would like to thank Mr. K. Bruce Gholson for expert technical assistance. We are also indebted to Dr. Kenneth Lanclos for the initial demonstration of the microinjection technique, to Dr. Stephen Subtelny of Rice University for permission to use his micropipette puller and microforge, and to Dr. Savio Woo for a generous supply of purified ovalbumin mRNA.

This investigation was supported in part by National Institutes of Health grant AM17307-02.

#### REFERENCES

- O'Malley, B. W., W. L. McGuire, P. O. Kohler, and S. G. Korenman. 1969. Studies on the mechanism of steroid hormone regulation of synthesis of specific proteins. *Recent Prog. Hormone Res.* **25**: 105-160.
- Palmiter, R. D. 1972. Regulation of protein synthesis in chick oviduct. I. Independent regulation of ovalbumin, conalbumin, ovomucoid, and lysozyme induction. *J. Biol. Chem.* **247**: 6450-6461.
- Means, A. R., J. P. Comstock, G. C. Rosenfeld, and B. W. O'Malley. 1972. Ovalbumin messenger RNA of chick oviduct: partial characterization, estrogen dependence, and translation in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **69**: 1146-1150.
- Palmiter, R. D., and L. T. Smith. 1973. Synergistic effects of oestrogen and progesterone on ovomucoid and conalbumin mRNA synthesis in chick oviduct. *Nat. New Biol.* **246**: 74-76.
- Rosenfeld, G. C., J. P. Comstock, A. R. Means, and B. W. O'Malley. 1972. Estrogen-induced synthesis of ovalbumin messenger RNA and its translation in a cell-free system. *Biochem. Biophys. Res. Commun.* **46**: 1695-1703.
- Chan, L., A. R. Means, and B. W. O'Malley. 1973. Rates of induction of specific translatable messenger RNAs for ovalbumin and avidin by steroid hormones. *Proc. Natl. Acad. Sci. U. S. A.* **70**: 1870-1874.
- O'Malley, B. W., L. Chan, S. E. Harris, J. P. Comstock, J. M. Rosen, and A. R. Means. 1974. Molecular mechanisms of steroid-mediated alterations in target cell growth and differentiation: induction of specific messenger RNAs. In *Control of Proliferation in Animal Cells*. B. Clarkson and R. Baserga, editors. Cold Spring Harbor Laboratories, N. Y. 85-96.
- Schultz, G., M. Beato, and P. Feigelson. 1973. Messenger RNA for hepatic tryptophan oxygenase: its partial purification, its translation in a heterologous cell-free system, and its control of glucocorticoid hormones. *Proc. Natl. Acad. Sci. U. S. A.* **70**: 1218-1221.
- Mainwaring, W. I. P., F. R. Mangan, R. A. Irving, and D. A. Jones. 1974. Specific changes in the messenger ribonucleic acid content of the rat ventral prostate gland after androgenic stimulation. Evidence from the synthesis of aldolase messenger ribonucleic acid. *Biochem. J.* **144**: 413-426.
- Sippel, A. E., P. Feigelson, and A. K. Roy. 1975. Hormonal regulation of the hepatic messenger RNA levels for  $\alpha_{\text{zu}}$  globin. *Biochemistry.* **14**: 825-829.
- Gurdon, J. B., C. D. Lane, H. R. Woodland, and G. Marbaix. 1971. Use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells. *Nature (Lond.)*. **233**: 177-182.
- Lane, C. D., G. Marbaix, and J. B. Gurdon. 1971. Rabbit haemoglobin synthesis in frog cells; the translation of reticulocyte 9 s RNA in frog oocytes. *J. Mol. Biol.* **61**: 73-91.
- Dumont, J. N. 1972. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* **136**: 153-179.
- Barth, L. G., and L. J. Barth. 1959. Differentiation of cells of the *Rana pipiens* gastrula in unconditioned medium. *J. Embryol. Exp. Morphol.* **7**: 210-222. Plates 1-3.
- Rosen, J. M., S. L. C. Woo, J. W. Holder, A. R. Means, and B. W. O'Malley. 1975. Preparation and preliminary characterization of purified ovalbumin messenger RNA from the hen oviduct. *Biochemistry.* **14**: 69-75.
- Rosenfeld, G. C., J. P. Comstock, A. R. Means, and B. W. O'Malley. 1972. A rapid method for the isolation and partial purification of specific eucaryotic messenger RNAs. *Biochem. Biophys. Res. Commun.* **47**: 387-392.
- Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. U. S. A.* **69**: 1408-1412.
- Weber, K., and M. Osborne. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**: 4406-4412.
- Orstein, L. 1962. Disc Electrophoresis. Eastman Kodak Co., Rochester, N. Y.
- Marbaix, G., and C. D. Lane. 1972. Rabbit haemoglobin synthesis in frog cells. II. Further characterization of the products of translation of reticulocyte 9 S RNA. *J. Mol. Biol.* **67**: 517-524.
- Campbell, P. N., D. McIlreavy, and D. Tarin. 1973. The detection of the messenger ribonucleic acid for the  $\alpha$ -lactalbumin of guinea-pig milk. *Biochem. J.* **134**: 345-347.
- Smith, M., J. Stavnezer, R. C. Huang, J. B. Gurdon, and C. D. Lane. 1973. Translation of messenger RNA for mouse immunoglobulin light chains in living frog oocytes. *J. Mol. Biol.* **80**: 553-557.
- Stevens, R. H., and A. R. Williamson. 1972. Specific IgM mRNA molecules from myeloma cells in heterogeneous nuclear and cytoplasmic RNA containing poly-A. *Nature. (Lond.)*. **239**: 143-146.
- Mach, B., C. F. Faust, P. Vasalli, and D. Rungger. 1973. Different size of the product of the 14S light chain mRNA translated in vitro and in amphibian oocytes. *Mol. Biol. Rep.* **1**: 3-6.
- Berns, A. J. M., M. Kraaikamp, M. Bloemendal, and C. D. Lane. 1972. Calf crystallin synthesis in frog cells:

- the translation of lens-cell 14S RNA in oocytes. *Proc. Natl. Acad. Sci. U. S. A.* **69**: 1606-1609.
26. Laskey, R. A., J. B. Gurdon, and L. V. Crawford. 1972. Translation of encephalomyocarditis viral RNA in oocytes of *Xenopus laevis*. *Proc. Natl. Acad. Sci. U. S. A.* **69**: 3665-3669.
  27. Lane, C. D., and J. S. Knowland. 1975. The injection of RNA into living cells: the use of frog oocytes for the assay of messenger RNA and the study of the control of gene expression. In *Biochemistry of Animal Development*. R. Weber, editor. Academic Press Inc., N. Y. Vol. 3. 145-181.
  28. Wu, Dixon, and J. B. Gurdon. 1974. Quoted by J. B. Gurdon. In *The Control of Gene Expression in Animal Development*. Harvard University Press, Cambridge, Mass. 58.
  29. Knowland, J. S., J. B. Gurdon, and R. A. Laskey. 1972. The Role of RNA in Reproduction and Development. Proceedings of the American Association for the Advancement of Science Symposium, December 28-30. N. C. Niu, and S. J. Segal, editors. North-Holland Publishing Co., Amsterdam. American Elsevier Publishing Co., Inc., N. Y. 110-120.
  30. Rollins, J. W., and R. A. Flickinger. 1972. Collagen synthesis in *Xenopus* oocytes after injection of nuclear RNA of frog embryos. *Science (Wash. D. C.)*. **178**: 1204-1205.
  31. Vassart, G., R. E. LeCocq, H. Brocas, and J. E. Dumont. 1974. Translation in *Xenopus* oocytes of messenger RNA coding for a beef heavy-chain thyroglobin. *Biochim. Biophys. Acta*. **353**: 261-264.
  32. DeNayer, Ph., B. Durieux, Fr. Nyssen-Gautier, and M. De Visscher. 1974. Synthesis of thyroglobulin subunits in oocytes injected with thyroidal ribonucleic acid. *Life Sci.* **14**: 625-630.
  33. Palmiter, R. D. 1972. Regulation of protein synthesis in chick oviduct. II. Modulation of polypeptide elongation and initiation rates by estrogen and progesterone. *J. Biol. Chem.* **247**: 6770-6780.
  34. Gurdon, J. B., J. B. Lingrel, and G. Marbaix. 1973. Message stability in injected frog oocytes: long life of mammalian  $\alpha$  and  $\beta$  globin messages. *J. Mol. Biol.* **80**: 539-551.
  35. Eppig, J. J., Jr., and J. N. Dumont. 1972. Amino acid pools in developing oocytes of *Xenopus laevis*. *Dev. Biol.* **28**: 531-536.
  36. Tristram, G. R., and R. H. Smith. 1963. The amino acid composition of some purified proteins. *Adv. Protein Chem.* **18**: 227-318.