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# Calf Crystallin Synthesis in Frog Cells: The Translation of Lens-Cell 14S RNA in Oocytes

**(messenger RNA/initiation factors/protein synthesis/acetyl methionine)**

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**ABSTRACT** **14S RNA isolated from calf-lens polyribosomes was injected into oocytes of the frog *Xenopus laevis*. Oocytes injected with 14S RNA and buffer contained a protein resembling the A2 chain of calf α-crystallin; oocytes injected with buffer alone contained no crystallin-like material. αA2 crystallin polypeptides were identified by various criteria: urea-gel electrophoresis under acidic and basic conditions, gel electrophoresis in sodium dodecyl sulfate, N-terminal analysis, and paper chromatography of methionine-containing tryptic peptides.**

**It is concluded that when it is injected into a living frog oocyte, the 14S RNA from lens tissue is reasonably stable and has the properties of an αA2 crystallin messenger. The messenger requires no lens cell-specific components for translation within the oocyte, and the translational machinery of the frog cell will accept messenger RNA from a totally different cell type from another species.**

**The A2 chains of α-crystallin extracted from lens tissue possess an acetylated N-terminal methionine residue; the N-terminal methionine of αA2 chains derived from frog oocytes injected with 14S RNA was also acetylated.**

Several interesting questions in developmental biology can be studied by combination of messenger RNA from one kind of cell with the translational apparatus of another cell type. Such experiments can provide proof of the identity of a messenger RNA, and can yield information regarding tissue-specific requirements and species- and cell-type specificities of the translation process.

When 9S RNA from rabbit reticulocytes is injected into frog oocytes, hemoglobin is synthesized, showing the absence of any requirement for tissue-specific factors and the presence within the oocyte of nonspecific translational apparatus (1), This paper describes the synthesis of A2 chains of α-crystallin in oocytes injected with 14S RNA from calf-lens tissue. For translation of the crystallin messenger (2, 3), there is no requirement for tissue-specific factors, and some, at least of the translational apparatus within the oocyte, are neither cell- nor species-specific. Similar conclusions were reached by Lane, Marbaix, and Gurdon (1) with rabbit hemoglobin messenger.

As in αA2 chains extracted from lens tissue (4), chains from oocytes injected with 14S RNA possess an N-terminal acetylated methionine residue. This observation is discussed in terms of the possibility that the protein-synthesizing machinery of different tissues possesses an acetylating mechanism that is able to recognize a certain aminoacid sequence.

Abbreviation: SDS, sodium dodecyl sulfate.

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## METHODS

*Handling of Oocytes.* The injection procedure and culture medium were as described (1), except that batches of 40 oocytes were incubated at 21° in medium containing [$^{35}$S]-methionine (26 Ci/mmol; 0.5 mCi/ml). 14S RNA was dissolved in the injection medium (88 mM NaCl–1.0 mM KCl–15 mM Tris·HCl, pH 7.6) at a concentration of 2 mg/ml.

*Isolation of 14S RNA from Calf-Lens Tissue.* Polyribosomes from calf lens were isolated as described (5), except that cortical, but not epithelial, tissue was used for the preparation. Polyribosomes were suspended (6) in a medium containing 6% (w/w) sucrose, 0.05 M Tris·HCl, and 1% sodium dodecyl sulfate (SDS). The final pH was 7.4. After incubation at 37° for 5 min, the polyribosomes were diluted twice with the same medium lacking SDS. 10–25 ml of sample, containing about 3 mg/ml of polysomal material, was applied to an exponential 8–28% sucrose gradient. After it was overlaid with 150 ml of buffer, the gradient was centrifuged at 50,000 rpm for 15 hr at 2° in a Bxxx IEC rotor. The gradient profile was monitored at 260 nm with a Gilford spectrophotometer, adapted to a 2-mm flow cell. All sucrose solutions used were boiled with 0.02% diethylpyrocarbonate for 30 min. The fraction corresponding to 14S RNA was precipitated by addition of 0.1 volume of 2 M potassium acetate, pH 5.0, and 2.5 volumes of cold ethanol; the solution was allowed to stand for 16 hr at −25°. RNA was pelletted and dissolved in the injection buffer.

*Homogenization of Oocytes.* Thawed samples of oocytes were homogenized in 10 μl per oocyte of a medium containing 0.05 M Tris·glycine, 0.08 M KCl, 0.05 mM methionine, and calf-lens α-crystallin (100 μg/ml). The final pH was 8.9. The homogenate was centrifuged at 3500 × *g* for 15 min at 4°. 0.04 Volumes of 0.2 M EDTA and 0.1 volume of 0.2 mg/ml pancreatic ribonuclease were added and, after incubation at 37° for 15 min, 0.2 volumes of 50% $Cl_3CCOOH$ was added; the precipitated protein was washed four times with 5% $Cl_3CCOOH$, once with ethanol, once with ethanol–ether 1:1, and once with ether. The material was dried at room temperature and subjected to electrophoretic analysis.

*Acrylamide Gel Electrophoresis.* Samples containing about 75,000 cpm of [$^{35}$S]methionine-labeled material were mixed with 25 μg of marker α-crystallin, and were electrophoresed (7) in acidic or basic urea gels. After staining and destaining, the gels were handled as described below. For SDS–gel electrophoresis, the precipitates were dissolved in a solution containing 10 mM sodium phosphate, pH 7.0–1% SDS–1%

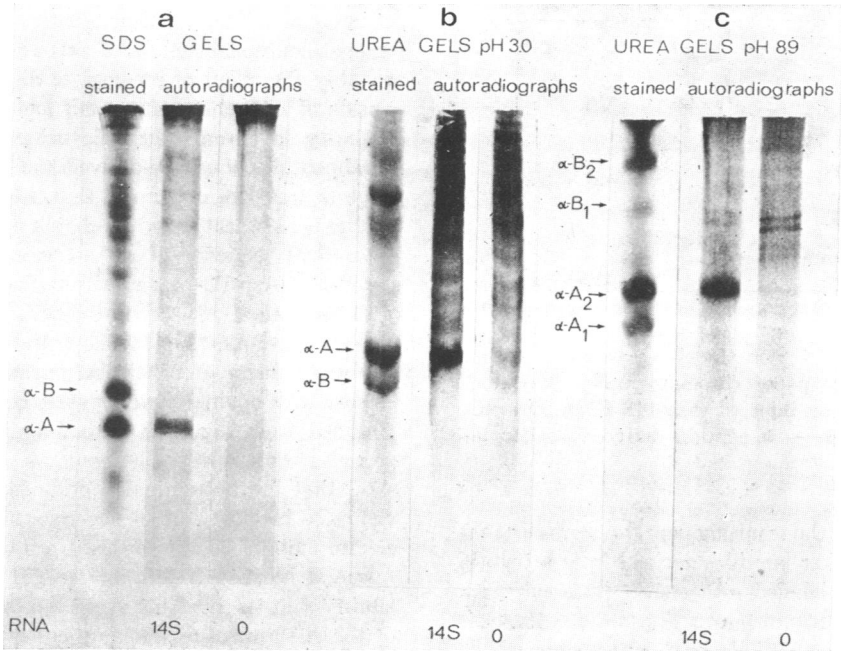


FIG. 1. Oocytes were injected and homogenized with marker α-crystallin; the supernatant was analyzed on polyacrylamide gels.

2-mercaptoethanol, and were heated at 100° for 3 min. Each sample contained about 75,000 cpm of [$^{35}$S]methionine, and electrophoresis was on 12.5% acrylamide gels, prepared according to the method of Weber and Osborn (8), in 0.6 × 0.8-cm glass tubes, at 5 mA/gel for 12 hr. The gels were stained with Coomassie blue, sliced, dried, and autoradiographed for 16 hr with Kodak Royal X-omat type RHP x-ray film.

*Tryptic Digestion of the αA2 Polypeptide.* αA2 chains were separated from the majority of the oocyte proteins by gel electrophoresis on basic urea gels. The gel segment containing the αA2 band was cut out, minced, dialyzed against 5 mM of 2-mercaptoethanol, and, after filtration to remove the gel, the solution was lyophilized. Part of the material so obtained was aminoethylated by the method of Raftery and Cole (9). After precipitation in 15% $Cl_3CCOOH$ and removal of the acid by acetone washing, the material was dissolved in 0.1 M $NH_4HCO_3$ and digested with trypsin (1:50, w/w) for 5 hr at 37°. The resulting peptides were lyophilized, dissolved in water, and subjected to descending paper chromatography on Whatman 3 MM paper eluted with butanol–acetic acid–pyridine–water 60:12:48:40. Tryptic peptides from [$^{35}$S]-methionine-labeled αA2 crystallin prepared by *in vitro* incubation of a lysate from calf lens were used as markers. These markers have the same chromatographic behavior as do the native methionine-containing peptides of αA2 (unpublished results). Chromatograms were dried and cut into 1-cm strips, which were counted in a liquid scintillation counter.

*Pronase Digestion of the αA2 Polypeptide.* Material from the αA2 band was digested (10) in 0.1 M $NH_4HCO_3$–1 mM $CaCl_2$ for 6 hr at 37°. The digest was lyophilized, dissolved in distilled water, and electrophoresed on Whatman 3 MM paper in acetic acid–pyridine–water 6:200:794 (pH 6.5) for 2 hr at 45 V/cm. Radioactivity was determined as described above for paper chromatography. Reference peptides were stained with platinic iodide (11).

## RESULTS

Oocytes were injected with calf-lens 14S RNA dissolved in injection medium, and were frozen after 18 hr of incubation with [$^{35}$S]methionine. Control oocytes were treated similarly, except that the 14S RNA was omitted from the injection mixture. Newly synthesized proteins from both batches of oocytes were then analyzed for their content of α-crystallin-like material.

### Identification of whole α-crystallin components

The radioactive oocyte proteins were first analyzed by SDS–gel electrophoresis, a technique known to resolve α-crystallin into two components, αA and αB, having molecular weights of 19,000 and 22,000, respectively (12). Fig. 1*a* shows that the presence of 14S RNA in the injection mixture is associated with the formation of radioactive molecules that coelectrophorese with the αA component of added marker α-crystallin.

Acidic urea gels also separate the acidic αA component from the basic αB component (7). Fig 1*b* confirms that the presence of 14S RNA in the injection mixture is associated with the formation of oocyte-derived material that coelectrophoreses with the αA component of marker α-crystallin. Basic urea gels resolve α-crystallin into its four constituent polypeptide chains, αA1, αA2, αB1, and αB2 (7). [$^{35}$S]-Methionine-labeled material from oocytes injected with 14S RNA analyzed on such a basic urea gel contained labeled material that coelectrophoresed with the A2 chain of marker α-crystallin. No radioactive material preferentially migrates to the αA1, αB1, or αB2 regions of the gel. If oocytes injected with 14S RNA and controls are labeled with a mixture of tritiated aminoacids (lysine, leucine, and histidine), the three types of gel analysis again reveal labeled αA2 crystallin chains in cells injected with messenger, but not in control cells.

The A2 chain of calf-lens α-crystallin normally contains one acetylated N-terminal methionine and one internal methionine residue (4). αA2 chains isolated from basic urea gels were

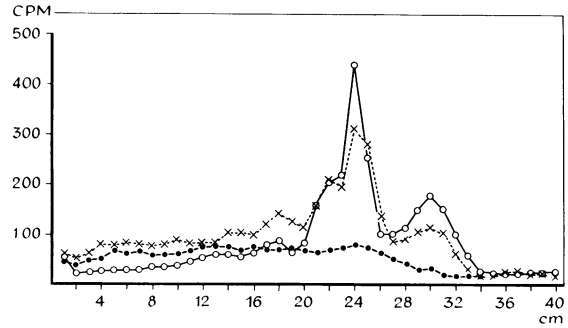


FIG. 2. Identification of the methionine-containing tryptic peptides of αA2 chains by paper chromatography. ×——×, peptides derived from oocytes injected with 14S RNA; O——O, peptides derived from lens; ●——●, peptides derived from oocytes injected with buffer only.

digested with trypsin, and the resulting peptides were chromatographed on paper. Marker peptides derived from a tryptic digest of [$^{35}$S]methionine-labeled αA2 chains from calf lens, were run on an adjacent strip of paper. Fig. 2 shows that the methionine peptides from oocyte-derived αA2 material have the same chromatographic mobility as do the reference peptides derived from calf-lens αA2 material. The fast-moving component shown in Fig. 2 is the N-terminal peptide; the slow-moving component is the internal peptide. The slow-moving peptide is partially resolved into a major and a minor component, the minor component representing the oxidized form of this methionine peptide: the two oxidation states of the fast-moving component are not resolved by this chromatographic solvent. Fig. 2 shows that the internal and N-terminal peptides from oocyte-derived material are not present in equal amounts; this inequality can also be seen in the [$^{35}$S]methionine-labeled αA2 reference peptides, and it is probably the result of incomplete digestion. The N-terminal peptide of αA2 chains from calf lens is acetylated (10); the similarity in chromatographic behavior between the N-terminal peptides of oocyte-derived and lens-derived αA2 chains suggests, but does not prove, that the N-terminal methionine residue is also acetylated in chains made in the oocyte. The N-terminal sequence of all α-crystallin polypeptides is *N*-acetyl-Met-Asp-Ile-Ala; subtilisin digestion releases a peptide of this sequence (13). Pronase digestion releases the dipeptide *N*-acetyl-Met-Asp. Fig. 3*a* shows the result of electrophoresis of the products of subtilisin digestion of [$^{35}$S]methionine-labeled αA2 chains from oocytes; Fig. 3*b* shows the results obtained from digestion with Pronase. Synthetic reference peptides were electrophoresed as standards. The results show that the N-terminal peptide from oocyte-derived αA2 chains is blocked, for in the case of Pronase digestion the electrophoretic conditions used are capable of resolving the free and blocked N-terminal peptides. To exclude the possibility that the blocking agent is a formyl group, one sample of the N-terminal dipeptide was heated with 0.5 N HCl for 30 min at 90° (14): there was no shift of radioactivity to the Met-Asp region of the chromatogram. We conclude, therefore, that frog oocytes programmed with 14S RNA from calf-lens synthesize material that is extremely similar, if not identical, to A2 chains of calf-lens α-crystallin, even to the extent that the oocyte-derived αA2 chains are *N*-acetylated.

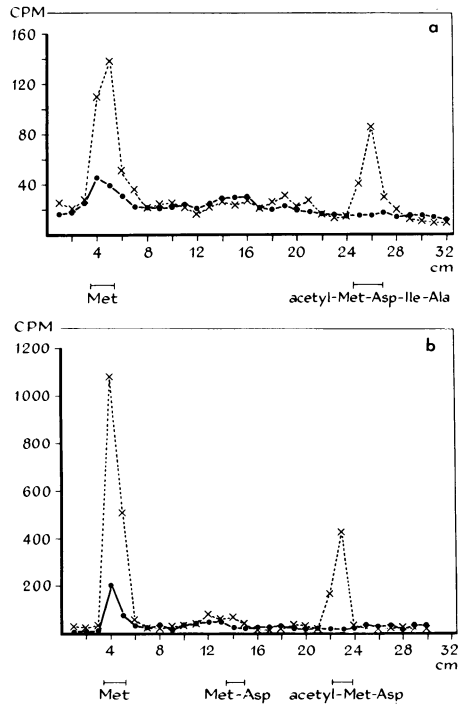


FIG. 3 (*a*). Identification of the N-terminal tetrapeptide of αA2 by paper electrophoresis. (*b*). Identification of the N-terminal dipeptide of αA2 by paper electrophoresis. ×——×, peptides derived from oocytes injected with 14S RNA; ●——●, peptides derived from oocytes injected with buffer only.

## DISCUSSION

Our results show that 14S RNA from calf lens, when injected into a frog oocyte, is not only spared from rapid degradation but is also translated, giving rise to material closely resembling calf αA2 crystallin.

α-Crystallin from calf lens is composed of two acidic polypeptide chains, αA1 and αA2, and two basic chains, αB1 and αB2. The material from oocytes injected with 14S RNA was shown, by SDS–gel electrophoresis, to have a molecular weight identical to that of the acidic chains of α-crystallin. Gel electrophoresis in acidic urea showed that the oocyte-derived material yielded molecules whose overall charge was equal to that of A chains from calf-lens α-crystallin. Gel electrophoresis in basic urea showed that oocytes injected with 14S RNA contain molecules of the same electrophoretic mobility as marker calf-lens αA2 chains. Paper chromatography of methionine-containing peptides showed that those from oocyte-derived αA2 chains were indistinguishable from those of lens-derived αA2 chains.§ Subtilisin and Pronase treatment of αA2 chains from oocytes released methionine peptides that were electrophoretically identical to acetyl-Met-Asp-Ile-Ala and acetyl-Met-Asp, respectively; these peptides correspond to the N-terminal sequence of α-crystallin (10, 13). As judged by all these analytical criteria, control oocytes contained no detectable amounts of crystallin-like substances.

Oocytes injected with 14S RNA contain, therefore, a substance that is extremely similar to A2 chains of calf α-crystallin; however, the criteria used would not necessarily reveal subtle differences in sequence (i.e., we have not yet measured the fidelity of translation).

The results indicate that the 14S RNA fraction from calf

§ Experiments are in progress to try to show that all tryptic peptides from the newly synthesized polypeptides are identical to the tryptic peptides from the native $\alpha A_2$ chain.

lens contains messengers coding for αA2 crystallin chains, in accord with information obtained from cell-free extracts (2, 3). However, the experiments described do not prove that oocytes injected with 14S RNA synthesize calf αA2 crystallin chains; it is conceivable that the 14S RNA elicits the synthesis of frog crystallin chains. However, this possibility is virtually ruled out by data obtained for the analogous situation of oocytes injected with rabbit reticulocyte 9S RNA (1) (15) (Marbaix, G. & Lane, C. D., paper in preparation).

Our results also contribute to the question of the species and cell-type specificities, and requirements of the translation process. Since calf-lens crystallin messenger RNA is successfully translated in frog oocytes, we can conclude that the components required to translate calf-lens αA2 crystallin messenger RNA are present in cells as unrelated as calf-lens cortex cells and frog oocytes. Thus, if messenger-specific components are required for the translation of crystallin messenger, then such components are present and available in frog oocytes. If it is assumed that such factors do exist in oocytes, then it is clear that their presence cannot be the only phenomenon determining the appearance of cell-type specific proteins during cell differentiation. The results strongly suggest, but do not prove, that the translational machinery of the frog oocyte is not cell-type specific:

This observation, showing that no additional factors are required for the translation of exogenous mRNA, is consistent with other experiments performed with whole oocytes (1) and with results obtained from various crude cell-free systems (2, 3, 16–18). Evidence for tissue specificity has only been found with purified cell-free extracts derived from terminally differentiated tissues (20, 21). These experimental results are not necessarily inconsistent, for it is possible that tissue specificity is masked in crude cell-free systems, and that the oocytes do not have such restrictive translational requirements as do the cells of terminally differentiated tissues.

The A2 chains of α-crystallin are N-terminally acetylated (4). Our results show that oocytes injected with 14S RNA give rise to *N*-acetylated αA2 crystallin chains. The same is true of 14S RNA-directed αA2 crystallin synthesis in a reticulocyte lysate (paper in preparation). Three possible acetylating mechanisms may be considered.

(*i*) The 14S messenger RNA, which has an estimated molecular weight of 360,000 (2), is large enough to code for two polypeptide chains, each of 20,000 molecular weight. One of these two chains may be an acetylating enzyme. Or, the αA2 polypeptide may itself have acetylating properties.

(*ii*) The 14S messenger may require acetyl-Met-$tRNA_f^{Met}$ for initiation.

(*iii*) Acetylation takes place after initiation with Met-$tRNA_f^{Met}$, and is determined by the N-terminal sequence.

The first possibility is rendered unlikely by the observation that 10S RNA from lens tissue directs the synthesis of *N*-acetylated α-crystallin B chains in a reticulocyte cell-free system (paper in preparation), while the aminoacid sequences of the B chains are, with the exception of the N-termini, totally different from those of the A chains. The second possibility is unlikely, for no acetylated Met-$tRNA_f^{Met}$ can be detected in lens tissue (unpublished results) or in reticulocytes (19).

The most obvious explanation is, therefore, that the protein synthesizing machinery of different tissues, from different species, possesses an acetylating mechanism that is able to recognize and acetylate a certain aminoacid sequence. One may speculate that all eukaryotic cells possess an acetylating mechanism, ribosome-bound or free in the cytoplasm, of this general nature.

The observed disparity between the size of the 14S message and the newly synthesized polypeptide may be explained in several ways.

(*i*) The 14S mRNA contain rather long untranslated regions. However, the low AMP content (6) makes the occurrence of long A-rich regions unlikely.

(*ii*) The 14S mRNA may be bicistronic.

(*iii*) The 14S mRNA may code for a longer precursor molecule, which is proteolytically trimmed to yield $\alpha A_2$.

Further experiments are necessary to clarify this disparity. The other lens messenger fraction, characterized by a sedimentation coefficient of 10 S, and with an average molecular weight of 260,000, codes for crystallin polypeptide chains of higher molecular weight than $\alpha A_2$ (3).

Our results also show that calf-lens $\alpha A_2$ crystallin chains are stable, at all stages of assembly, in frog oocytes. Moreover, the translation of the crystallin messenger and the stability of the products formed lend support to the idea that the oocyte system may prove to be a generally useful microassay for eukaryotic mRNA molecules.

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